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Fishery and Sea Resources

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Review of Loggerhead Turtle (*Caretta Caretta*) By-catch and Technical Mitigation Measures in the Mediterranean Sea

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Abstract

Incidental catch by fishing gear is one of the major threats to the survival of the loggerhead turtle (*Caretta caretta*) in the Mediterranean Sea. The current paper reviews the turtle bycatch assessment and the gear parameters responsible for turtle capture and mortality, taking into account the possible mitigation measures tested in the Mediterranean Sea. Drifting longlines, bottom trawls and set nets (without considering illegal drift nets) are demonstrated to have the greatest impact on Mediterranean turtles. Nevertheless the type of impact strongly depends on fishing gear characteristics. Different types of fishing gear may induce different mortality rates and may affect different sea turtle ecological phases (pelagic or demersal). The bycatch assessment in different Mediterranean areas was reviewed evidencing the need of a method for homogenising the estimates of turtle bycatch. Most information available is focused on drift longlines but for some mitigation measures, Authors obtained different results in different areas: circle hooks have the potential to reduce turtle mortality only in certain fisheries and areas larger hooks are less likely to be swallowed by turtles due to physical constraints of the mouth, reducing the mortality rate and the catch of juveniles branchlines, once ingested, appear to be one of the major causes of sea turtle mortality; squid bait, which consistently catches more turtles than mackerel, and lightsticks, which strongly attract turtles, should be banned, at least in some areas and seasons. Only two studies were carried out in the bottom trawl where the Turtle Excluder Devices (TEDs) were tested with very good preliminary results. For set nets no practical solutions are available at this time.

1 Introduction

Human activities (incidental catch, pollution, habitat degradation etc.) are considered as the major threats to the survival and to the general decline of marine turtle populations in the Mediterranean Sea [1]. The Barcelona Convention (UNEP MAP RAC/SPA, 2007, [2]) acknowledged that the impact of fishing activities is one of the most important anthropogenic mortality factors for sea turtles in the Mediterranean Sea. Several countries regularly fish in this basin thus the fishing effort is a key factor to take into account in considering the sea turtle conservation [3].

Loggerhead-turtle (*Caretta caretta*) is the most abundant marine turtle in the Mediterranean Sea and the knowledge of its biology represents a key point to evaluate the impact of different fishing activity in different areas. The loggerhead is known to nest in the Mediterranean, especially on the beaches of the East side (Cyprus, Egypt, Greece, Israel, Italy, Lebanon, Libya, Syria, Turkey and Tunisia, Figure 1). Moreover, additional specimens migrate from Atlantic population to Mediterranean Sea through the strait of Gibraltar during the first half of the year [4, 5], Figure 1).

Loggerhead life is characterized by three main ecological phases: the pelagic phase, when loggerheads feed on pelagic preys; the demersal phase, when they swim close to the bottom and feed on benthic species; and finally an intermediate neritic phase, when loggerheads shift from pelagic–oceanic to benthic–neritic foraging habitats [6], Figure 1. The bathymetry and environmental characteristics of different areas determine the distribution and abundance of marine turtles. Loggerheads exhibit high fidelity to migratory routes, foraging areas and wintering sites, between and within years and after successive breeding migrations [7], Figure 1.

Therefore, different types of fishing gear (towed or passive, on the bottom or in the water column) can produce different captures and mortality rates [8] and may affect different ecological phases (Figure 2).

2 Assessment of loggerhead sea turtle bycatch

More than 60,000 turtles per year are estimated to be accidentally caught in the Mediterranean as a result of fishing practices [12]. Direct mortality rates is estimated to be from 10% to 50% and even 100% of individuals caught, depending on fishing gear, area, season etc. Moreover, the delayed mortality, when turtles are released, is mostly unknown. On the other hand [13] highlights an even worse picture suggesting that over 150,000 captures per year may take place in the Mediterranean by trawlers, longliners and set netters, with possibly over 50,000 deaths per year by interaction alone.

2.1 Drifting longline

Drifting longline targeting swordfish (*Xiphias gladius*), albacore (*Thunnus alalunga*) and bluefin tuna (*Thunnus thynnus*) is considered as the most dangerous fishing gear for marine turtles in the Mediterranean Sea, in terms of catch per year [8, 14, 1], Table 1. The incidental capture of loggerhead turtles in pelagic phase mainly extends from spring to late autumn, with most captures occurring during the summer (Figure 1 and Figure 2).

Casale [13] estimated that over 50,000 specimens are probably caught with pelagic longlines with a mortality of 40% mainly in Spain, Morocco, Italy, Greece, Malta, Libya (Table 1). [15] considered at least 60,000-80,000 captures per year in the Mediterranean while [16] found an annual catch of about 35,000 specimens only for the Central-Western Mediterranean Sea. Spain is the country with the highest number of turtle catch per year, especially around the Balearic Islands (Table 1, Figure 2). The high concentration of sea turtles in the western basin is due to the Mediterranean population but also to the entrance of specimens from the Atlantic Ocean via Gibraltar (Figure 1). Moreover, results indicated that longline targeting swordfish is responsible for more abundant incidental catch than bluefin tuna and albacore longlines [17].

Italian waters are populated by turtles migrating from the Eastern to the West-

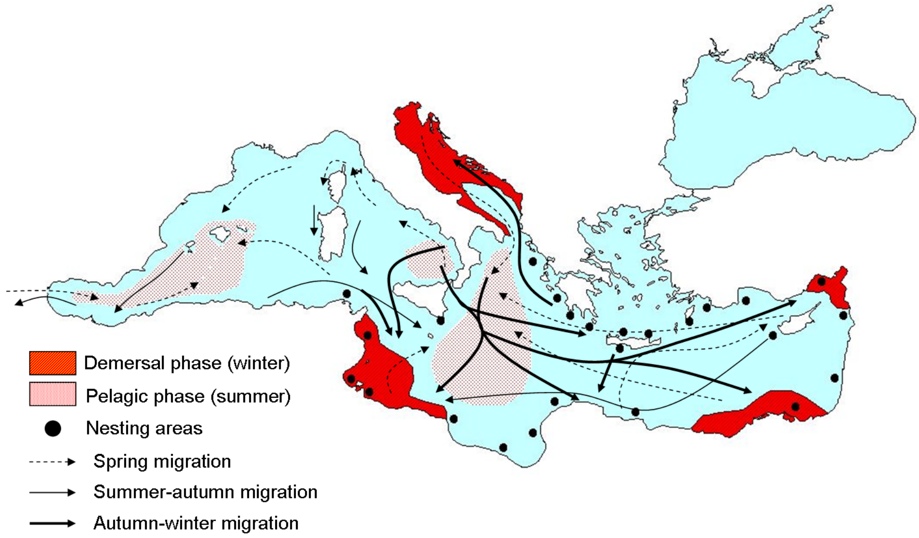

Figure 1: Loggerhead migrations in the Mediterranean Sea: the main routes, nesting beaches, pelagic and demersal areas are shown [9, 7, 10, 11].

ern Mediterranean basin and vice versa through the strait of Messina and the strait of Sicily (Figure 1). The two corridors are characterized by high fishing pressure and in this area loggerhead sea turtle was the second most abundant fished species after swordfish in longline fishery [18]. Jribi and Bradai [19] recorded that most of incidental catches in Zarzis (Tunisia) occurred during summer (Table 1). Data collected in Morocco were very similar [20] while the bycatch data available for Algeria [10] seem to be underestimated (Table 1).

Bycatch due to foreign industrial longline fleets operating in the Mediterranean waters is widely unknown and this could lead to an even worse situation. Data on annual catches are available for other non-EU countries but in some cases there is a little concern on their validity.

2.2 Bottom trawl

Bottom trawl, the second most impacting fishing gears for sea turtle (Table 1), mainly impacts turtles in demersal phase, since they prefer coastal shallow waters where they feed [21, 22]. In some places (Gulf of Gabés, Northern Adriatic Sea, Southern Turkey and Egypt), the continental shelf, which is the feeding habitats for juveniles and sub-adults turtles, is large enough and turtles in demersal phase are used to spend winter time in these areas. Mediterranean bottom trawlers are estimated to catch around 30,000 specimens per year altogether (Table 1), with a mortality of 25% (Figure 2). Moreover it is well known that the same turtle can be caught more than one time.

North Adriatic Sea with its shallow waters ($<$100 m) and rich benthic communi-

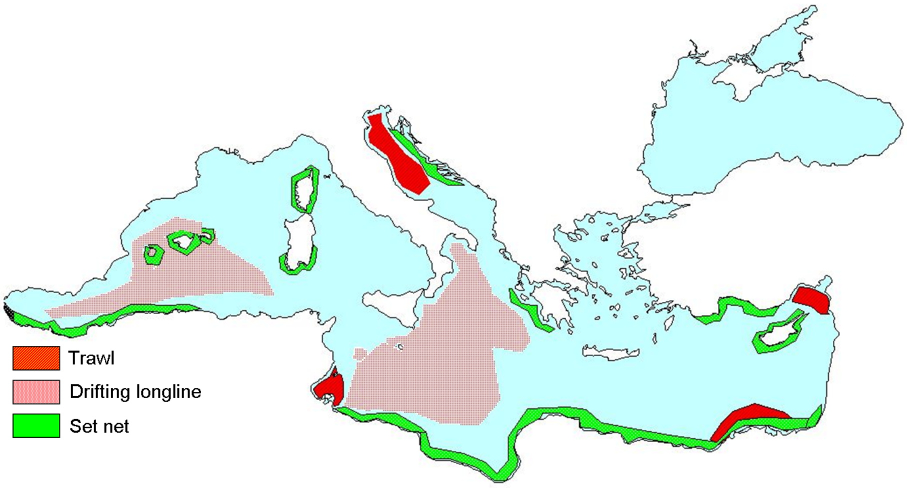

Figure 2: Turtle bycatch in Mediterranean by gear type and area.

ties is considered as one of the most important feeding habitat in the whole Mediterranean, mainly for the population nesting in Greece (Figure 1) and the bycatch estimates for this area are high [3]. A study carried out in the continental shelf south of Lampedusa Island showed considerable catch rates of loggerhead turtle, due to the high fishing pressure (Table 1, [23]). Loggerhead bycatch estimates in the Gulf of Gabès (Tunisia) are comparable with those reported for the North Adriatic (Table 1, [24]), although the estimates for the whole Tunisia gave an even worse picture [19]. Sea turtle bycatch estimated by [25] in Egypt was slightly lower than that observed in the Gulf of Gabès. In the Eastern Mediterranean coast of Turkey, which represents an important nesting and feeding area, [26] observed that turtles captured were mostly juveniles (81%) and most turtles were alive when taken from the trawls.

2.3 Other gears

Illegal drift nets are still used in some countries (i.e. illegal spadara net used in Italy to catch swordfish masked as legal "Ferrettara"), and the amount of bycatch is supposed to be very high. According to [27], Italian drift nets in the Ionian Sea were estimated to catch around 16,000 turtle/year. Moreover drift nets are legal in some non EU countries shifting the bycatch problems from the North to the South of basin. Bottom longlines are generally set at a depth of 200-700 m which not arouses concern however, when these gears are used at a much shallower depth, they cause numerous captures of marine turtles, mainly juveniles. Demersal longlines could be responsible for about 35,000 capture events, mainly in the north African continental shelf, Alboran Sea, the Levantine basin and Aegean [13].

The quantification of captures by fixed nets is very difficult because of the very high number of small boats along the Mediter-

ranean coasts. Over 30,000 captures per year are estimated mainly in coastal areas of Tunisia, Libya, Greece, Turkey, Cyprus, Croatia, Italy, Morocco, Egypt, France [13], Figure 2. Juveniles are frequently caught nearby nesting areas in Greece, Turkey and Cyprus. [28] reported that in Mediterranean French coast loggerhead sea turtles are mainly captured with trammel nets and bottom trawl.
Very little information is available for pelagic pair trawl, even if in the North Adriatic an annual estimate reports that about 1,550 turtles per year are caught accidentally [29] with all turtles released alive. The purse seines seem to represent a minor problem.

3 Technical parameters affecting turtle bycatch and

Loggerhead sea turtle bycatch strongly depends on different parameters. The most important factor is the fishing effort: number of vessels, engine power, Gross Tonnage, time at sea, dimension of the gears (length, high etc). The mortality rate largely depends on gear type, practices on board before and after the catch and on capability of surviving to forced apnoea.
In the longline technical details of hooks can be defined by: shape, dimensions, material (steel, inox), point (with barb or not), shape of eye flat or twisted (Figure 3).
In the hooking process, the most important parameters are: the overall hook width, which can be correlated with turtle mouth dimension, its gap, which ensures deeper penetration of the point and better holding power of the fish, its shape which can influence the hooking position.
The direct mortality induced by longlines appears to be low (Table 1) but the delayed mortality, widely unknown, is suspected to be very high. The low direct mortality can be justified by the fact that the hooked turtles maintain enough power to raise the lines to the surface and to breathe. The post-release mortality strongly depends on the hooks position in the digestive tract. In particular, if the hook is swallowed in the lower oesophagus or in the stomach the turtle has a very low chance of surviving. On the contrary the mortality of turtles with a hook in the mouth or higher oesophagus seems to be less important, even if a hook in the mouth could compromise the feeding performance [22]. Studies made by rescue centres showed a high post-release mortality, both in the short- and in the long-term. The parameters barb size and hook length can affect the capacity of the turtle to disengage: some studies showed that the use of barbless hooks implied a small reduction in unhooking time, leading to an improvement of survival rate.
In addition to the hook, the branchlines, which is the piece of line attached to the hook, can easily cause turtle death after many days, especially if it is long enough to be affected by intestinal peristalsis [30]. Unfortunately fishermen are used to cut the branchline from the deck, while the turtle is still in the water, leaving lethal branchlines longer than 1 m. Albacore and bluefin tuna longlines generally produce higher direct mortality than swordfish longlines. This is probably because of the gear structure and hook size but it could be also due to the fishing depth or to the distance from the coast. Turtle mortality could be strongly affected by the depth of the main line setting. Loggerhead sea turtles spend most of their time at less than 40 m and they do not dive deeper than 100 m. Thus the main

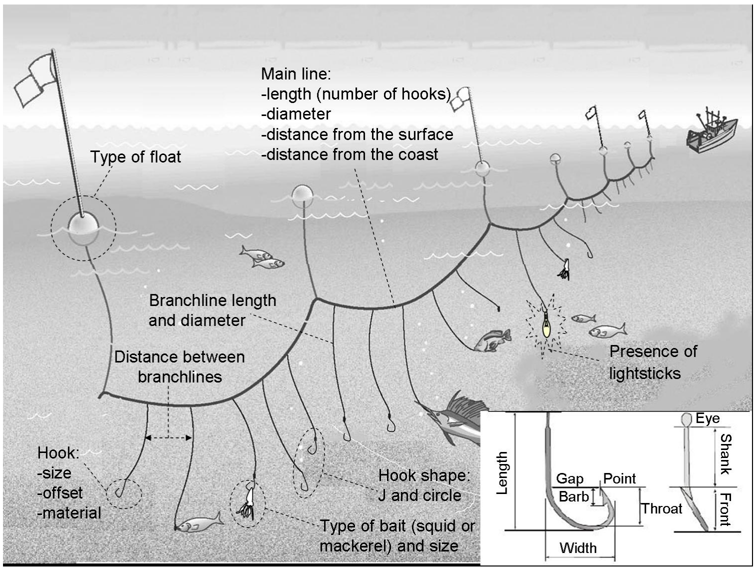

Figure 3: Longline technical parameter affecting turtle bycatch.

interaction depth with longline is the upper 20 m of the water column. The potential mortality rate in bottom longlines about (40%) seems to be concentrated on juveniles because bigger specimens are able to drag the main line with its weights up to the surface, while juveniles cannot reach the surface to breathe [13]. Finally turtle mortality seems to be correlated to the time setting: the longer the set time, the more turtles captured die. Loggerhead bycatch in bottom trawl probably occur during towing operations when turtles are foraging on the bottom. Direct mortality is very low even if a single turtle can be recaptured more than one time. Mortality by trawling is due to forced apnoea, thus longer or faster tows are responsible of higher mortality rates.

As it concerns passive nets it is realistic to consider that this fishing gear has the potential to be more harmful than pelagic longlines and bottom trawl. Studies on gillnets and trammel nets found that these gears are responsible of high turtle direct mortality (from 50 to 100%, Table 1). Drowning is the main reason for the sea turtle mortality since turtles get entangled in nets when trying to feed on fish previously captured (Table 1). To this, environmental parameters could affect sea turtle mortality: high water temperature (such as in the North Africa countries) associated with high metabolic rates, can strongly reduce the resistance to forced apnoea.

4 Mitigation measures

The identification of methods aimed at reducing or preventing sea turtle bycatch is a high priority for fishery managers. The most obvious and simplest approach to solve the bycatch problems is the reduction of the fishing effort, even if this is often not a practical or viable option for economic, social and sustenance reasons. A summary of the main mitigation measures tested in the Mediterranean Sea is shown in Table 2.

4.1 Drifting longline

Several measures have recently been proposed to reduce sea turtle capture and mortality (see for a review [31]) but few studies have been performed in the Mediterranean Sea. Among these solutions three are particularly promising: reducing the hook size, setting the hooks deeper in the water column and changing the hook shape.

Hook size. The hook size influences the probability that a hook is swallowed and the turtle becomes hooked internally, increasing the delayed mortality after the release. The reduction in the hook size is the easiest solution because larger hooks are less likely to be swallowed by turtles due to physical constraints of the mouth. In the Balearic Islands [32] found that the hook size is the most important cause for the deep-hooking. They also found that large hooks reduce the incidence of hooking injuries, with a small reduction in catch rate. Similar results were obtained in Tunisia and the Ionian Sea, where the smaller hooks of bottom longlines seemed to be more easily swallowed in the digestive tract in comparison with hooks of surface longlines, which remained in the mouth.

Hook shape. The shape greatly affects the hook position in the mouth and the capacity of a turtle to disengage from the hook. As it concerns the Mediterranean, one of the most important mitigation measures tested is the change in hook shape from traditional "J" shape to a "circular" one.

The efficacy of circle hooks in reducing turtle bycatch and throat hooking is clearly demonstrated only in certain fishing grounds. Some authors found that circle hooks tend to be located mostly externally in the jaw or mouth as opposed to deeper hooking.[33] found that circle hooks can effectively reduce the number of immature loggerhead sea turtles accidentally captured by up to 70% without affecting the capture rate of target species (swordfish). They also found that all hooked turtles were released alive, irrespective of the type of hook. This seems to confirm the very low direct mortality rate due to shallow-set longline gear activities. They also showed that 81% of the hooking occurred in the mouth, while 19% were swallowed, all of which were on J hooks.

On the contrary the results obtained in a project carried out in the Atlantic and Mediterranean areas [34] do not support the promotion of shifting from J hooks to circle hooks, as these hooks did not consistently reduce turtle catch rates and had negative impacts on swordfish catches.

In Spanish [35] compared circle and J shape hooks but they did not find any significant difference in turtle bycatch and in tuna catches. [36] noticed that circular hooks seemed to shift the bycatch problem from turtles to cetaceans and sharks.

Bait types. In non Mediterranean experiments (U.S. and Pacific data) mackerel bait reduced turtle bycatch compared to squid bait (82%) and increased the catch of swordfish compared to squid bait. This was probably due to the fact that fish bait (mackerel) tends to come free of the hook while the turtle takes small bites from it. On the contrary squid remains more firmly attached, requiring the turtle to take larger bites in order to swallow the bait increasing the chances of becoming hooked. In captivity studies [37] found that mackerel (Scomber spp.) bait smell was an important component for the detection of bait by

turtles. Some authors [35] showed that turtle bycatch was lower with mackerel baits, whereas the target species (swordfish) had no important difference. Results obtained in the EU project UE-FISH/2005/28-A [34] showed that the combination of hook and bait type that resulted in the lowest bycatch of turtles and the highest catches of swordfish was J hooks with mackerel bait.
Depth setting. In the Balearic Islands tests were carried out positioning longlines at a different depth [37]. They found a reduction in turtle bycatch in the deeper longline but, on the other hand, they also found a reduction in the catch target species. In the same area [38] observed that probability of catching at least one loggerhead was related not only to the setting depth but also to the distance of the fishing-ground to the coast.
In the Ionian Sea preliminary results of project Life Nature 2003 – NAT/IT/000163 seem to indicate that most sea turtle bycatch happened when hooks are set between 10 and 15 m deep. Other studies [39] showed that the maximum depth at which the marine turtles were caught was 60 m for swordfish longline and 20 m for albacore longline.
Sensory stimuli. One approach to reduce sea turtle interactions with longline fisheries can also take into account the behaviour of sea turtles and the factors that lead them to interact with fishing gear. An overview of sound, chemical, and light detection in sea turtles is provided by [40].
Fishermen have often suggested that hooks set closer to the floats have a higher probability of catching turtles accidentally.
Experiments carried out in Italy aimed at evaluating turtle behaviour with respect to lit as well as unlit white floats [41]. The results seemed to indicate that the distribution of the turtles in the tanks was not affected by the presence of floats either lit or unlit. Moreover, the effect of bait colours and bait odour was tested: colour attractiveness demonstrated to be partially age dependent. Young specimens never attacked a red-coloured sheath, whereas subadults apparently prefer this colour. Some other studies [42] showed that blue bait was not effective in reducing sea turtle capture rate than untreated bait.
Swimmer and Brill [42] highlighted the importance of olfactory and acoustic stimulus in turtle reaction. Chemical cues can play a key role in the sea turtle bite/no bite decision once a food item has been visually located.
[43] demonstrated that loggerheads had an ability to distinguish between fishing lures based on odour.
Recent behavioural experiments indicate that lightsticks used in many longline fisheries attract sea turtles. The participants to the Working Group on bycatch/incidental catches (Rome, Italy, 15-16 September 2008) recommended to seriously considering the opportunity of banning lightsticks and any light source in pelagic longline fishery.
Considering the acoustic stimulus experiments carried out in the Cattolica “Delphynursery” the most frequent turtle behaviour at all frequencies was “no response” and this results, even if based on a small sample, together with the increased level of acoustic pollution in the Mediterranean did not encourage continuation of this type of experiments.

4.2 Bottom trawl

In order to reduce the time of submergence and than the turtle mortality, a specific technical modification was proposed in the early 1980s: the Turtle Excluder De-

vice (TED). TED is a sort of grid, which diverts large objects or animals like turtles towards a special exit positioned before the codend. TEDs have demonstrated to be very effective mainly in prawn trawl fisheries; for this reason several countries adopted TEDs as mandatory management measure.
Some authors believed that TEDs available at present are probably not a realistic solution for Mediterranean mixed bottom trawl fisheries. Conversely in two recent experiments, carried out in the Mediterranean trawl fisheries by [44] and [45], the authors found that TEDs can be properly proposed as management tool for the conservation of marine turtle population in the Mediterranean Sea, at least in certain periods and areas. They obtained very good results because sea turtles were excluded by the Supershooter TED, and unwanted incidental catches were also excluded. The Supershooter TED also reduced the debris and improved fish quality in the catch. This might imply a reduction of additional sorting operations on board, increasing time and costs. Authors, in agreement with the doubts expressed by [23], stressed the fact that additional tests should be conducted in order to evaluate the loss of large fish.

5 Discussion

The review allows to conclude that surface longlines, bottom trawls and illegal driftnets are the major threats to the survival of this species, even if the impact of fixed nets (gillnets and trammel nets), due to their high direct mortality, should be carefully considered. Nevertheless, turtle bycatch assessment reported in several papers seem to be unreal. This is mainly due to the methods applied for the data standardization. Authors often assumed that CPUE was homogeneous by area, season and boat, which is not exactly so. We believe it is incorrect to extrapolate to all boats and all year the results of a fraction of one season and fleet. Thus, a method for homogenising the data collection through the calibration of the procedures for the bycatch estimation would be very useful in the Mediterranean Sea, in order to provide fishing managers with reliable data.
Bottom trawls mainly impact turtle population during winter time while longlines and fixed nets have their main influence from spring to autumn with most captures occurring in the summer. Finally the assessment of turtle bycatch in some non-EU countries, mainly in those countries where nesting beaches are observed, would be essential for the conservation of the sea turtle population.
The following recommendations and remarks can be summarized for the Mediterranean.
Regarding the longline, the efficacy of circle hooks in reducing the turtle bycatch is widely investigated but contrasting results have been obtained. Further investigation of post-hooking mortality in loggerhead sea turtles would be very useful to determine the real effects of J and circle hooks. Fishermen stated that it is more difficult to release turtles from circle hooks than J hooks, which could also affect turtle survival and requires further investigation. Thus, it is possible to conclude that circle hooks have the potential to reduce the turtle mortality only in certain fisheries and areas, but rigorous field tests should be conducted before requiring circle hooks as a mandatory measure.
In combination with hooks the branchlines appear to be the major source of sea turtle mortality. A relatively short piece of branchline left in the mouth of a released turtle can easily cause death if ingested and further research on branchline characteristics (material, length, thickness etc.)

should be carried out in order to reduce turtle mortality.
Changing the bait from squid to mackerel has been demonstrated to be effective in reducing incidental capture of loggerhead without affecting target species catch. We believe that a very simple, relatively cheap and effective method to reducing incidental catches would be using mackerel bait at least during those periods when turtles are most abundant.
Moreover, we strongly suggest taking into consideration the possibility of banning the lightsticks and any light source in pelagic longline fishery which can strongly attract loggerhead sea turtles.
As it concerns dyeing baits, the importance of physical factors (i.e. light penetration and colour absorbance with the depth, currents, oceanographic factors, temperature etc.) makes very difficult to adopt different bait colours as a mitigation measure. Moreover, the reaction to different colours strongly depends on individual age as well as other factors, such as smell. Another important factor is the isolation of a single turtle in a captive environment. In the light of these problems we believe that tank tests should be considered with caution.
Experiments with acoustic deterrent demonstrate that habituation to acoustic signals is an important issue with respect to the feasibility and long-term effectiveness of an acoustic deterrent. Results showed that there is a low possibility that an acoustic signal could selectively deter sea turtles from interacting with longline gear without affecting commercial species. This does not encourage continuing these types of experiments.
Some discrepancies concerning fishing practices and scientific strategies were observed in several experiments carried out in various countries and fleets. The lack of standardization might explain the discrepancies. We believe that technical parameters affecting turtle bycatch and mortality should only be studied one at a time (hook size, hook shape, set depth, distance from the coast etc.) to avoid inconclusive results.
For bottom and mid-water trawl we think TEDs could probably represent a suitable solution in these fisheries but only when properly matched to fishing conditions (i.e. TED angle, construction materials, floatation, position and size of the exit hole, webbing flap etc.). The introduction of TEDs may reduce turtle mortality by avoiding the multiple submergences of a turtle. Additionally, TEDs could reduce the amount of discard in the codend catch leading to an improvement of fish quality and to a reduction of the sorting time. However, there might be a risk in increasing the losses of large commercial flat species. In the light of bycatch estimates we recommend to assess the impact of pelagic trawl on turtle population more in depth; moreover, we suggest trying TEDs in particular fishing areas and fisheries such as the prawn fisheries in Sicilian, Turkish and Tunisian waters. Towing time would probably need to be to 10 min or less in order to achieve the negligible mortality of $<1\%$. Obviously in our opinion this is not a practical solution.
Direct mortality associated with passive nets seems to be very high in comparison with other fishing gears but no practical solutions other than changing mesh size or twine thickness are available at this time in the Mediterranean Sea for set nets. Nevertheless, we consider the use of high hanging ratio, which makes the nets tighter, and the use of gillnets instead of trammel nets, could reduce turtle entanglements. Further studies to develop excluder devices in set nets would be necessary. However, measures other than technical solutions should be considered to reduce interactions with this gear, such as spatial and temporal measures. Finally, we strongly support the banning of driftnets in all countries of the Mediterranean due to the very high bycatch

rate of these nets. As a general result the direct mortality observed at gear retrieval is often very low for most fishing practices except for fixed nets. However the post-release mortality is suspected to be very high. We believe that further studies with video-camera, satellite tags etc. focused on the delayed mortality would be very useful in order to understand the efficacy of the mitigation measures.
Furthermore, some simple modification to common fishing practices would be useful. The increase of setting depth for longlines has been found to decrease the overall catch rates of turtles but it has led to increase mortality but also to a reduction in the catches of target species. The distance from the coast is a parameter that fisheries managers should take into account for the conservation of sea turtle population.
Loss of hooks, bait, branch lines and other components of the gear and loss of time (to repair or replace the gear) are economic concerns needing to be solved. In any case fishermen cooperation is essential for the survival of sea turtles after catch. Comatose specimens can survive or die, depending on the circumstances; turtles caught are generally hauled on board in a very weak or comatose state and should not be released immediately because they cannot swim to the surface to breathe and in these cases the probability of drowning is very high. Therefore, awareness campaigns (handling practices) and tools (dehooking devices) given to fishermen so that they can cut branchlines and remove hooks whenever possible are valuable as mitigating measures. Thus this problem can be substantially reduced by simply keeping the turtles onboard and allowing them to recover. Fishermen acts at this time can greatly affect the probability of surviving [33].
In summary, reducing loggerhead bycatch in the Mediterranean Sea will only be successful through a multidisciplinary approach of taking into consideration changes to fishing gear and practices, management policies, turtle reaction behaviour to different stimuli and the continual education and updating for fishermen.

Current paper is taken from: "Lucchetti Alessandro, Sala Antonello. An overview of loggerhead sea turtle (*Caretta caretta*) bycatch and technical mitigation measures in the Mediterranean Sea. Rev Fish Biol Fisheries DOI 10.1007/s11160-009-9126-1".

GEAR TYPE	AREA	CATCH / YEAR	DIRECT MORTALITY	REFERENCE
Drifting longline	Entire Mediterranean	50,000	0-4% (40% potential)	Casale 2008
	Entire Mediterranean	60,000-80,000	Potential mortality 17-42%	Lewison et al. 2004; NMFS 2001
	Western-central Mediterranean	35,000		Panou et al. 1992
	Spain	22,000-35,000	0.36-7.7% (20-30% potential)	Aguilar et al. 1995; Tudela 2000, Carreras et al. 2004
	(Balearic Islands: 15,000-18,000; Aguilar et al. 1995; Camiñas 1988; Camiñas et al. 2001; Mayol et al. 1988)			
	Spain		0.54- 4.24%	Camiñas et al. 2006
	South Sicily (Italy)	2,148	>30% potential	Casale et al. 2007a,b
	Lampedusa Island (Italy)	245		Casale et al. 2007a
	Ionian sea (Italy)	1,084-4,447	0% (potential mortality high)	De Florio et al. 2005
	Ionian sea (Greece)	280-3,181		Panou et al. 1999; SGRST-SGFEN 2005
	Aegean and South Ionian seas (Greece)	1,145-5,474		Kapantagakis and Lioudakis 2006
	Cephalonia (Greece)	50		Panou et al. 1992
	Malta	1,500-2,500		Gramentz 1989
	Cyprus	2,000		Godley et al. 1998
	Morocco	3,000		Laurent 1990
	Algeria	250-300		Laurent 1990, Camiñas 2004
	Tunisia	486-4,000	0% (9.1% potential)	Echwikhi et al. 2006; Salter, 1995; Demetropoulos, 1998; Jribi et al. 2008
Bottom trawl	Entire Mediterranean	30,000	5% (20-25% potential)	Casale et al. 2004; Casale 2008; Laurent et al. 1996; Lazar and Tvrkovic 1995; Oruc 2001
	Italy	8,500	14% (57% potential)	Casale et al. 2004; 2007a; De Metrio and Casale 2001
	Lampedusa Island (Italy)	4,056		Casale et al. 2007a
	North Adriatic	4,273	9.4% (43.8% potential)	Casale et al. 2004
	France		3.3-3.7%	Laurent 1991; Delauguerre 1987
	Croatia	2,500	low	Lazar and Tvrtkovic 1995
	Thracian sea	0-418		Margaritoulis et al. 2001
	Ionian sea (Greece)	0-448		Margaritoulis et al. 2001
	Tunisia (whole continetal shelf)	14,000		Jribi and Bradai 2008
	Gulf of Gabés (Tunisia)	2,500-5,500	3.3%	Bradai 1992; Jribi et al., 2004
	Egypt	2,269- high	1-10%	Nada and Casale 2008; Laurent et al. 1996
	Turkey	High	1.6% (13% potential)	Oruç A (2001); Oruç et al. 1996
Drift Nets	Italy	16,000	20-30%	De Metrio and Megalofonou 1988
	Ligurian and Tyrrhenian sea (Italy)	low		Di Natale 1995
	Spain	117-354	3.3%	Aguilar et al. 1995
	Spain	236		Silvani et al. 1999
Bottom longline	Entire Mediterranean	35,000	Potential mortality 40%	Casale 2008
	Lampedusa Island	257		Casale et al. 2007a
	Tunisia	733-2,000	0.53%-12.5% (33% potential)	Echwikhi et al. 2006; Jribi et al. 2008; Bradai 1993
	Egypt	2,218		Nada and Casale 2008
Fixed nets	Entire Mediterranean	30,000	>50% (60% potential)	Casale 2008
	Balearic islands (Spain)	209	50-100%	Carreras et al. 2004
	Corsica (France)	low	93.3-75%	Laurent 1996; Delaguerre 1987
	France	10-100-low	50-100%	Laurent 1991
	Italy		50%	Argano et al. 1992
	Slovenia-Croatia	657-4,038	50% - 73%	Lazar et al. 2006
	Cyprus	500	10%	Godley et al. 1998
	Tunisia	920-2,000	5%	Bradai 1993
	Egypt	754		Nada and Casale 2008
	Turkey	1,328	10%	Godley et al. 1998
Pelagic pair trawl	North Adriatic	1,550		GFCM-SAC 2008
	Turkey	high (5 trawlers catch around 100 loggerheads)		Oruç A (2001)
Purse seine	Egypt	37		Nada and Casale 2008
Small scale fishery fixed nets, purse seines, bottom and surface longlines etc.)	Tunisia	5,000		Bradai 1995

Table 1: Assessment of turtle bycatch and direct mortality in Mediterranean by gear type.

GEAR TYPE	MITIGATION MEASURE	AREA	ACTION	REFERENCE
Drifting longline	Hook size	Mediterranean	Smaller turtles are caught by BLL (smaller hooks)	GFCM-SAC 2008
		Ionian Sea (Italy)	Larger turtles are caught by SWO-LL (larger hooks)	De Florio et al. 2005
		Balearic Islands (Spain)	Smaller hooks caused deep-hooking	Alòs et al. 2008
		Tunisia	Smaller hooks (BLL) caused deep-hooking	Jribi et al. 2008
	Hook shape: J vs circle hook	Atlantic - Mediterranean	Results do not support move from J to circle hook	project UE-FISH/2005/28-A
		Italy	Circle hooks reduced turtle bycatch; circle hooks were not easily swallowed	Piovano et al. 2009
		Spain	Hook type is not the main factor for turtle bycatch reduction	De La Serna et al. 2008
		Spain	Inconclusive results	Parga 2008
		Spain	Circle hook can shift the problem from turtle to cetaceans or sharks	Camiñas and Valerias 2001
		Western Mediterranean	No evident differences	Rueda and Sagarminaga 2008
			Circle hook only reduce leatherback turtle bycatch	Casale 2005 (review of Watson et al. 2003; 2004)
			Circle hook effective in reducing turtle bycatch in several countries, circle hooks reduce the rate of hook ingestion	Several authors (i.e. Watson et al., 2004; NGO web-sites etc)
	Bait Type	Spain	Mackerel bait instead of squid bait reduce turtle bycatch	Rueda and Sagarminaga 2008
		Atlantic - Mediterranean	Mackerel bait instead of squid bait reduce turtle bycatch	project UE-FISH/2005/28-A
			Mackerel bait instead of squid bait reduce turtle bycatch	GFCM-SAC 2008
	Depth setting	Atlantic - Mediterranean	Deep longline: reduction in turtle bycatch, reduction in target species	Rueda and Sagarminaga 2008
		Ionian Sea (Italy)	Most of turtle bycatch between 10-15 m depth	Project Life Nature 2003 - NAT/IT/000163
		Mediterranean	SWO-LL catch turtle at a depth < 60m; ALB-LL catch turtle at a depth < 20m	Laurent et al. 2001
	Sensory stimuli	Italy (tank tests)	Floats presence or absence does not influence turtle behaviour	Piovano et al. 2002
		Italy (tank tests)	Odour play a key role in the bite-no bite decision	Piovano et al. 2004
		Spain	Lightsticks attract sea turtle	Rueda and Sagarminaga 2008
		Italy (tank tests)	Acoustic tests: inconclusive results	Piovano et al. 2002
Bottom trawl	TED (Turtle Excluder Device)	Italy	Effective in reducing turtle bycatch, discard and debris; possible problems with loss of large fish	Lucchetti et al. 2008
		Turkey	Effective in reducing turtle bycatch	Atabey and Taskavak 2001

SWO-LL: Swordfish longline
ALB-LL: Albacore longline
BLL: Bottom longline

Table 2: Principal bycatch mitigation measures tested in the Mediterranean sea. SWO-LL: Swordfish longline. ALB-LL: Albacore longline. BLL: Bottom longline.

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Diamond and Square-Mesh Codends Selectivity in Multi-Species Mediterranean Bottom Trawl Fisheries

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Abstract

Mediterranean demersal trawl fisheries traditionally operate using small diamond-shape meshes in the codend, which tend to retain almost all animals. We investigated the effect of mesh configuration (diamond and square) on the size selectivity of nine commercial species commonly captured in the Mediterranean demersal trawls. Two codends having meshes with the same opening (ca. 39 mm, stretched) but different mesh configuration (DM38: diamond-mesh and SM38: square-mesh configuration) were fished daily and alternately on the same trawl. Selectivity was measured using the covered codend technique, the cover being supported by circular hoops. The results were analysed taking into account the between-haul variation in selectivity. Two other important external variables were identified: the trawling depth and the codend catch, which for some species influenced between-haul variation, but there was no evidence of a coherent effect on selectivity parameters.

Current results demonstrated a substantial improvement in selectivity with square-mesh. The selectivity of 40mm diamond-mesh codend has been reported to be rather poor because a large proportion of the codend catch is composed by juveniles (i.e. virginal and early maturing) and smaller than the minimum landing size (MLS) or first maturity size. With the exception of the flatfish (*Arnoglossus laterna*), the effect of a change of mesh configuration from diamond- to square-mesh on size selectivity positively affected the retention length at 50% (L50). Square mesh codend appeared to protect individuals under the MLS of *Nephrops norvegicus* and *Mullus barbatus* even if for some other species such as *Merluccius merluccius*, the increase in L50 with square-mesh would not avoid the catch of juveniles.

1 Introduction

The bycatch has become a priority issue for global fisheries management during the last two decades. The majority of interest in bycatch has focused on demersal trawl fisheries because conventional otter trawls are generally non-selective fishing gears and so catch a wide range of unwanted species and undersized individuals. Mediterranean bottom trawl fisheries commonly imply the capture of a large number and variety of commercially important species. In this area the bottom trawl may simultaneously catch target species, such as whiting (*Merlangius marlangus*), Norway lobster (*Nephrops norvegicus*), hake (*Merluccius merluccius*), squid (*Loligo vulgaris*), short-finned squid (*Illex coindetii*), monkfish (*Lophius spp.*), red mullet (*Mullus bar-*

batus) or other species. Currently, Mediterranean stocks are mainly managed and conserved by regulations by defining closed areas and seasons, Minimum Landing Sizes (MLS) and Minimum Mesh Sizes (MMS). Nevertheless it is recognised that in multi-species fisheries a MMS in the codend appropriate for one species could be unsuitable for many others [1].

Traditionally the Mediterranean bottom trawl codend is made of small diamond meshes (in some cases minimum mesh opening less than 40 mm stretched) resulting in a very poor selective fishing gear [2, 3]. At the moment, in Italy the MMS of trawl codends is 40 mm stretched mesh size, resulting in the capture of large amounts of fish below the MLS. Improving trawl net selectivity is therefore of prime importance. The use of square-mesh codends has been specified as a conservation requirement in the ICES regions [4]. Relatively little scientific work, however, has been done to assess the selectivity of square-mesh codend in the highly variable multi-species conditions prevailing in the Mediterranean demersal trawl fisheries [1].

In Mediterranean, some works have assessed the codend selectivity for some important species in demersal trawl fisheries; however, selectivity parameters have been traditionally estimated for pooled data within all hauls, while few studies used the general framework, introduced by Fryer in 1991 [5], for modelling codend selectivity data in which between-haul variation is modelled by allowing the selectivity curves to vary randomly between hauls about a mean selectivity curve with a given probability distribution.

The main goal of current study was undertaken to analyse the effect, according to [5], of inserting a 40 mm square-mesh codend in a commercial Italian demersal trawl, on selectivity of some commercially important fish species and some species with unknown selectivity. In fact, in order to avoid further increases in mortality rates for juveniles and to substantially reduce the amount of discards of dead marine organisms by fishing vessels, Council Regulation (EC) No 1967/2006 [6], concerning management measures for the sustainable exploitation of fishery resources in the Mediterranean establishes that it is appropriate to provide for increases in mesh sizes for trawl nets used for fishing for certain species of marine organisms and for the mandatory use of square-meshed netting.

2 Materials and methods

2.1 Research vessel and trawl gear

The gear employed in the sea trials was a typical Italian commercial trawl as used in the Central Adriatic, entirely made up of knotless polyamide (PA) netting that was approximately 58 m long from the wing tips to the codend, with 600 meshes in the top panel at the footrope level. It was equipped with a 60.3 m footrope comprising a 38 mm combined rope weighted with 80 kg of leads over its whole length (Figure 1). All rigging components of the gear were identical with those commonly adopted in commercial practice in Central Adriatic demersal trawl fisheries.

Two codends were made using the same netting, having meshes with ca. 40 mm nominal mesh size (ms), but in one codend the netting was rigged in square-mesh configuration.

In the current work, we used a diamond-codend with 280 meshes (nm - number of

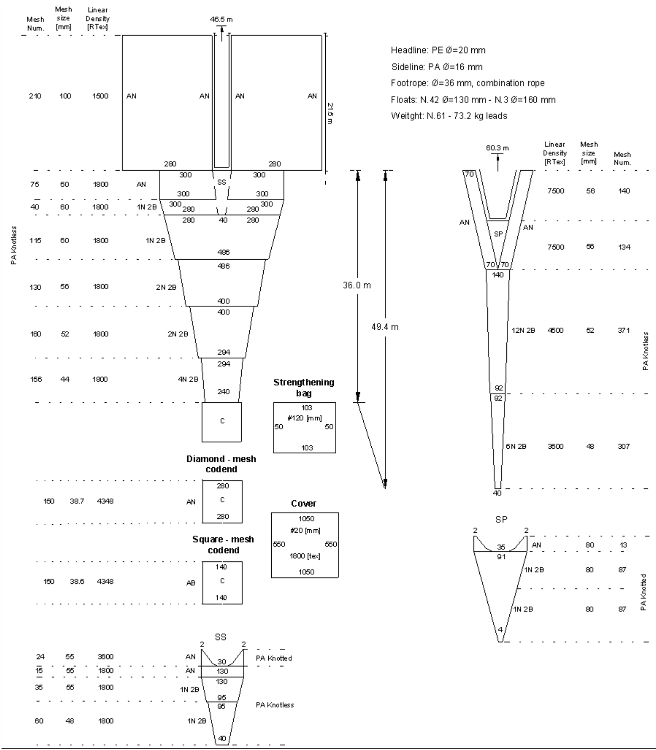


Figure 1: Design of the trawl adopted for the study during the first (30/08/04 - 07/09/04) and second sea trials (22/09/04 - 06/10/04).

meshes) having nominal mesh size (ms) of 40 mm and a fractional mesh opening (fmo) of 0.25. Therefore, the actual fishing circumference (afc) is: acf = 0.25*40*280=2800 mm. To find out the number of meshes of the square-mesh codend (nms) to match at such circumference, it is enough to divide it by the nominal square-mesh size (or bar): nms = 2800/20=140 meshes.

2.2 Sea trials and gear behaviour performance

Samplings were conducted on the Italian research vessel RV "Andrea" (1350x2 HP at 2300 rpm; length over all 29.15 m and Gross Tonnage 285 GT). Sea trials were carried out in the course of two subsequent fishing cruises on two different fishing grounds of the Central Adriatic normally exploited by local fishermen. The first cruise took place from 30/08/04 to 07/09/04 at about 15-21 m of depth, approximately 5 nm off Ancona, and the second from 22/09/04 to 06/10/04 in the Ortona depression also named Western Pomo pit, at a depth of about 70 m.

The two codends were alternated daily on the same trawl. Commercial practice was followed with regard to trawling speed and tow time.

SPECIES	CODEND	L50 [cm]	SE [cm]	SR [cm]	SE [cm]	SF	v_1	v_2	{Ri}			
									R_{i11}	R_{i12}	R_{i22}	R_{i11}
AL	DM38	**8.30**	0.088	**1.18**	0.114	**2.15**	-15.403	1.855	0.020	0.024	0.067	0.008
	SM38	**7.61**	0.139	**0.77**	0.087	**1.97**	-21.626	2.842	0.086	0.018	0.017	0.019
IC	DM38	**4.90**	0.198	**0.97**	0.114	**1.27**	-11.069	2.260	0.274	0.023	0.048	0.039
	SM38	**8.38**	0.392	**1.90**	0.248	**2.17**	-9.691	1.157	1.004	-0.530	0.311	0.153
MM	DM38	**8.26**	0.212	**1.74**	0.179	**2.13**	-10.440	1.264	0.107	-0.081	0.061	0.045
	SM38	**14.17**	0.370	**3.64**	0.222	**3.67**	-8.544	0.603	0.536	0.068	0.009	0.137
MB	DM38	**7.76**	0.201	**1.86**	0.086	**2.00**	-9.184	1.184	0.409	-0.109	0.056	0.041
	SM38	**10.91**	0.166	**1.43**	0.132	**2.83**	-16.737	1.534	0.267	0.110	0.156	0.027
NN	DM38	**1.46**	0.122	**0.28**	0.029	**0.38**	-11.412	7.798	0.037	0.003	0.000	0.015
	SM38	**1.91**	0.086	**0.37**	0.045	**0.49**	-11.200	5.869	0.039	-0.016	0.008	0.007
PE	DM38	**7.56**	0.278	**2.43**	0.135	**1.95**	-6.840	0.905	0.512	-0.173	0.066	0.077
	SM38	**9.67**	0.305	**1.36**	0.096	**2.50**	-15.623	1.616	0.384	-0.023	0.007	0.093
PL	DM38	**1.20**	0.043	**0.24**	0.049	**0.31**	-10.859	9.078	0.007	0.002	0.008	0.002
	SM38	**1.49**	0.027	**0.26**	0.029	**0.39**	-12.635	8.474	0.004	0.000	0.003	0.001
TM	DM38	**9.71**	0.256	**2.75**	0.184	**2.51**	-7.751	0.799	0.561	0.077	0.150	0.066
	SM38	**13.12**	0.417	**2.43**	0.351	**3.40**	-11.855	0.904	0.613	-0.278	0.468	0.174
TMC	DM38	**8.11**	0.390	**2.07**	0.263	**2.10**	-8.597	1.060	0.551	-0.173	0.054	0.152
	SM38	**11.26**	0.234	**1.65**	0.174	**2.92**	-15.011	1.333	0.309	0.029	0.063	0.055

Table 1: Direct estimate of the selectivity parameters for the diamond- (DM38) and square-mesh (SM38) codends. Mean values (in bold) and respective Standard Errors (SE) of the retention length at 50% (L50) and Selection Range (SR) are reported for: *Arnoglossus laterna* (AL), *Illex coindettii* (IC), *Merluccius merluccius* (MM), *Mullus barbatus* (MB), *Nephrops norvegicus* (NN), *Pagellus erythrinus* (PE), *Parapenaeus longirostris* (PL), *Trachurus mediterraneus* (TM) and *Trisopterus minutus capelanus* (TMC). SF: Selection factor and v_1, v_2: maximum likelihood estimators of the selectivity parameters; R: variance matrix measuring the within-haul variation; D: measures the between-haul variation in the parameters v. Definitions of selection parameters: a) The 50% retention length (L50) of a codend for a particular species is the fish length at which 50% of the species entering the codend is retained. b) The selection range (SR) is the length difference between the 75% retention length and 25% retention length. c) The selection factor (SF) of a codend for a particular species is the ratio of the 50% retention length and the mean measured mesh opening.

The actual mesh openings, measured using an ICES mesh gauge with 4 kg tension, were 38.70 ± 0.73 mm for the diamond- (DM38) and 38.65 ± 1.09 mm for the square-mesh (SM38) codend.
Gear performance (i.e., door spread, horizontal and vertical net openings) was measured on all hauls using the SCANBAS SGM-15 system (SCANMAR, Norway). A laptop automatically controlled data acquisition and provided for real time correct system functioning through a customized software. The main goal of these measurements was to obtain for each haul detailed, real time data on gear performance.

Selectivity was measured using the covered codend technique, where a cover with a nominal mesh opening of 20 mm is supported by circular hoops to keep it clear of the codend and minimize masking effects [7]. The cover, made from the same PA netting, was approximately 1.5 times larger and longer than the codend, as recommended by [8].

2.3 Selectivity data analysis

For each haul, the retention probability r(l) in the codend was modelled by means of

Species		alpha parameters							
		α_1 (L_{50}, Constant)	α_2 (SR, Constant)	α_3 (L_{50}, Mesh)	α_4 (SR, Mesh)	α_5 (L_{50}, Catch)	α_6 (SR, Catch)	α_7 (L_{50}, Depth)	α_8 (SR, Depth)
AL	*Estimate*	**8.267**	**1.152**	**-0.643**	**-0.361**	-	-	-	-
	SD	0.108	0.104	0.164	0.151	-	-	-	-
	t-Value	76.348	11.062	-3.930	-2.396	-	-	-	-
	dof	29	29	29	29	-	-	-	-
	p-value	0.000	0.000	0.000	0.023	-	-	-	-
IC	*Estimate*	**4.890**	**0.966**	**3.710**	**0.856**	-	-	-	-
	SD	0.272	0.139	0.418	0.230	-	-	-	-
	t-Value	17.986	6.936	8.878	3.715	-	-	-	-
	dof	21	21	21	21	-	-	-	-
	p-value	0.000	0.000	0.000	0.001	-	-	-	-
MM	*Estimate*	**8.302**	-	**5.812**	**1.895**	-	-	-	**0.011**
	SD	0.237	-	0.340	0.297	-	-	-	0.001
	t-Value	35.101	-	17.078	6.371	-	-	-	9.920
	dof	13	-	13	13	-	-	-	13
	p-value	0.000	-	0.000	0.000	-	-	-	0.000
MB	*Estimate*	**8.342**	**1.854**	**2.864**	**-0.522**	**-0.006**	-	-	-
	SD	0.227	0.078	0.232	0.107	0.002	-	-	-
	t-Value	36.809	23.919	12.348	-4.880	-3.493	-	-	-
	dof	34	34	34	34	34	-	-	-
	p-value	0.000	0.000	0.000	0.000	0.001	-	-	-
NN	*Estimate*	**1.835**	-	**0.363**	**0.160**	**-0.006**	**0.004**	-	-
	SD	0.148	-	0.132	0.055	0.003	0.001	-	-
	t-Value	12.368	-	2.742	2.931	-2.479	4.668	-	-
	dof	12	-	12	12	12	12	-	-
	p-value	0.000	-	0.018	0.013	0.029	0.001	-	-
PE	*Estimate*	**7.655**	**3.644**	**2.022**	**-1.173**	-	-	-	**-0.071**
	SD	0.273	0.490	0.434	0.143	-	-	-	0.026
	t-Value	28.055	7.435	4.656	-8.195	-	-	-	-2.745
	dof	16	16	16	16	-	-	-	16
	p-value	0.000	0.000	0.000	0.000	-	-	-	0.014
PL	*Estimate*	**1.189**	**0.709**	**0.306**	-	-	**-0.0004**	-	**-0.003**
	SD	0.038	0.135	0.050	-	-	0.0002	-	0.001
	t-Value	31.123	5.265	6.153	-	-	-2.588	-	-3.195
	dof	16	16	16	-	-	16	-	16
	p-value	0.000	0.000	0.000	-	-	0.0198	-	0.006
TM	*Estimate*	**9.694**	**2.595**	**3.518**	-	-	-	-	-
	SD	0.276	0.178	0.498	-	-	-	-	-
	t-Value	35.138	14.581	7.060	-	-	-	-	-
	dof	28	28	28	-	-	-	-	-
	p-value	0.000	0.000	0.000	-	-	-	-	-
TMC	*Estimate*	**8.284**	**1.772**	**2.958**	-	-	-	-	-
	SD	0.305	0.128	0.390	-	-	-	-	-
	t-Value	27.127	13.792	7.585	-	-	-	-	-
	dof	18	18	18	-	-	-	-	-
	p-value	0.000	0.000	0.000	-	-	-	-	-

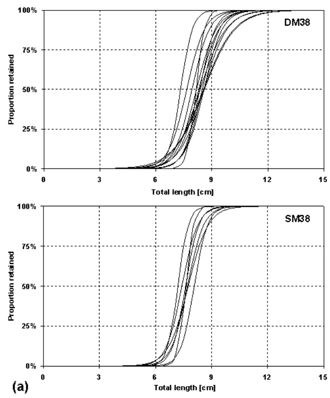
Table 2: Alpha parameter estimates, standard deviation (SD), t-value, degrees of freedom (dof) and p-value for the species studied. α is the vector that determines the direction and magnitude of the influence of the explanatory variables on selectivity parameters (L50 and SR).

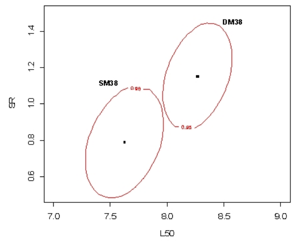
the logistic selectivity curve:

$$r(l) = \frac{e^{v_1+v_2 l}}{1+e^{v_1+v_2 l}}, \qquad (1)$$

where $r(l)$ is the probability that a fish of length l is retained, given that it entered the codend [9], and $\hat{v} = (v_1, v_2)^T$ is the vector of the selectivity parameters. The model of between-haul variation proposed by [5] was then used to investigate the between-haul variation of the selectivity parameters v_1 and v_2 by twine thickness, allowing a mean curve to be estimated for each codend mesh.

Finally the selectivity data were modelled according to [5] by estimating the individual contribution of some explanatory variables to the selectivity parameters. All possible linear combinations of the selectivity parameters as functions of the explanatory variables mesh configuration (mi), fishing depth (di) and codend catch size (ci) were tested. All were adjusted as continuous variables except for mesh configuration,





(b)

$$E\begin{pmatrix} L50 \\ SR \end{pmatrix} = E\begin{pmatrix} \alpha_1 + \alpha_3 m_i \\ \alpha_2 + \alpha_4 m_i \end{pmatrix}$$

Codend type	*pred.L50* [cm]	*pred.SR* [cm]	N.obs.	{D} R_{i11}	R_{i12}	R_{i22}
DM38	8.27	1.15	18	0.0117	0.0041	0.0109
SM38	7.62	0.79		0.0151	0.0046	0.0119

Figure 2: *Arnoglossus laterna* (scaldfish). (a) Selectivity curves for individual hauls (thin lines) and mean curve (thick line) according to [5]. (b) Confidence regions (95%) for expected mean REML values of Selection Range (pred.SR) plotted against the 50% retention length (pred.L50) calculated by the best model, based on the lowest value of Akaike's Information criterion (AIC). DM38: diamond-mesh codend; SM-38: square-mesh codend.

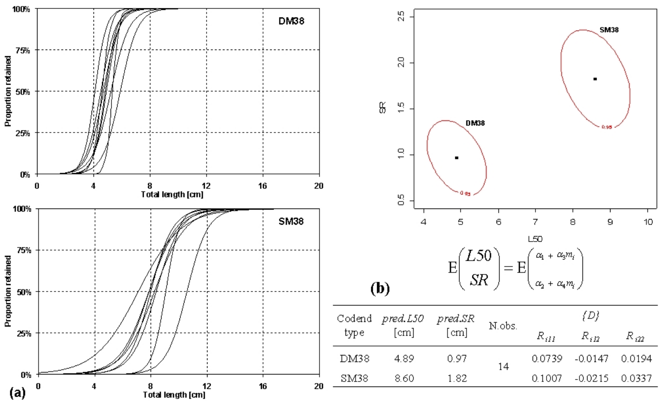
which was adjusted as a two-level factor (DM38=0; SM38=1). The choice of the model best fitting the data was based on the lowest value for Akaike's Information Criterion-AIC [10].

The haul-by-haul maximum likelihood of selectivity parameters for individual hauls was estimated using the CC2000 software [11]. Models, including between-haul variation, were estimated using the EC-Modeller software (Constat, 1995), which adopts the REML method (REsidual Maximum Likelihood) proposed by [5].

Traditionally the results of a selectivity analysis is a logistic curve were the inflexion point coincide with the L50 or 50% retention length.

3 Results

Overall, 21 valid hauls were performed in the first cruise and 27 in the second. The collected data allowed analysis of the selection characteristics for 9 species: scaldfish (*Arnoglossus laterna*), broad-tail shortfin squid (*I. coindettii*), European hake (*M. merluccius*), red mullet (*M. barbatus*), Norway lobster (N. norvegicus), common pandora (*Pagellus erythrinus*), deepwater rose shrimp (*Parapenaeus longirostris*), Mediterranean horse mackerel (*Trachurus mediterraneus*) and poorcod (*Trisopterus minutus capelanus*). The REML parameter estimates of each codend obtained from direct analysis (no fixed effects) are shown in Table 1. Individual haul and mean curves are shown in Figure 2a to Figure 10a. The estimated alpha parameters of all species are listed in Table 2, along with their standard devia-

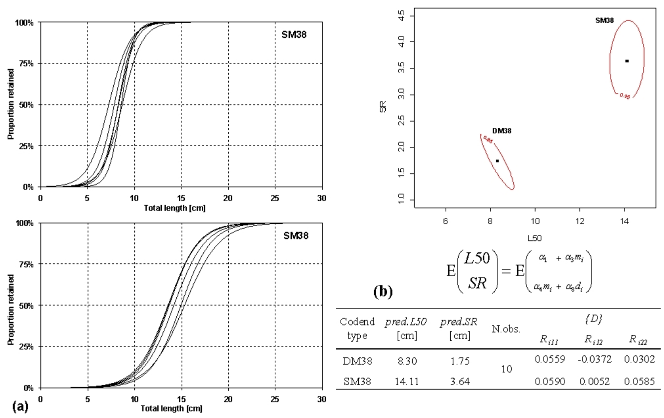


$$\mathrm{E}\begin{pmatrix} L50 \\ SR \end{pmatrix} = \mathrm{E}\begin{pmatrix} \alpha_1 + \alpha_3 m_i \\ \alpha_2 + \alpha_4 m_i \end{pmatrix}$$

Codend type	*pred.L50* [cm]	*pred.SR* [cm]	N.obs.	{D} R_{i11}	R_{i12}	R_{i22}
DM38	4.89	0.97	14	0.0739	-0.0147	0.0194
SM38	8.60	1.82		0.1007	-0.0215	0.0337

Figure 3: *Illex coindettii* (broad-tail shortfin squid). For the explanations see caption Figure 2. Total length refers to the Mantle length.

tion and t-values, which indicates the relative importance of the variables in the models. Of all the external variables, mesh configuration (mi) was consistently significant ($p<0.001$) in the between-haul variation for all species. With the exception of scaldfish (*A. laterna*), square-mesh configuration positively affect L50 (Table 2), in contrast there is no evidence of a coherent significant effect on SR. Scaldfish was the most abundant flatfish species and showed a size range of aproximately 4-16 cm (total length, TL, as for all fish species). Both the diamond- (DM38) and square-mesh codend (SM38) demonstrated good selection properties below 7 cm, as also revealed by the high proportion escaping from the codend (98% and 94% in the DM38 and SM38, respectively) and retained by the cover. Such a proportion is important as regards fish that may be landed legally in Italy (MLS: 7 cm, DPR 1639, 1968). However, escape of fish above MLS is of little concern to crews, given the very low market value of scaldfish around this size. Individual haul and mean selectivity curves for both codends are shown in Figure 2a. Both L50 and SR were negatively affected by mesh configuration (Table 2). Merely for this flatfish species the square-mesh was less selective and a highly significant ($p<0.001$) decrease in expected L50 from 8.27 cm to 7.62 cm was calculated in correspondence with the use of square-mesh codend (SM38). Expected SR followed a similar trend, being lower (0.79 cm) for the square-mesh codend and increasing to 1.15 cm for the diamond-mesh codend (Figure 2b). In Mediterranean, broad-tail shortfin squid (*I. coindettii*) is not subjected to any MLS and may be always landed legally. For several hauls it was one of the most abundant species (more than 10% of the total weight). In this study the size of broad-tail shortfin squid ranged between 3.0-22.5 cm and 4.5-18.0 cm, respectively for the DM38 and SM38 codend. Only mesh configuration appeared to positively affect ($p<0.001$) both L50 and SR (Table 2), which the first increased from 4.89 cm (DM38) to 8.60 cm (SM38) and SR from 0.97 cm to 1.82 cm (Figure 3b). All indi-

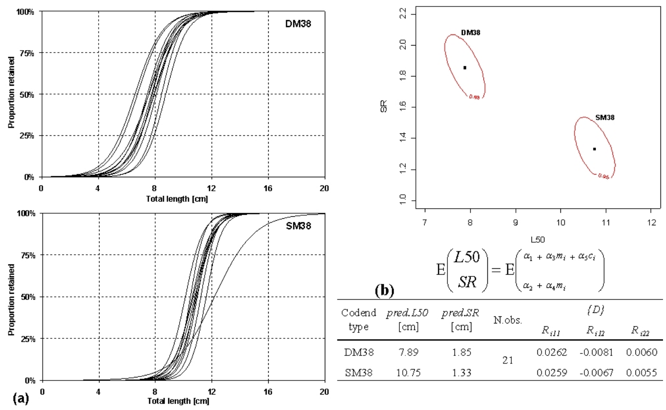


$$E\begin{pmatrix} L50 \\ SR \end{pmatrix} = E\begin{pmatrix} \alpha_1 + \alpha_3 m_i \\ \alpha_4 m_i + \alpha_8 d_i \end{pmatrix}$$

Codend type	*pred.L50* [cm]	*pred.SR* [cm]	N.obs.	{D} R_{i11}	R_{i12}	R_{i22}
DM38	8.30	1.75	10	0.0559	-0.0372	0.0302
SM38	14.11	3.64		0.0590	0.0052	0.0585

Figure 4: *Merluccius merluccius* (European hake). For the explanations see caption Figure 2.

viduals of European hake (*M. merluccius*) equal or above 20 cm, which is the MLS [6] and a large proportion of those below 20 cm (79% and 62% in the DM38 and SM38, respectively) were retained in the codends. In the present study the expected L50, calculated using Fryer's model (Figure 4b), declined from 14.11 cm to 8.30 cm when the DM38 codend replaced the SM38 codend, while SR decreased significantly from 3.64 cm to 1.75 cm (Figure 4b). Notably, for this species the model best describing the data (Table 2) showed that fishing depth also significantly ($p<0.001$) influenced SR (α_8 = 0.011). Red mullet (*M. barbatus*) was the most abundant species and, for several hauls, the catch was more than 70% of the total weight. In the Mediterranean Sea, red mullet is subject to an MLS of 11 cm [6]. Both the expected L50 and SR estimates, according to Fryer's model (1991), demonstrate a highly significant ($p<0.001$) increase in the selectivity parameters with the use of square-mesh (Table 2). The change from diamond- to square-mesh increased L50 from 7.89 cm to 10.75 cm, while SR decreased significantly from 1.85 cm to 1.33 cm (Figure 6b). The model best describing the data (Table 2) showed that total catch size also negatively ($p<0.01$) affected L50 (α_7 = -0.006); therefore, a mean catch size of 73 kg was used to calculate the expected L50s in the model and to compare the two codends. N. norvegicus is very abundant in the Pomo pit area, therefore it was very advantageous to carry out here the selectivity of this species. In the Mediterranean Sea, Norway lobster is subject to an MLS of 2 cm of Carapace Length (CL) or 7 cm of Total Length (TL) [6]. Both the expected L50 and SR estimates, according to Fryer's model (1991), demonstrate a highly significant ($p<0.01$) increase in the selectivity parameters with the use of square-mesh Figure 6b. The change from diamond- to square-mesh increased L50 from 1.56 cm (CL, DM38) to 1.92 cm (CL, SM38), which is really close to the MLS of this species. However, SR increased significantly from 0.19 cm (DM38) to 0.35 cm (SM38) (Figure 6b).

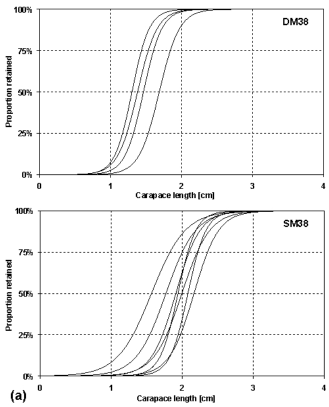


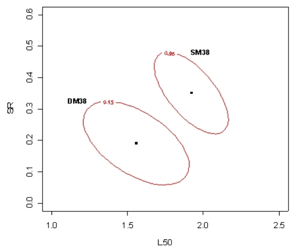
$$\mathrm{E}\begin{pmatrix} L50 \\ SR \end{pmatrix} = \mathrm{E}\begin{pmatrix} \alpha_1 + \alpha_3 m_i + \alpha_5 c_i \\ \alpha_2 + \alpha_4 m_i \end{pmatrix}$$

Codend type	*pred.L50* [cm]	*pred.SR* [cm]	N.obs.	{D} R_{i11}	R_{i12}	R_{i22}
DM38	7.89	1.85	21	0.0262	-0.0081	0.0060
SM38	10.75	1.33		0.0259	-0.0067	0.0055

Figure 5: *Mullus barbatus* (Red mullet). For the explanations see caption Figure 2.

The model, best describing the data (Table 2), showed that total catch size negatively ($p<0.05$) affected L50 and positively the SR ($p<0.01$); therefore, the mean catch size of 44 kg was used to calculate the expected L50s in the model and to compare the two codends. The MLS of common pandora (*P. erythrinus*) in the Mediterranean is 12 cm [6]. Use of the square-mesh resulted in a significant ($p<0.001$) improvement in L50, which increased from 7.65 cm to 9.68 cm when the DM38 codend was replaced by the SM38 codend (Figure 7b). Also in the SR there was an improvement with the use of square-mesh, in this case the expected SR followed an opposite trend, being higher (2.54 cm) for the DM38 codend and decreasing to 1.37 cm for the SM38 codend (Figure 7b). For this species the model best describing the data (Table 2) showed that fishing depth negatively ($p<0.01$) influenced SR (α_8 = -0.071). Deepwater rose shrimp (P. longirostris) was caught at depths more than 150 m. Selectivity estimates and the selectivity curves, based of Fryer's model are presented in Figure 8a. Compared to the other species, the model best describing the data was more complex (Table 2). Both codend catch size and fishing depth have played a significant role for SR, whilst L50 has been affected only by mesh configuration, particularly the values estimated for L50s were 1.19 cm and 1.50 cm, respectively for the diamond- and square-mesh codend (Figure 8b). The MLS for this species is 2.0 cm carapace length [6]. Estimates of SRs were 0.24 cm, with overlapping confidence intervals (Figure 8b). In Mediterranean, this shrimp is not subjected to any MLS, however considering that the size at 50% of maturity ranges between 2.0 cm and 2.8 cm, the use of both codends tends to reduce the spawning potentiality of the stock, determining the direct (by capture) death of a great amount of shrimps, the most part still immature. Individual haul and mean curves for T. mediterraneus are shown in Figure 9a. Retention was very high in the diamond-mesh codend, particularly a fraction of more than 45% of the individuals below the MLS of 12 cm [6] entered was retained, whilst in the square-mesh codend only 3% of the undersized





(b) $$E\begin{pmatrix} \text{L50} \\ \text{SR} \end{pmatrix} = E\begin{pmatrix} \alpha_1 + \alpha_3 m_i + \alpha_5 c_i \\ \alpha_4 m_i + \alpha_6 c_i \end{pmatrix}$$

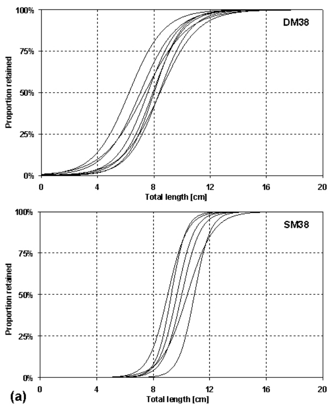
Species	Codend type	*pred.L50* [cm]	*pred.SR* [cm]	N.obs.	{D}		
					R_{i11}	R_{i12}	R_{i22}
NN	DM38	1.56	0.19	10	0.0120	-0.0025	0.0017
	SM38	1.92	0.35		0.0059	-0.0020	0.0017

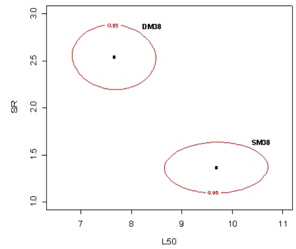
Figure 6: *Nephrops norvegicus* (Norway lobster). For the explanations see caption Figure 2.

individuals entered was retained. Only mesh configuration had a highly significant ($p<0.001$) effect on selectivity, however it affected only L50 (Table 2), while the estimates of SRs were 2.59 cm with overlapping confidence intervals. L50 estimates of 9.69 cm and 13.21 cm were obtained for the DM38 and SM38 codends respectively, with non-overlapping confidence intervals (Figure 9b). Given the very low market value of poor-cod (*T. minutus capelanus*) of MLS or similar size (7 cm; DPR 1639, 1968), escape of fish above the MLS is not a cause for concern to crews. Mesh configuration appeared to affect significantly ($p<0.001$) only L50 (Table 2), which decreased from 11.24 cm (SM38) to 8.28 cm (DM38) (Figure 10b). SRs were 1.77 cm with overlapping confidence intervals (Figure 10b).

4 Discussion and conclusions

The present study provides information on the 40 mm diamond mesh and 40 mm square mesh codends performances in the Mediterranean demersal trawl fishery. It highlights the existing problem regarding the very poor selection of the traditional diamond mesh used by bottom trawlers in multi-species fisheries of the Mediterranean sea, as already showed in recent studies [2, 12]. To deal with the multi-species characteristics of this fishery, the comparison was focused on the main commercial species of this area. Some of them were already studied in other Mediterranean areas [13, 14, 15, 16, 17, 12, 18], however in many cases the low captures and the poor selectivity prevented to escape enough specimens and the estimation of size selectivity parameters using Fryer's methodology was unsuitable. In these situations, selectivity curves have been traditionally estimated for pooled data within all hauls for which controlled parameters





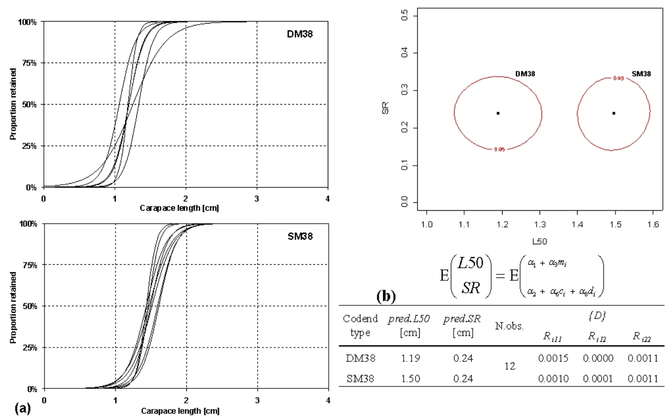
$$E\begin{pmatrix} L50 \\ SR \end{pmatrix} = E\begin{pmatrix} \alpha_1 + \alpha_3 m_i \\ \alpha_2 + \alpha_4 m_i + \alpha_8 d_i \end{pmatrix}$$

(b)

Codend type	*pred.L50* [cm]	*pred.SR* [cm]	N.obs.	{D} R_{i11}	R_{i12}	R_{i22}
DM38	7.65	2.54	12	0.0744	-0.0014	0.0129
SM38	9.68	1.37		0.1142	0.0009	0.0080

Figure 7: *Pagellus erythrinus* (common pandora). For the explanations see caption Figure 2.

remain unchanged, however the conclusions must be considered no more than an indication as any variance estimates will surely be underestimated [5]. Worthy of mention is that in some of those studies (see Table 3), selectivity was measured using the covered codend technique where a cover was not supported by circular hoops as suggested by Main and Sangster (1991) and likely this does not prevented masking effects. Furthermore, the parameter estimates for European hake (*M. merluccius*) obtained by [12], are extreme results and may be caused both by the large mesh size used in the control codend and by discrepancies between the applied paired-gear method and the other covered codend experiments (see Table 3). In fact, [19], [20] and [21] pointed out that the reliability of selection parameters based on data from sea trials depends both on the structure of the data collected and on the methods of measuring gear selectivity. Recent studies confirmed that the methods referred to as paired-gear methods [9] may lead to biased results and overrepresentation of extreme parameter estimates (Frandsen et al., 2006a, 2006b), [21]. We noted that many experiments did not provided the gear design and it was difficult for us to explain the variability observed in the reviewed selectivity results. Usually selectivity data are normally presented for a specific gear. Different gears of the same type may vary in size, design and construction, material used, rigging and operation, and all may have an effect on selection [22, 23, 24]. However, where the information was available we take note of missing measurement of the mesh openings [17, 13, 18] and the results were based upon nominal mesh sizes. In some others the authors did not assessed the selectivity of the codend sensu-strictu, but they measured the combined selectivity of the extension and the codend as the cover enclosed both the two (i.e. [15]). In the current studies, using the information from the seasonal trawl surveys carried out along the Italian coasts we decided a suitable location of areas and period to assess the selectivity of the main commercial species according to

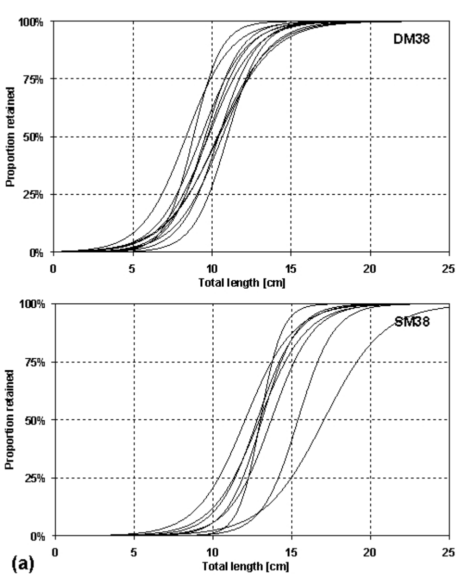


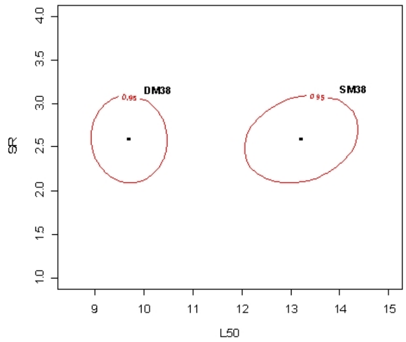
$$\mathrm{E}\begin{pmatrix} L50 \\ SR \end{pmatrix} = \mathrm{E}\begin{pmatrix} \alpha_1 + \alpha_3 m_i \\ \alpha_2 + \alpha_6 c_i + \alpha_8 d_i \end{pmatrix}$$

Codend type	*pred.L50* [cm]	*pred.SR* [cm]	N.obs.	{D} R_{i11}	R_{i12}	R_{i22}
DM38	1.19	0.24	12	0.0015	0.0000	0.0011
SM38	1.50	0.24		0.0010	0.0001	0.0011

Figure 8: *Parapenaeus longirostris* (deepwater rose shrimp). For the explanations see caption Figure 2.

[5]. Data analysis showed that the different mesh configurations (square and diamond) did not seem to influence the gear performance and the general behaviour of the net. The data analysed in this study were obtained in the course of two different cruises, but the selectivity results for each species were derived only from one or the other within the same area. The selectivity of 40-mm diamond-mesh codend has been reported to be rather poor because a significant proportion of the codend catch is immature and smaller than the minimum landing size or first maturity size. For all the species considered in this paper, the square-mesh codend plays a role which is as important as mesh size. However square-meshes were found to be unsuitable for flat and/or deep-bodied fish as these escape more readily from diamond-meshes. In particular in this study, better values of L50 were found for all species with the exception of scaldfish (*A. laterna*). In agreement with other studies [15, 16, 17], we confirm that all species, with the exception of flat fish, make the best use of the square-mesh opening, either because of their body shape or because of forcing the mesh to penetrate their body through. In conclusion, enforcement of installation of square-mesh codends in Mediterranean demersal trawl fisheries can be a suitable alternative to decrease the capture of immature individuals. The Council Regulation (EC) No 1967/2006, concerning management measures for the sustainable exploitation of fishery resources in the Mediterranean, establishes in Annex III the Minimum Landing Sizes (MLS) of marine organisms. However, the increase in L50 with square-mesh would not avoid some of the existing contradictions in allowing the use of codend mesh which leads to lower L50 than the MLS, such as the MLS established for European hake (*M. merluccius*)) which will not correspond to the selectivity of 40 mm square-mesh. According to [17], we believe that the improvement of the resources state, by increasing selectivity, depends upon the survival rate of the escaped individuals. In this sense, the data available is scarce and even null in the case





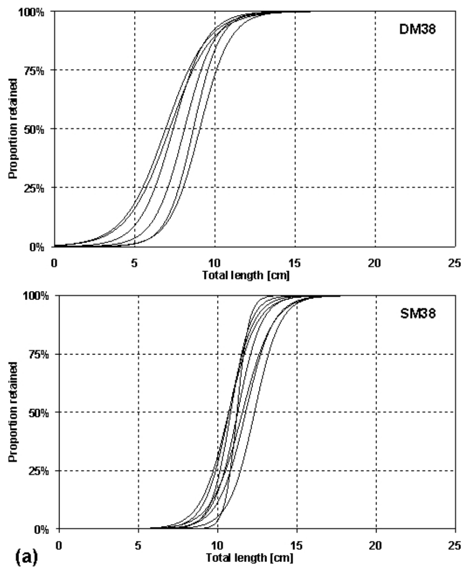
$$\mathrm{E}\begin{pmatrix} L50 \\ SR \end{pmatrix} = \mathrm{E}\begin{pmatrix} \alpha_1 + \alpha_3 m_i \\ \alpha_2 \end{pmatrix}$$

(b)

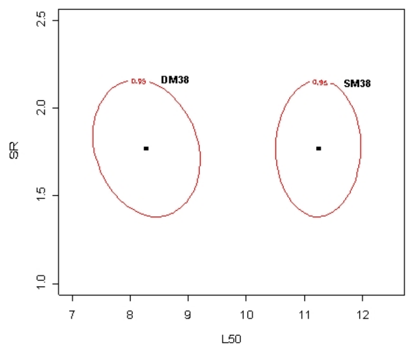
Codend type	*pred.L50* [cm]	*pred.SR* [cm]	N.obs.	{D} R_{i11}	R_{i12}	R_{i22}
DM38	9.69	2.59	17	0.0761	-0.0017	0.0317
SM38	13.21	2.59		0.1705	0.0163	0.0317

Figure 9: *Trachurus mediterraneus* (Mediterranean horse mackerel). For the explanations see caption Figure 2.

of many Mediterranean species. Therefore we encourage the promotion of research programs on different fishing technology aspects in order to know the survival rates for Mediterranean fisheries and to assess the actual improvement that could be achieved with a change of mesh configuration. In according with [12] the results described in this paper allowed us to conclude that square mesh codend is not a definitive solution of the low selection properties of the traditional bottom trawl net but it could represent a reasonable, simple and inexpensive solution waiting for the implementation of more sophisticated tools, such as the separator grids, recently experimented by Sala and Lucchetti in the Adriatic sea.



(a)



(b)

$$\mathrm{E}\begin{pmatrix} L50 \\ SR \end{pmatrix} = \mathrm{E}\begin{pmatrix} \alpha_1 + \alpha_3 m_i \\ \alpha_2 \end{pmatrix}$$

Codend type	*pred.L50* [cm]	*pred.SR* [cm]	N.obs.	{D}		
				R_{i11}	R_{i12}	R_{i22}
DM38	8.28	1.77	12	0.0933	-0.0076	0.0165
SM38	11.24	1.77		0.0584	0.0005	0.0165

Figure 10: *Trisopterus minutus capelanus* (poor-cod). For the explanations see caption Figure 2.

SPECIES	MLS [cm]	Reference	Area	Cover	MI	MS [mm]	CMC	L50 [cm]	SR [cm]	SF	Diff.SF
Arnoglossus laterna	-	Current paper	NAS	HCC	FM	39	DM	**8.30**	**1.18**	**2.15**	-8%
(scaldfish)						39	SM	**7.61**	**0.77**	**1.97**	
Illex coindettii	-	Current paper	NAS	HCC	FM	39	DM	**4.90**	**0.97**	**1.27**	71%
(broad-tail shortfin squid)						39	SM	**8.38**	**1.90**	**2.17**	
Merluccius merluccius	20	Petrakis and Stergiou, 1997	WAS	CC	PD	40	DM	**13.79**	**7.06**	**3.45**	9%
(European hake)						40	SM	**15.10**	**5.68**	**3.78**	
		Ordines et al., 2006	WMS	CC	PD	40	DM	**10.60**	**3.30**	**2.65**	43%
						40	SM	**15.20**	**3.30**	**3.80**	
		Sardà et al., 2006	NWM	AH40	PD	36	SM	**18.47**	**5.07**	**5.13**	-
		Bahamon et al., 2006	NWM	HCC	FM	40	DM	**10.10**	**3.10**	**2.51**	58%
						40	SM	**16.00**	**3.20**	**3.97**	
		Guijarro and Massutí, 2006	WMS	CC	PD	40	DM	**11.60**	**0.80**	**2.90**	32%
						40	SM	**15.30**	**2.20**	**3.83**	
		Current paper	NAS	HCC	FM	39	DM	**8.26**	**1.74**	**2.13**	72%
						39	SM	**14.17**	**3.64**	**3.67**	
Mullus barbatus	11	Tokaç et al., 1998	EAS	HCC	PD	36	DM	**11.02**	**1.76**	**3.06**	7%
(red mullet)						36	SM	**11.82**	**1.58**	**3.28**	
						40	DM	**12.19**	**2.15**	**3.05**	8%
						40	SM	**13.20**	**1.85**	**3.30**	
						44	DM	**13.50**	**2.65**	**3.07**	9%
						44	SM	**14.67**	**2.89**	**3.33**	
		Sardà et al., 2006	NWM	AH40	PD	36	SM	**10.40**	**3.90**	**2.89**	-
		Current paper	NAS	HCC	FM	39	DM	**7.76**	**1.86**	**2.00**	41%
						39	SM	**10.91**	**1.43**	**2.83**	
Nephrops norvegicus	2	Bahamon et al., 2006	NWM	HCC	FM	40	DM	-	-	-	-
(Norway lobster)						40	SM	**2.20**	**0.65**	**0.55**	
		Guijarro and Massutí, 2006	WMS	CC	FM	40	DM	-	-	-	-
						40	SM	**2.66**	**0.34**	**0.67**	
		Current paper	NAS	HCC	FM	39	DM	**1.46**	**0.28**	**0.38**	31%
						39	SM	**1.91**	**0.37**	**0.49**	
Pagellus erythrinus	12	Ordines et al., 2006	WMS	CC	PD	40	DM	-	-	-	-
(common pandora)						40	SM	**10.40**	**2.00**	**2.60**	
		Current paper	NAS	HCC	FM	39	DM	**7.56**	**2.43**	**1.95**	28%
						39	SM	**9.67**	**1.36**	**2.50**	
Parapenaeus longirostris	2	Guijarro and Massutí, 2006	WMS	CC	FM	40	DM	**1.66**	**0.38**	**0.42**	22%
(deepwater rose shrimp)						40	SM	**2.02**	**0.23**	**0.51**	
		Current paper	NAS	HCC	FM	39	DM	**1.20**	**0.24**	**0.31**	25%
						39	SM	**1.49**	**0.26**	**0.39**	
Trachurus mediterraneus	15	Ordines et al., 2006	WMS	CC	FM	40	DM	**13.70**	**2.10**	**3.43**	11%
(Mediterranean horse mackerel)						40	SM	**15.20**	**3.00**	**3.80**	
		Current paper	NAS	HCC	FM	39	DM	**9.71**	**2.75**	**2.51**	35%
						39	SM	**13.12**	**2.43**	**3.40**	
Trisopterus minutus capelanus	-	Petrakis and Stergiou, 1997	WAS	CC	PD	40	DM	**13.73**	**5.50**	**3.43**	-14%
(poor-cod)						40	SM	**11.85**	**5.95**	**2.96**	
		Bahamon et al., 2006	NWM	HCC	FM	40	DM	**9.20**	**3.00**	**2.28**	41%
						40	SM	**13.00**	**3.00**	**3.23**	
		Current paper	NAS	HCC	FM	39	DM	**8.11**	**2.07**	**2.10**	39%
						39	SM	**11.26**	**1.65**	**2.92**	

Table 3: Review of selectivity studies in multi-species Mediterranean demersal trawl fisheries. Selection parameter estimates: mean values of the retention length at 50% (L50), Selection Range (SR) and Selection factor (SF) are reported for diamond- (DM38) and square-mesh (SM38) codends. MLS: Minimum Landing Size. MI: method for the estimation of the selectivity (FM=Fryer's method; PD=pooled data). MS: Codend mesh size. CMC: Codend Mesh Configuration (DM38=Diamond-Mesh; SM38=Square-Mesh codend). Definitions of selection parameters: The 50% retention length (L50) of a codend for a particular species is the fish length at which 50% of the species entering the codend is retained. The selection range (SR) is the length difference between the 75% retention length and 25% retention length. The selection factor (SF) of a codend for a particular species is the ratio of the 50% retention length and the mean measured mesh opening.

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Physical Impact and Performance of Otterboard: Comparison Between Model Testing and Full-Scale Sea Trials

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Abstract

A new door has been designed to reduce hydrodynamic drag coefficient and increase spread of door commonly used in the Mediterranean commercial demersal trawl fisheries. Flume tank testing and engineering sea trials provide data which allow us to illustrate the performance and impact on the seabed of an existing door and a new door design. In the flume tank, each model was tested over a range of attack angles and for a limited range of otterboard heels. Curves of spreading-, drag- and down-force coefficients have been calculated. In the case of sea trials in order to extract the hydrodynamic coefficients an analysis has been applied and a mathematical model was used to calculate attack angle functions. From analysing the differences between engineering sea trials and flume tank tests we have deduced some conclusions about additional ground contact forces on sea trials that affect the performance of the doors. Moreover, a comparison between reaction forces of the flume tank and the estimation of reaction forces at sea has been given. Finally, this study allowed us to notice important differences between traditional and experimental otterboards.

1 Introduction

The otterboard is a key component for effective and efficient use of an otter trawl. The otterboard selected must open the trawl to the correct wing-end spread but also have the minimum physical impact possible, combined with stable shooting and handling. Many modern trawl doors are the result of initial designs, improved through practical trials until they work well enough to be used commercially. Modern door designs are more advanced and sophisticated as a result of increasing fuel costs and the necessity to minimize impact on the environment. Meeting these challenges has led to significant improvements in the way new otterboards are designed and tested. Manufacturers' experience in design and adjustment is important, but flume-tank testing first and research sea trials afterwards can help to ensure greater efficiency as well as lower impact by providing both quantitative and qualitative data on spreading, drag and reaction forces for different angles of attack, giving accurate data on the most efficient operating regime. We have found that most of them do not take into account the differences between model and full-scale tests. As full-scale otterboards can dig into the sea bed, in agreement with [1] we believe that additional ground contact forces apply to the otterboard and, especially on soft ground and at low towing speeds, the spread of the doors could well be higher due to the extra spreading force produced by the ground shear. In this respect the current paper illustrates the performance and impact on the seabed of an existing door and a new door design for demersal fisheries, with the main purpose of discussing the differences between engineering sea trials and the flume-tank tests and the differences between both trawl doors.

2 Materials and methods

In order to specify the basic design of traditional doors, a review of common commercial door specifications was made before the scale door trials. A typical otterboard Cambered vee type (termed AR door), commonly used in the commercial Mediterranean demersal trawl fisheries, was selected as the reference door (Table 1). A new experimental door (Clarck-Y door) has been developed by the door manufacture Grilli sas (Italy) in collaboration with Prosilas sas (Italy) and CNR-ISMAR of Ancona (Table 1). The experimental door was designed to try to reduce hydrodynamic drag coefficient and increase spread, and its design was based on the most advanced hydrodynamic concepts in improving the water flux on the upper part of the trawl door to avoid vortices, which are the cause of increased drag and cavitations. Since the trawl doors mostly operate under approximately steady-state conditions, the steady-state hydrodynamic coefficients are their most important hydrodynamic properties. To find these coefficients as accurate as possible, flume-tank experiment was performed at the North Sea Center flume-tank in Hirtshals (Denmark). This made it possible to find the hydrodynamic forces for various combinations of orientation angles. The hydrodynamic forces are assumed to be pressure-induced and, therefore for a given angle of attack, proportional to the square of the trawl door velocity relative to the water. It is, therefore, sufficient to do measurements for a single velocity. The two large door models, AR and Clarck-Y, were designed, produced and tested in the flume tank. The scaling of the physical models was based on the normal scaling rules. The linear scale factor used here is defined as the quantity in the full-scale trawl divided by the corresponding quantity in the model. In general terms, reductions to dimensions of a linear nature are made throughout the model by the amount of the basic factor. The factor concerning drag resistance, which is dependent on surface area for its value, varies proportionally with the square of the velocity of water flow. Weight and buoyancy forces that rely on volume for their value are reduced by the cube of the basic scale. Both the AR and Clarck-Y models were tested in the flume-tank for a limited range of otterboard heels with the intention to make some quantitative measurements of the performance (otterboard spreading- and drag-forces) and of the reaction force on the seabed. The total downward force of a door on the seabed (equal to the reaction force) is the resultant of the door weight in water, the downward hydrodynamic force, the upward force in the warp and the downward force in the bridle. Because the model is held in a fixed position, two angles of heel (0 and 30 degrees outward) have been tested. The spreading- and drag-force measurements have been made using in-line load cells, whereas to measure reaction loads new load cells have been developed to fit onto the door shoes. Two reaction load cells were required on each door in

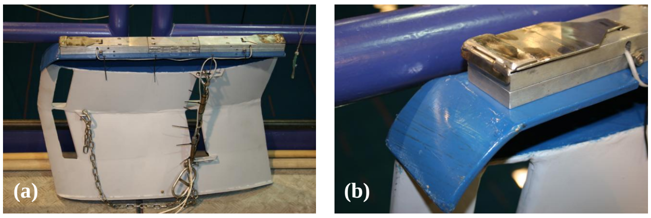


Figure 1: Button load cells in place of existing shoe (a) and (b) particular of the lever plates and ridge bars acting on the load cell.

Parameter	*AR*		*Clarck-Y*	
	ST	FT	ST	FT
L	1.800	0.710	1.800	0.710
H	1.050	0.480	1.060	0.480
A_p	1.764	0.338	1.709	0.332
W_T	310	16.67	275	18.05

L[m]: Length of otterboard; H[m]: Height of otterboard; $A_p[m^2]$: projected area of otterboard; W_T[kg]: weight of otterboard in water.

Table 1: Characteristics of the traditional Cambered Vee otterboard (AR), which is commonly used in the commercial Italian demersal trawl fisheries and of the experimental otterboard (Clarck-Y). ST: full-scale, FT: scaled door.

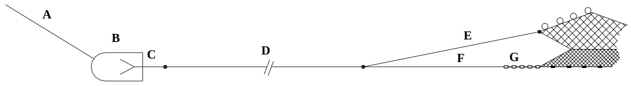
case the door under test pitched the nose up or down and lifted one off the bottom. Button load cells were used to satisfy the need for a compact low profile shape Figure 1. Both the full-scale AR and Clarck-Y otterboards Figure 2 were tested in the Adriatic Sea, using the Italian Research Vessel "G. Dallaporta". All rigging components of the gear were identical with those commonly adopted in commercial practice in Mediterranean demersal trawl fisheries Figure 3. Sea trials were conducted in the course of three sea cruises on two different fishing grounds with depth ranges of 25-30 m and 60-70 m. The first and the third cruises (termed ST3.8[1] and ST3.8[3] respectively) took place from 31/05/07 to 05/06/07 and from 03/03/08 to 13/03/08 respectively at about 27 m of depth with a towing speed of 3.8 knots. The second cruise (termed ST3.2[2]) was conducted from 16/10/07 to 18/10/07 at a depth of about 66 m with a towing speed of 3.2 knots. Overall, 12 valid hauls of the first cruise, 9 of the second and 8 of the third were analysed. In order to determine the effects of the sea current, at least two tows on reciprocal courses were made for each gear arrangement tested. After the first two cruises we realized that the Clarck-Y door had poor spreading and shooting behaviour and hence instability might have occurred. Therefore, in the third cruise the attachment of the chain backstrop brackets was moved 23 cm forward to try getting larger spreading. The otterboard to be used first was chosen randomly at the beginning of each trip, then the two otterboards were alternated on the same trawl. Adverse weather conditions prevented the

Figure 2: Particular of the Cambered vee AR (on the left) and Clarck-Y (on the right) otterboards. Pictures on the top show the SCANMAR angle sensor mounted on the otterboard for measuring heel- and pitch-angles of the door.

same number of hauls from being performed with both otterboards. During all the hauls done, the SCANBAS SGM-15 system (SCANMAR, Norway) was used to measure the gear performance: door spread, horizontal net opening, heel and pitch door angles. Moreover, two MICREL (France) underwater force sensors were inserted just in front and in the backside of the port-door to measure the drags ahead and behind the otterboard. All the instruments were linked by RS232/485 serial ports to a personal computer, which automatically control the data acquisition and provide the correct functioning of the system in real time through an appropriately developed Microsoft Visual Basic 6.0 program. In order to compare full- and scaled-otterboards, we balanced the forces and then the spreading-, drag- and down-forces of full-scale otterboard have been obtained. These forces in the case of scaled door are known as a function of attack- and heel-angle with zero pitch-angle, however attack-angle of the full-scale door was unknown, therefore we also propose a model to calculate the attack-angle at sea trials. A more detailed description of the analysis is given in [2].

3 Results

Data from the flume-tank and the experimental sea trials, together with the results obtained by the model, are summarized in Table 2. In the sea trials we have measured the door spread (HDS[m]), the horizontal net opening (HNO[m]), the heel- (ϕ [deg.]) and the pitch-angle (θ [deg.]) of the door, the warp attachment position to the otterboard (so named hole number HF on Table 2) as well as the tensions exerted to the otterboard by the warp (W[kg]) and by the bridle (B[kg]). We also have reported the results obtained with our analysis applied to the sea trials data: drag-, spreading- and down-force coefficients, efficiency and the corresponding attack angles (α[deg.]). In



	Description	Sea Cruise		
		ST3.8[1]	ST3.2[2]	ST3.8[3]
A	Warp (∅ 16 mm)	200 m	400 m	200 m
B	Otterboard	*AR, Clarck-Y*	*AR, Clarck-Y*	*AR, Clarck-Y*
C	Combination rope (∅ 22 mm)	6.5 m	6.5 m	6.5 m
D	Combination rope (∅ 22 mm)	100 m	110 m	100 m
E	Wire (∅ 14 mm)	52.5 m	8 m	52.5 m
F	Combination rope (∅ 36 mm)	50 m	5 m	50 m
G	Chain	3.30 m	3.30 m	3.30 m

Figure 3: Main details of the gear rigging adopted during the first (ST3.8[1]), second (ST3.2[2]) and third (ST3.8[3]) sea cruises.

Table 2, the values of the reaction force, Rz [kg] are also shown. For each door type, flume tank tests were carried out at either two angles of heel (0 and 30 degree outward) over a range of angles of attack from 30° to 49°. 0° heel is considered to be when the door is upright, or for a vee door (as for the case of AR and Clarck-Y) when the top plate is vertical. Considering the results at sea, which revealed for the heel a range of operation between -2° and 14°, in the current paper only tests at 0° heel have been analysed and compared with sea trials. The dimensions of models were measured and projected areas evaluated using the video analysis software (Image Pro Plus, 2005) on the basis of the outline shape of the otterboards (Table 1). Hydrodynamic coefficients (CD, CL and CZ) and down-force coefficient (C'z) are reported in Table 2 and shown in Figure 4 as function of attack angle (α[deg.]). In the range of attack angles studied, the door AR has a higher drag and lift components than Clarck-Y door. The drag component for the AR door increases as the angle of attack increases, conversely the Clarck-Y door is hold steady (Fig. 4). Examining the spreading component curve Figure 4, it can be seen that CL presents a maximum for AR door while it decreases monotonously for the Clarck-Y door. In general for both door models the efficiency looks similar and a reduction in the angle of attack will, therefore, always give better efficiency (CL/CD) as it can be seen in Figure 4. In Figure 4 we have also plotted, for both doors, the hydrodynamic down-force coefficient (Cz) and the down-force coefficient (C'z). The graph for the two door models shows that C'z decreases with the attack-angle, while Cz does not seem to depend on the attack angle. The Reference vee AR door produces smaller Cz in absolute value, ranging around -0.40, than the Clarck-Y door, which is approximately -0.50 (see Table 2 and Figure 4). Moreover, for a given attack angle, the absolute values of the down-force coefficient and consistently the reaction forces are greater in the AR door (Table 2 and Figure 4). The performance of each full-scale otterboard at sea was ascertained over a range of angles of attack from 22° to 43° (Table 2). These angles, calculated on the basis of the model proposed in [2], were achieved by adjusting the warp attachment position

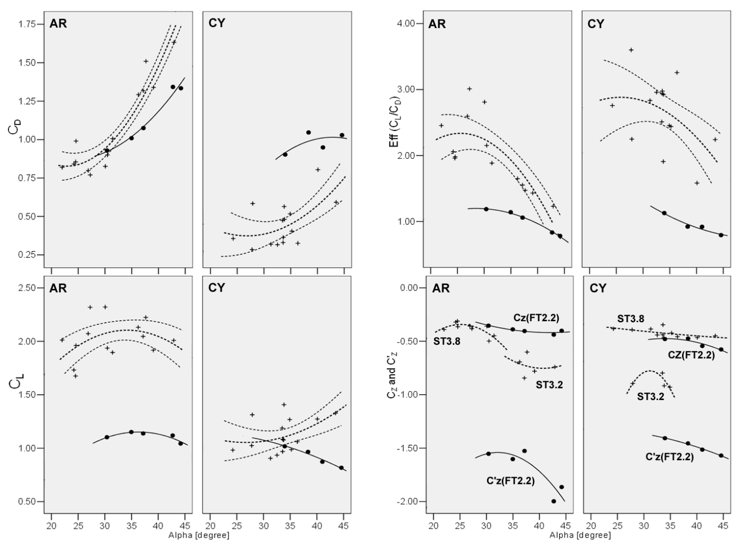


Figure 4: Drag-force coefficient, (CD) lift-force coefficient, CL efficiency coefficient, Eff(CL/CD) hydrodynamic down-force coefficient, CZ and down-force coefficient, C'Z, with angle of attack, Alpha: comparison between the experimental flume-tank (circle points and continuous lines) and full-scale (cross points and dotted lines) obtained on the Cambered vee AR (AR) and Clarck-Y (CY) otterboards. In the last graph on the right, the hydrodynamic down-force coefficient, CZ(FT2.2), obtained in the flume-tank experiment at 2.2 kn has been reported together the C'Z data, C'Z(FT2.2). The C'Z data attained during the sea trials at towing speed of 3.2 (ST3.2) and 3.8 kn (ST3.8) have been also underlined.

to the otterboard (HF) and in the cruise ST3.8[3] by also modifying the attachment of the chain backstrop brackets which was moved 23 cm forward. The testing procedure adopted gives accurate and consistent results to define the performance of trawl doors in sea trial conditions. Coefficients of drag-, lift- and down-forces (CD, CL and C'z respectively) for each cruise are shown in Figure 4 as a function of attack angle, the confidence region is due to the sea cruise variability. Results for the cambered vee AR door show higher values of both drag- and lift-coefficient than the experimental Clarck-Y door (Table 2 and Figure 4). Behaviour of drag coefficient in both doors presents some differences: in AR door it rises steeply with attack angle while in Clarck-Y it increases steadily. The lift coefficient tendency is different in both doors: it presents a maximum for the AR door but it increases with the attack angle for the Clarck-Y door (Figure 4). Apparently, for a given attack angle, the Clarck-Y showed an evident higher efficiency (Figure 4), however a poor shooting behaviour and lower performance in the door spread (see Table 2) was noticed at sea and some-

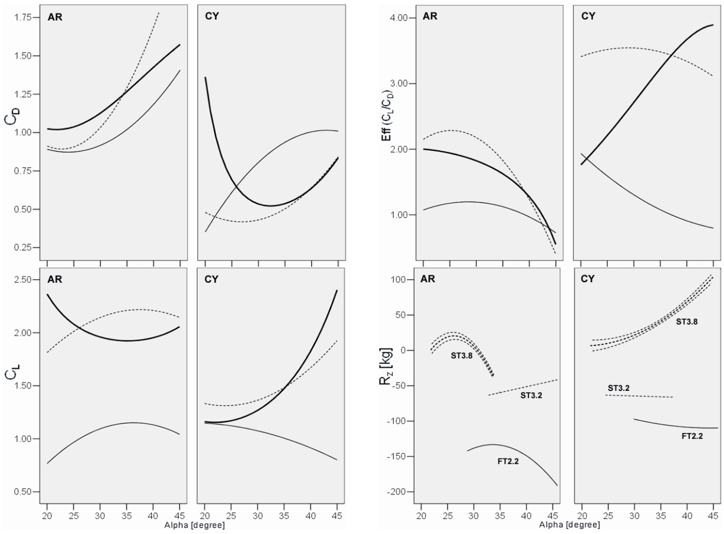


Figure 5: Statistical models at 0° of heel for the drag-force coefficient (CD), lift-force coefficient (CL), efficiency coefficient (Eff(CL/CD)) and reaction force (RZ[kg]) with angle of attack (Alpha): comparison between the flume-tank (continuous line) and full-scale (dotted line) obtained on the Cambered vee AR (AR) and Clarck-Y (CY) otterboards. The bold line represents the ratio between the full-scale and the flume-tank test. For RZ[kg], the data of the flume-tank experiment carried out at 2.2 kn (FT2.2) and full-scale attained at towing speed of 3.2 (ST3.2) and 3.8 kn (ST3.8) have been underlined.

times the otterboard tended to be unstable (heterogeneous measurements of door spread and tensions). Probably for this reason the drag of the Clarck-Y was very low compared to the AR door (Figure 4) and hence the higher efficiency (Figure 4). Fine adjustment of the attachment of the chain backstrop brackets, and consequently of the angle of attack, carried out just before the third cruise proved to be necessary as the instability disappeared and the door spread improved (Table 2), conversely in such conditions, the Clarck-Y provided evidence of lower performance than AR (Table 2). In Figure 4, we can observe as down-force coefficient C'z is towing speed dependent and, for a given speed, it is similar in the two otterboards. The absolute value of C'z ranges between 0.31-0.50 at the towing speed of 3.8 kn, and it reaches higher absolute values (0.60-0.93) at 3.2 kn. Table 3 shows in details the comparison between the two full-scale doors. In this table, for each warp attachment position (HF), estimated values of attack-, heel- and pitch-angles and corresponding drag, lift and efficiency coefficients for both the doors have been summarized. In both doors the attack-, heel- and pitch-angles increase as warp towing point (HF) moved aft. Moreover, the differences in attack angles between consecutives towing points

FC	Door	φ [deg.]	HF	α [deg.]	C_D [-]	C_L [-]	Eff [-]	C'_z [-]	C_z [-]	HDS [m]	HNO [m]	W [kg]	B [kg]	δ [deg.]	θ [deg.]	R_z [kg]
ST3.8[1]	AR	3.5	1	22.0	0.82	2.01	2.45	-0.39	-	81.7	21.38	2028	1713	5.0	8.9	-22.1
		3.1	1	24.2	0.84	1.73	2.06	-0.32	-	77.4	20.61	1892	1565	6.1	9.7	5.4
		2.8	2	24.6	0.99	1.96	1.98	-0.36	-	78.5	20.65	1973	1618	5.7	9.2	-8.6
		3.3	2	24.5	0.86	1.68	1.96	-0.31	-	77.3	20.61	1851	1518	6.3	9.7	9.1
	CY	3.8	2	27.8	0.28	1.02	3.60	-0.39	-	51.3	15.18	1630	1507	5.1	10.4	3.0
		7.3	2	31.3	0.32	0.91	2.83	-0.39	-	50.8	14.99	1475	1340	5.7	13.3	17.5
		8.4	3	32.5	0.32	0.93	2.96	-0.44	-	53.3	16.18	1446	1315	5.0	14.0	2.5
		4.4	3	33.6	0.36	1.08	2.98	-0.35	-	53.5	15.49	1653	1501	5.6	12.2	38.8
		5.6	4	36.3	0.33	1.06	3.26	-0.46	-	54.6	16.51	1542	1410	4.7	14.1	9.9
		6.6	4	35.2	0.40	0.99	2.44	-0.42	-	54.4	16.55	1476	1316	5.3	13.9	17.8
		7.8	4	33.6	0.33	0.97	2.94	-0.44	-	51.1	15.91	1558	1422	4.8	13.8	6.8
		5.9	3	24.3	0.36	0.98	2.76	-0.38	-	53.9	17.52	1529	1381	5.5	9.4	-5.8
ST3.2[2]	AR	9.5	3	37.2	1.32	2.05	1.55	-0.84	-	98.5	28.76	1499	1194	4.0	13.6	-112.1
		5.4	3	36.3	1.29	2.13	1.65	-0.69	-	92.8	28.73	1713	1400	4.6	11.8	-77.9
		10.0	4	39.1	1.34	1.92	1.43	-0.78	-	97.4	28.65	1450	1134	4.7	14.5	-94.6
		4.0	4	37.7	1.51	2.22	1.47	-0.60	-	97.2	28.19	1715	1349	5.4	11.4	-52.8
		7.0	5	42.9	1.63	2.01	1.23	-0.74	-	98.1	28.63	1549	1160	4.8	13.9	-80.5
	CY	9.3	4	33.8	0.48	1.41	2.93	-0.92	-	55.1	16.33	1628	1509	2.2	13.3	-107.9
		13.0	4	34.9	0.52	1.27	2.45	-0.93	-	61.4	18.83	1327	1203	2.6	15.1	-107.1
		13.6	3	33.5	0.47	1.19	2.51	-0.80	-	65.8	21.04	1251	1130	3.6	14.9	-84.6
		13.2	3	27.9	0.58	1.31	2.25	-0.89	-	62.2	19.12	1357	1218	2.9	11.2	-114.8
ST3.8[3]	AR	0.6	2	26.9	0.80	2.07	2.59	-0.36	-	90.2	22.28	1825	1524	6.0	9.2	-3.5
		-1.2	2	27.3	0.77	2.32	3.01	-0.38	-	86.0	21.30	1982	1708	5.6	8.2	-10.7
		3.8	2	30.5	0.90	1.94	2.15	-0.50	-	92.3	22.81	1685	1355	4.9	11.0	-45.5
		4.7	2	30.1	0.83	2.32	2.81	-0.36	-	94.3	23.65	1939	1629	5.7	12.1	0.9
		2.6	2	31.4	1.01	1.90	1.88	-0.45	-	92.4	23.12	1718	1341	5.3	10.8	-28.0
	CY	13.8	2	33.8	0.56	1.08	1.91	-0.47	-	78.2	19.41	1147	930	5.7	17.1	-1.8
		12.7	3	40.1	0.80	1.27	1.58	-0.47	-	76.7	18.59	1321	1035	5.4	18.7	19.5
		14.4	4	43.5	0.59	1.33	2.24	-0.45	-	75.8	18.83	1364	1145	5.4	21.6	36.2
FT	AR	0.0	-	44.2	1.33	1.04	0.78	-1.86	-0.40	-	-	-	-	-	-	-167.5
			-	42.7	1.34	1.12	0.83	-2.00	-0.44	-	-	-	-	-	-	-178.5
			-	37.2	1.07	1.14	1.06	-1.53	-0.40	-	-	-	-	-	-	-128.5
			-	35.0	1.01	1.15	1.14	-1.60	-0.39	-	-	-	-	-	-	-139.0
			-	30.4	0.93	1.10	1.19	-1.55	-0.35	-	-	-	-	-	-	-137.3
	CY	0.0	-	44.6	1.03	0.82	0.79	-1.57	-0.58	-	-	-	-	-	-	-110.2
			-	41.0	0.95	0.87	0.92	-1.51	-0.54	-	-	-	-	-	-	-107.7
			-	34.0	0.90	1.02	1.13	-1.41	-0.48	-	-	-	-	-	-	-103.0
			-	38.3	1.05	0.97	0.92	-1.46	-0.47	-	-	-	-	-	-	-108.9

Table 2: Results obtained in the sea cruises: ST3.8[1], ST3.2[2], ST3.8[3] and flume-tank tests (FT). Traditional- (AR) and experimental doors (CY) were daily alternated. Heel angle of otterboard (ϕ) warp attachment position to the otterboard (HF) angle of attack of otterboard (α), drag force coefficient (CD), spreading force coefficient (CL), efficiency of otterboard (Eff), down-force coefficient (C'Z), and hydrodynamic down-force coefficient (CZ), horizontal door spread (HDS), horizontal net opening (HNO), warp tension (W), bridle tension (B), warp pitch angle (δ) pitch angle of the otterboard (θ), reaction force (Rz).

are not constant and, in fact, these differences are smaller as towing point moves aft (or as hole number increases). Comparing both doors, we noticed the Clarck-Y worked with bigger heel- and pitch-angles than AR door. Of special interest for this study is the performance of the full-scale door spread due to its importance to door manufacturers and fishermen (see in Table 3 the estimated values of door spread calculated as a function of CL). It can be seen that, for all the tested conditions, the horizontal door spread of the full-scale traditional AR door was higher than that of the experimental Clarck-Y door, even though not more than 26%. In order to compare door results from flume-tank and sea trials we have plotted in Figure 5 the statistical models of drag- and lift-force coefficients, efficiency and reaction force Rz, for the full-scale curves the confidence region is due to the sea cruise variability. The regression curves shown in Figure 5 have been obtained by General Linear Models (GLM) procedures. The angle-of-attack and the heel of the door have been included in the models as a covariate and the door-type and type-of-experiment (sea cruises ST3.8[1],

Door	HF	α [deg.]	φ [deg.]	θ [deg.]	C_D [-]	C_L [-]	Eff [-]	HDS [m]
AR								
	1	22.2	2.3	9.8	0.83	1.87	2.25	76.5
	2	28.6	3.0	10.2	0.91	2.07	2.29	93.9
	3	34.2	4.7	11.2	1.12	2.14	1.91	99.3
	4	39.1	7.0	12.6	1.41	2.10	1.49	96.6
	5	43.1	9.5	14.3	1.74	2.02	1.16	89.3
CY								
	1 *	24.5	7.0	12.7	0.36	1.06	2.99	56.2
	2	33.8	9.1	13.7	0.38	1.12	2.91	59.7
	3	40.1	11.9	15.5	0.51	1.25	2.45	68.9
	4	43.5	13.8	16.9	0.61	1.35	2.20	76.1
	5 *	44.0	14.1	17.2	0.63	1.37	2.17	77.3

Note: for the *Clarck-Y* door, the values of the warp attachment position to the otterboard (HF) have been calculated on the basis of the results obtained with the modified otterboard used in the ST3.8[3] cruise.
(*) The warp attachment positions to the otterboard (HF) nr. 1 and 5 have been extrapolated from the other tested HF.

Table 3: Estimated values of full-scale traditional- (AR) and experimental (CY) doors for each warp attachment position to the otterboard (HF). Angle of attack of otterboard (α), heel angle of otterboard (ϕ), pitch angle of the otterboard (θ), drag force coefficient (CD), spreading force coefficient (CL), efficiency of otterboard (Eff), horizontal door spread (HDS).

ST3.2[2], ST3.8[3] or flume-tank) as factors. The use of further variables did not substantially improve the approximation of data. Considering that only the flume-tank tests at 0° heel have been analysed, the curves have been produced by zeroing the heel term. It should be noted that flume-tank curves of CD, CL correspond to a pure hydrodynamic effect while the coefficients from sea trials have been affected by the seabed effect also. For the AR otterboard, practically the same tendencies between the model and full-scale experimental trials were found in all the curves with angle of attack. For the drag- and lift-force coefficients, we obtained for this door the expected results: sea trials result in higher values than the flume-tank ones (Figure 5), this is coherent with the fact that with real seabed there is an additional friction and ground shear effect. To be precise the maximum values obtained in sea trials are for the drag and lift, around 50% and 100% respectively more than flume-tank ones (see bold lines in Figure 5). While for the Clarck-Y there is no evidence of coherent tendencies as abovementioned in section 3.2. For both doors, the graph for efficiency in Figure 5 shows that, for a given attack angle, efficiency at sea is higher than in flume-tank, in particular the maximum ratio is 1.9 and 3.9 for AR and Clarck-Y door respectively. To compare the effect of seabed in vertical direction we have estimated the values of reaction force Rz. In Figure 5, the continuous lines are the scaled values of reaction forces measured in the flume-tank and dotted lines are the full-scale ones, discerned for towing speeds 3.2 and 3.8 kn (ST3.2 and ST3.8 respectively). Comparing the full-scale doors, the reaction forces with angle of attack have inconsistent tendencies. We observe that reaction force, starting now the discussion of it will be in absolute value, depends strongly on the tow-

Door			φ [deg.]	HF	α [deg.]	θ [deg.]	C_D [-]	C_L [-]	Eff [-]
AR									
	C_{LMAX}	FT(0)	0.0	-	36.3	-	1.05	1.15	1.09
		ST(H0)	0.0	2	29.3	8.6	1.12	2.30	2.05
		ST(H)	4.7	3	34.2	11.2	1.05	2.10	2.00
	C_L=Eff	FT(0)	0.0	-	34.1	-	1.00	1.14	1.14
		ST(H0)	0.0	2	26.7	8.6	1.00	2.24	2.24
		ST(H)	4.2	3	32.8	10.9	1.00	2.10	2.10
	Eff_{MAX}	FT(0)	0.0	-	28.5	-	0.89	1.06	1.20
		ST(H0)	0.0	1	21.5	8.6	0.74	1.76	2.38
		ST(H)	2.5	2	25.6	10.0	0.85	1.98	2.34
CY									
	C_{LMAX}	FT(0)	0.0	-	-	-	-	-	-
		ST(H0)	0.0	3	39.7	11.0	0.45	1.55	3.44
		ST(H)	-	-	-	-	-	-	-
	C_L=Eff	FT(0)	0.0	-	39.3	-	1.00	0.93	0.93
		ST(H0)	0.0	-	-	-	-	-	-
		ST(H)	16.5	6 *	47.4	19.2	1.00	1.48	1.48
	Eff_{MAX}	FT(0)	0.0	-	-	-	-	-	-
		ST(H0)	0.0	2	31.5	11.0	0.40	1.41	3.56
		ST(H)	7.2	2	25.8	12.8	0.37	1.05	2.88

C_{LMAX}: maximum spreading force coefficient; C_L=Eff: optimum condition at a given angle of attack when C_L=Eff and with different attack-angles decrease one of the two; Eff_{MAX}: maximum efficiency of the otterboard.

Note: (*) the warp attachment position to the otterboard, HF=6, does not exist (see Fig. 5). The statistical model estimated, for that angle of attach (α), a backward attachment position to get larger attack-angle.

Table 4: Estimated values of traditional- (AR) and experimental (CY) doors for the flume-tank, FT(0) sea cruises at 0° of heel, ST(H0) and when heel is free to vary, ST(H). Heel angle of otterboard (ϕ) warp attachment position to the otterboard (HF), angle of attack of otterboard (α), pitch angle of the otterboard (θ), drag force coefficient (CD), spreading force coefficient (CL), efficiency of otterboard (Eff).

ing speed, in particular Rz increases when towing speed decreases. Furthermore, the reaction force is, in general, smaller at sea than in the flume-tank tests, probably due to the small towing speed used in flume-tank tests (TS=2.2 kn). Comparing both doors in flume-tank tests at a given angle of attack and for the same towing speed, the reaction of AR is higher than the Clarck-Y. Furthermore, Clarck-Y is more sensitive to changes of towing speed and it seems that it has a lower impact than AR door when towing speed is higher. Positive values of Rz have been observed for Clarck-Y in all trials with a towing speed of 3.8 kn, this means that this door might has been flown at this speed, likely due to the poor warp and backstrop attachments. In Table 4 are summarized at which attack angle both doors had the maximum lift (CLMAX) and the maximum efficiency (EffMAX) both in flume-tank and sea trials. In order to get the optimum door performance condition, we have found the attack angle at which we have the concurrence of optimum efficiency and lift (i.e. efficiency equals lift, CL=Eff). For the sea trials, the results are presented for two cases: zeroing the heel term, ST(H0), and without any constraint of the heel, ST(H). In each case the corresponding warp towing point (HF) have been also calculated. For AR door, in general the differences of the estimated attack angle values between sea trials at 0° heel and flume tank tests is around 7 degrees (Table 4). The warp attachment position to the otterboard with maximum lift at sea was found at HF=3, with a corresponding attack angle of 34.2º. At the same HF, this otterboard works in optimum condition (CL=Eff) and attack angle of 32.8° (Table 4). Meanwhile the maximum efficiency was reached at sea with the HF=2 and a correspondent α=25.6°. For Clarck-Y door, in some cases it was not possible to find these values (see Figure 4). The optimum behaviour of this door at sea was reached at HF=6 (α=47.4°), which does not exist. Therefore, the statistical model applied estimated a fictitious backward warp attachment position to get optimum condition of Clarck-Y door. As a result of the model we have also found the location of the fictitious center of pressure, affected by ground effect. Usually in flume-tank the longitudinal distance is around 40% (see [1]) in sea trials we found it was around 60% for both AR and Clarck-Y. With respect to zp, in AR is located in the upper door plate above the cord, at 66% of the height, while in Clarck-Y it is on the lower plate, at approximately 55% of the height.

4 Discussion and conclusions

This paper illustrates the performance and impact on the seabed of an existing door (termed AR door) and a new door design (Clarck-Y door) for demersal fisheries, discussing the differences between engineering sea trials and flume-tank tests and also the differences between both trawl doors. We provided some results related to behaviour of otterboards such as the drag-, lift- and down-coefficient hydrodynamic coefficients and the angle of attack. A valuable indicator of the impact of the otterboard on the seabed such as the reaction force of the otterboards, was also calculated. The current paper is not new only because it gives details on two unstudied otterboards but it also undertakes a more detailed and rigorous analysis using sea trials data, which is why it is been credited. In flume-tank tests both doors not only presented a similar behaviour with attack angle but also similar magnitude of drag-, lift- and efficiency coefficients. Meanwhile comparing both doors in sea trials there are important differences, for instance, AR door works with a higher drag- and lift-force as well as a larger spread but lower efficiency (CL/CD) than Clarck-Y door. As a main result of this work, we estimated values of attack-, heel- and pitch-angles and the corresponding horizontal door spread, drag-, lift- and efficiency coefficients in sea trials condition for each warp attachment position to the otterboard. This is useful information both for door manufacturers and fishermen: the maximum lift and the optimum behaviour estimated for the AR otterboard were for the third attachment warp position. For Clarck-Y door, the estimated optimum condition might have been reached at a fictitious aft warp attachment position (i.e. moving further 55mm backward to the existing last one). Finally, we can extract some conclusions of the doors' impact on the seabed studying the reaction force. The reaction force was, in general, smaller at sea than in the flume-tank tests, probably due to the small towing speed used in flume-tank tests. In the flume-tank test the reaction has been measured and we observed, for a given attack angle, it is smaller in Clarck-Y door than AR door. For sea trials data, a prediction of reaction force has been calculated considering an equivalent hydrodynamic of the flume-tank experiment. In sea trials the estimation of reaction force was strongly dependent on towing speed, in particular the doors' impact decreases when towing speed increases. In particular, Clarck-Y presents positive values of reaction force when towing speed is around 3.8 kn, which means a poor warp and backstrop rigging. Although, computational modelling is a valuable complementary tool to assess behaviour of an otterboard [3], and it can estimate door performances and loads, design defects are clearly visible in flume tank and do not require more questioning, while viewing the results of the simulation can be sometimes more complex. For this reason, scaled prototypes in flume-tank experiments have been extensively used to predict the behaviour of the fishing gear [4]. However, it is difficult to achieve a dynamic similarity between the prototype and the full-scale gear. Comparing flume-tank test data and sea trials data from otterboard we also noticed important differences. This difference is important in AR door (a factor of 2) and using only flume-tank test results it is not possible to predict the real spread of the door at sea. Moreover, it is noticeable that comparing flume tank data and sea trials, AR door has similar trends versus attack angle of drag, lift and efficiency while for the Clarck-Y door there is no evidence of coherent tendencies. In addition, the optimum attack angle (maximum lift and maximum efficiency) for Clarck-Y door is very different in sea trials and flume tank. Meanwhile for AR door the optimum behaviour is reached at an attack angle of 32.8º (warp towing point HF=3) which is only 1.3 degrees smaller than in the flume tank. The current paper shows that data from the flume-tank do not always seem to reflect the real performance of doors at sea, where there is a valuable impact on the seabed. It is well known that at sea other variables like towing speed, heel or pitch, soft seabed may also affect the hydrodynamics and behaviour of the otterboards [1]. Notwithstanding the foregoing, there was generally a better agreement between the result from the flume tank tests and the full-scale trials with the traditional AR door: the full-scale door and the model reacted in similar ways. But the behaviour of the Clarck-Y door at sea corresponded poorly with that obtained in the flume-tank. However, we foresee future works with this door because the promising door performance was in general not verified.

5 Acknowledgements

This study was conducted with financial support from the Commission of the European Communities, Project "DEGREE: Development of fishing Gears with Reduced Effects on the Environment", contract no. SSP8-CT-2004-022576) and with support from DGICYT (Spain) under the grant DPI2008-01408. These supports are gratefully acknowledged. We are indebted to Dominic Rihan and Tan Huan (BIM, Ireland), Nigel Ward and Adrian Strickland (SEAFISH, England), Kurt Hansen (SINTEF, Denmark) for their enormous work and effort in the flume-tank trials. We would like also to thank our colleague Vito Palumbo (CNR-ISMAR, Italy) for his support in the fieldwork and Justine Garden for helpful revision of the manuscript. Finally, we are grateful to Mr. Roberto Grilli (Grilli sas) and Eng. Giulio Menco (Prosilas sas) for having developed and built the two doors for the flume-tank and sea trials.

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A New Observing System for the Collection of Fishery and Oceanographic Data

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Abstract

Fishing vessels represent a great opportunity to carry out repeated and continuous observations on both fishing and environmental parameters in coastal areas. In 2003, a Fishery Observing System (FOS) was developed by ISMAR- CNR, within the framework of the EU research program Mediterranean Forecasting System: Toward an Environmental Prediction (MFSTEP), with the aim to exploit this great potential. Eight fishing vessels belonging to Adriatic fleet, targetting anchovy and sardine, were equipped with FOS instrumentation in order to collect geo-referenced catch data, associated with in-situ temperature and depth.
The system is nowadays still working in the Adriatic Sea, collecting very important data for the evaluation of the fishing effort and the influence of the environment on the abundance distribution of the target species.

1 Introduction

Real time estimates of stock abundance and its spatial and temporal distributions are very difficult to obtain. Acoustic surveys and landings information are the traditional tools used to achieve an evaluation on fish populations' abundance [1, 2, 3]. These methods showed to be effective for scientific purposes and a posteriori evaluations. On the other hand, acoustic surveys do not adequately cover the stock spatial extent and their temporal coverage is gappy because of the difficulty to carry out surveys regularly. Moreover landings data provided at a later time are useless for management and operational purposes, considering the fact that in these contexts, data should be delivered in near real time.
In order to overcome the limits of the above mentioned methods, in the year 2003, within the framework of the EU research program "Mediterranean Forecasting System: Toward an Environmental Prediction" (MFSTEP), ISMAR - CNR developed a Fishery Observing System (FOS hereafter). The main objective of the FOS was that to obtain a continuous monitoring of the fish abundance distribution, by means of geo and time referenced abundance indexes (Catch Per Unit of Effort - CPUE). In addition, relationships between the environmental conditions and the distribution of the target species could be inves-

tigated thanks to this instrument, putting in relation the CPUE index and detailed sets of environmental descriptors, obtained both from MFSTEP models and in situ measurement. In general, understanding how the environment influences the distribution of fish stocks represents a complicated matter. Many studies have tried to answer this question and to explain how environmental variability can affect recruitment and fish distribution [4, 5]. The problem is even more complex considering the fact that the relationships between climate/environment and stocks are not stationary [6, 7]. To address this complicate issue, long time series of both environmental and abundance data, collected at comparable temporal and spatial scales, are necessary. The FOS, developed in August 2003 and still working, is as well oriented toward this long term objective.
Data collection by means of the FOS started in 2003 in the Adriatic Sea, an epicontinental basin of the Mediterranean where fishing activity is very intense.
Anchovy (*Engraulis encrasicolus*, L.) was chosen as the main target species due to its commercial importance in the Mediterranean Sea and in particular in the northern and central Adriatic Sea, where it reached annual estimated catches ranging between 20000 and 30000 tonnes during the period 1976-2006, and up to 44.000 tonnes in the three-year period 2006-2008 [8, 9]. The Adriatic Sea is then one of the most important fishing areas for this species.
The analysis of the big amount of data collected by the FOS since 2003 is still underway. Results relative to the observational period October 2003- August 2005 are presented and discussed in [10].

2 The MFSTEP FOS

The FOS was built taking into account the compromise between a good quality of the collected data and the impact that the instruments used for this purpose might have on the fishing activity. The FOS underwent a number of improvements and in the last version it consists mainly in three components: an electronic logbook (EL), a GPS (Figure 1a) and a temperature and pressure recorder (Figure 1c). The software used to collect and store data on the EL was developed ad hoc for this application (the interface used to input fishery data is showed in Figure 1b). Each single component of the FOS generates a file: catch and position data are stored on the EL, temperature and depth data are stored in the memory of the sensor. Temperature data are collected every time the fishing gear is hauled. Ultimately, all the data are stored in a database and collated in the post processing stage.
The EL is basically a computer with a touch screen as user interface. It is very compact so that it occupies little space and can easily be installed on the control deck of a fishing vessel. It has six powered serial communication ports (two DB9 and four RJ45) and six 2.0 USB connections. The EL powers the GPS, and then each time the EL is turned on, the GPS as well starts to send position data to the EL memory.
The fishermen input directly the daily catch information on the EL by means of dedicated software, expressly developed in order to be as user friendly as possible. The skipper enters information regarding selected species, indicated directly by the software; for each species total catch for haul, estimate of the mean size of individuals per catch (only for anchovy and sardine) and discard (in terms of catch and size) are requested. The unit measure used

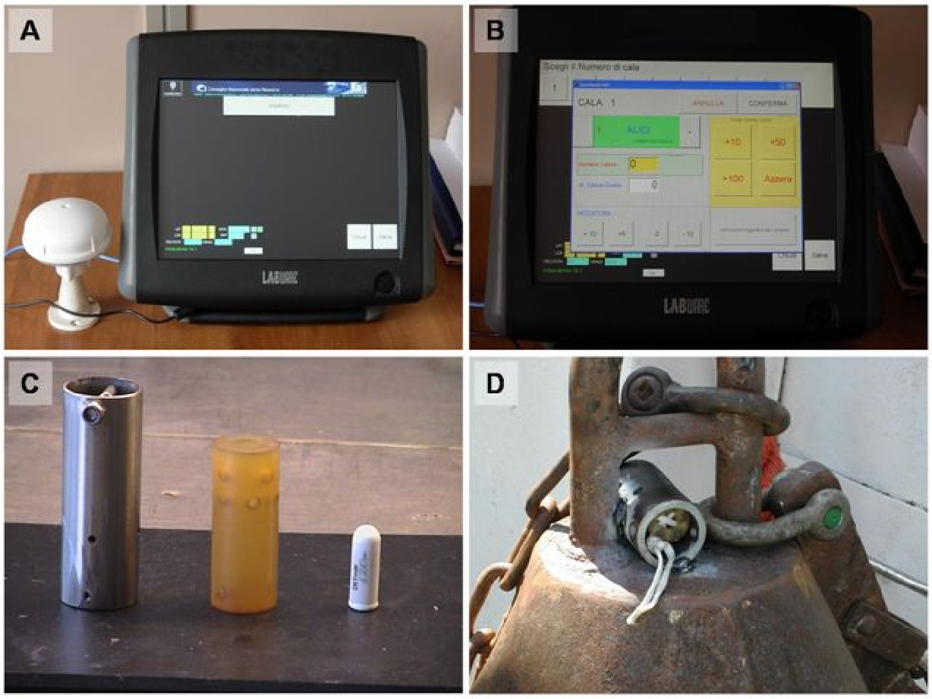

Figure 1: Fishery Observing System (FOS) elements.

for the estimate of the catch is "boxes of fishes" considering anchovies the average size is expressed in number of individuals per kilogram, while sardine average size may be selected between two categories (large or small). The chosen measure units are those usually adopted by fishermen of the Adriatic fleet to indicate catch and size for these two species. In the post-processing phase, catches are converted in kg using a known conversion factor between boxes and kg. This conversion factor may differ from harbour to harbour.

The last component of the FOS is a temperature and pressure sensor (TPs hereafter Figure 1c). The probes used, manufactured by Star Oddi Company (Iceland), are very compact (few centimetres in length). The latter feature is very important considering the fact that the sensor has to be attached to the fishing gear (an example of TPs installed on the weight used to make a pelagic trawler net sink, is showed in Figure 1d) small instruments have in fact the advantage to have a narrow impact on the gear and to be easily positioned in a safe way. Two types of TPs are used: DST Milli and DST Logic. The first is a standard product made by Star Oddi, while the second was produced following some indications suggested to the company during the development phase of the FOS. The DST Milli, once switched on, collects data continuously, even when the boat is not working this aspect limits the duration of memory and batteries. Furthermore, after some

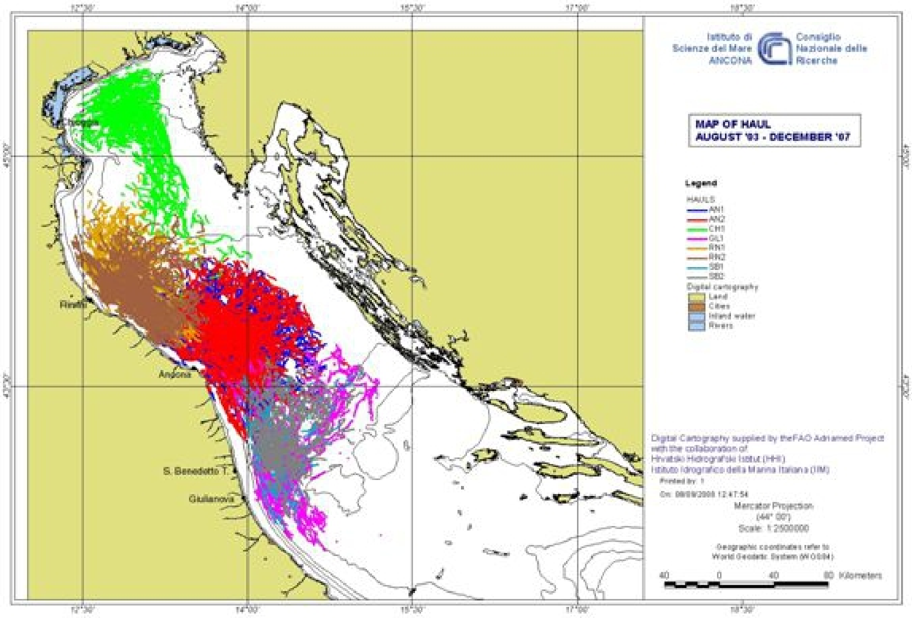

Figure 2: Map of the hauls monitored from October 2003 to December 2007.

comparisons with standard oceanographic probes, the response time to temperature variations of DST Milli resulted too slow especially where temperature gradient are strong (e.g. in correspondence to the thermocline).
The DST Logic was conceived in order to fix the mentioned problems indeed the new TPs collect data only when the pressure overcomes a threshold value (factory defined), the memory space has been expanded, and the temperature response time has been significantly increased with respect to the DST Milli.

3 FOS application to the Adriatic Sea

Starting from August 2003, the FOS was installed on eight boats belonging to the fleets of Chioggia, Rimini, Ancona, San Benedetto del Tronto and Giulianova degli Abruzzi harbours (Figure 2). Four vessels were equipped within the first 3 months from the beginning of the project. The remaining four were provided with a FOS a year and half later, thanks to national funding of the General Fishery Directorate of the Italian Ministry of Agriculture. At the present time still two vessels per harbour are monitored, with the exception of Chioggia and Giulianova (where only one vessel is monitored). This distribution ensured a good spatial coverage above all in

the central part of the Adriatic Sea.
The monitored vessels are pelagic trawlers (volante in Italian) and work all along the year with the exception of the boats from Giulianova and San Benedetto, which change fishing gear at the end of May and until approximately the half of October work as light attraction purse seines (lampara in Italian). Since August 2003, data from about 18000 hauls were collected.
The map in Figure 2 shows the routes of the hauls monitored from October 2003 to December 2007. Even though the monitored vessels belong to different harbours, working then on different fishing grounds, these fishing areas overlap. Furthermore, considering the fact that the area exploited by the fishing fleet of one harbour is approximately the same for each single vessel belonging to this fleet, conversely it can be assumed that each single area exploited by one vessel is representative of the fishing ground of the entire fleet of the considered harbour. These two aspects have strong implications on the standardization of CPUE index [10].
In addition, CPUE can also be defined as a function of different environmental conditions and the results obtained for this project ([10] for a complete description) suggest that oceanographic features can determine abundance distribution. Especially in the area of Giulianova (central Adriatic Sea), conditions were found to be more favourable to fish aggregation. For instance, areas of convergence could be found here [11, 12], both where the southward long shore current encounters the descending branch of the Middle Adriatic gyre and in correspondence of the frontal zone which separates the coastal fresh water from off-shore saltier waters. Bottom topography can also be an important factor in determining areas of strong gradient such as shelf break fronts. Such conditions could contribute to fish aggregation in the Giulianova and San Benedetto areas. Furthermore, the Adriatic Sea is an epicontinental basin where the contribution of the Po River run-off, strongly influences both the physical and ecological features of the environment. The importance of the correlation between anchovy abundance and recruitment with river freshwater outputs was already proved in previous studies [13, 14] and river effects were reported to be noteworthy in semi-enclosed basin [1]. These aspects can play a crucial role in determining fish aggregation, especially in the northern sector of the Adriatic Sea where the Po River outflow strongly influences the dynamic and sea water properties [11].

4 FOS data set

At the end of each haul, catch data (species, number of boxes and sizes), position data (tracks of the vessel's course steered during the trawling phase for volante or mean position of the vessel while fishing for lampara), depth of the trawl (in form of pressure data) and temperature of the water are available. All these information are included in separate files which have to be merged in order to provide geo and time referenced catch data associated to depth and temperature. To merge the three different files, an ad hoc database (developed in MS Access®) is used.
The merging is done for each volante and lampara haul depth data obtained by TPs allow the selection of the initial and final coordinates of the haul. All positions included in this time interval are selected. Catch data are input by the fishermen in the EL sequentially every fishing day; thus the order of the hauls and their date allow to

merge daily catch data with TPs and position data.
Temperature and pressure data are seldom missing due to damaging or loss of the TPs sensors. Besides, TPs data cannot be associated with geographic position, when the GPS does not receive the signal.

5 Conclusions

Fluctuations in the physical features of the environment may produce relevant changes in the marine ecosystem. The fish distribution is obviously influenced by these fluctuations. Therefore it is easy to understand the relevance of appropriate observational tools able to provide a better understanding of the links between environment and fish resources. The FOS discussed hereby represents a first and successful attempt toward this goal. Thus an application of such a monitoring system in other important fishing areas of the Mediterranean Sea would be crucial in order to reach an effective fishery management.
At present time, one of the limits of the FOS is the deferred availability of the data: once a month the ISMAR-CNR operator visits the vessels in order to download the TPs data set. This time interval could be fine for fishery management purposes but is too long for operational applications. In a very next future, the TPs will be upgraded with new sensors able to transmit the collected data directly to the EL at the end of each haul. The EL is already arranged to be able to send data by GPRS to a data center, thus the TPs upgrade will allow obtaining a complete data set in near real-time.
The new TPs probes will also be able to produce more reliable pressure and temperature data, and to collect salinity and possibly other oceanographic (e.g., fluorescence and/or turbidity) data.
Another focal point in the FOS application is the collaboration of the fishermen. Hence the necessity to deepen the relationships with the fishery sector creating stronger synergies. Interactions between the research community and the fishing industry [15] as well as with the regulatory authorities would allow consistent step towards the institution of an operational fishery and oceanographic framework aimed to a better management of the resources.

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Recruitment of Anchovy and Sardine in the Adriatic Sea and Environmental Factors

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Abstract

Anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) are important fishery resources in the Adriatic Sea. Since 1975, CNR-ISMAR-Ancona has been conducting research on these stocks in the central and northern Adriatic Sea, particularly on their dynamic. The level of recruitment to the stock has been estimated by means of population dynamics methods for both species and was analyzed to identify possible effects related to changes in some environmental variables, in particular Po River flow rate, wind stress and North Atlantic Oscillation (NAO) index. At this aim, different types of regression models were applied. A positive correlation was found between anchovy recruitment and Po River flow rate and quadrant specific wind stress. On the other hand, the influence of Po River seemed to be weaker for sardine, while a positive correlation between NAO index and recruitment was found for this species. Moreover, about the role of Po River runoff on anchovy, useful elements were also obtained from some investigations on the growth pattern of young stages preceding recruitment. Further investigations in both stock assessment and biology could provide a better understanding of the influence of environment on the recruitment of Adriatic anchovy and sardine, allowing also an improvement in stock management, e.g. forecasts.

1 Introduction

Anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) are of very high ecological importance within the Adriatic ecosystem: anchovy is a key prey, while sardine is important both as prey and predator [1, 2]. Moreover, both species are important fishery resources in the Adriatic Sea and the stocks are shared by different countries [3, 4, 5]: the adults are fished all the year round by the fleets of Italy, Croatia and Slovenia, with two gears being used: purse seine and pelagic trawl. On the other hand, the fishery of sardine late larvae and juveniles (”bianchetto”), mainly concentrated along the Apulian coast between Manfredonia and Bari, takes place between January and April [6, 7]. Many studies have been carried out to describe the migration pattern and the distribution of these species in the Adriatic Sea. For sardine, two main spawning grounds were described [8], in the northern and southern Adriatic, respectively; the analysis carried out by [9] showed the lack of genetic heterogeneity in the Adriatic stock, so that the morphometric and meristic differences found by previous studies seem to be mainly due to environmental factors rather than to the existence of two genetically distinct stocks. Anchovy spawning activity takes place in the coastal waters of the western Adriatic between the Gulf of Trieste and the Gargano Promontory [8, 10] and the largest number of eggs occurs in the Gulf of Trieste and off the river Po Delta [11]. Growth rates, morphometric and allozyme frequency analyses suggested the existence of two distinct stocks: one in the northern Adriatic, between Chioggia and Ancona, and the other one in the area between San Benedetto and Vieste. However, this feature is still unclear and needs further studies [5]. These issues are of great importance from a management point of view: in fact, in a closed basin like the Adriatic Sea, agreement between countries exploiting the same stocks is crucial. Since 1975, CNR-ISMAR-Ancona has been conducting research on the biology and assessment of the stocks of anchovy and sardine in the central and northern Adriatic Sea. Stock assessment has been carried out by means of population dynamics and acoustic methods. In the present work, emphasis is given to the former ones. These methods are based on mathematical models and always require catch data, along with information on other features like, for example, the component of mortality not due to fishing activity. One of the methods mainly used for the two stocks [3, 4, 12, 13] is represented from Virtual Population Analysis or VPA, which requires catch data distributed into age classes [14]. For both anchovy and sardine, total catch (i.e. from fleet of both western and eastern side of Adriatic) and stock biomass estimated by VPA are compared over years in Figure 1 [15]. For anchovy, split year data were used, assuming the first of June as the birth date, consistently with the biology of Adriatic anchovy (e.g. split 1976 is from calendar first of June 1975 to the 31st May 1976). The annual biomass at sea of these stocks estimated by VPA display strong fluctuations along the last three decades. Anchovy progressively decreases from the end of the 1970s to 1987, the year of lowest abundance and fishery crisis, followed from partial recovery and a peak in recent years; sardine abundance, on the other hand, shows a continuous decrease of the population since the middle of the 1980s [3, 4, 16, 17, 18, 13]. These patterns of abundance over years are not very different form those obtained by means of the echo-surveys carried out by the same CNR-ISMAR-Ancona [18]. Likely the decreasing trends are imputable not only to the fisheries. In fact, the stock dynamics can be influenced by physical oceanographic factors [19, 14, 20]. From this point of view the Adriatic Sea is emblematic because of the great number of environmental variables that affect daily and seasonally the main characteristics of this basin (e.g. temperature, salinity, food availability, circulation). The general circulation is cyclonic with important influence, especially in the northern sub-basin, exerted by Po River runoff and dense water formation processes. Winds are able to induce relevant changes in current patterns at several spatial and time scales, with relevant differences between winter and summer wind regimes. Besides that, the great surface related to the small volume makes this basin strongly variable regards several parameters: e.g. temperature in winter it is characterized by a vertical homogeneity and by a pronounced horizontal thermal gradient (both from South to North along its major axis and from the western to the eastern coast along its minor axis), while in summer the situation reverts (there are limited and variable horizontal thermal gradient, and an intense vertical gradient). The river runoff, that affect mainly the western side of the basin, causes a great variability in the salinity pattern as well as in the concentration of nutrients and in the circulation of the surface layers. During winter, river waters are confined near the western coast, whereas during summer thermal stratification and a weaker wind regime allows for a wide horizontal distribution of these river waters inside the basin [21]. Environment can influence growth, reproduction and survival of young stages preceding recruitment, with consequences on the stock abundance and, thus, strong implications for the management.

2 Materials and Methods

As said previously, the basic information for VPA is represented from catch at age data. In the present case, the age frequency distributions of both anchovy and sardine catch were obtained on annual basis, combining the corresponding length frequency distributions and age-length keys; the age of fish was determined by otolith readings [14, 3, 4]. For these stocks, the natural mortality rate M has been usually assumed as constant over time and age class [18]. VPA provides, for each year and age class, the fishing mortality rates F (thus F+M = Z total mortality) and the numbers of individuals at sea, so that recruit and spawner abundance can be also estimated. The age class 0, formed by individuals that have not yet reached the first birthday, was taken to quantify recruits for both species. It's important to remember that the "bianchetto" catch data do not enter into the model: it is a particular issue that needs a different approach. For anchovy, statistical analysis were performed to evaluate how much the recruitment strength could be related with the corresponding parental stock (i.e. spawning biomass), and different environmental variables, such as surface (2 m) air temperature, sea surface atmospheric pressure, quadrant specific wind stress, Po River flow rate and NAO index [16]. Three types of regression models were employed: a simple linear model, used to relate recruitment to spawners or to just one of the environmental variables; a multiple and linear model, relating recruitment to spawners and one or major than one environmental variables and, finally, a nonlinear type represented from the Beverton and Holt stock-recruitment model and from an alternative version modified *ad hoc* to take into account at least one environmental variable [16]. For sardine, the environmental variables mainly investigated were NAO index and Po River flow rate and simple linear models were used [17]. For both species, both normal and log normal distributions were evaluated to model the statistical error in regression analysis [14]. About the study of biology of these two species in the Adriatic Sea, in the present context, it is particularly worth noting that investigations on growth of early life stages of anchovy and sardine were carried out between June 2007 and February 2008 in two nursery areas along the Adriatic coast, off the Po River delta and in the Gulf of Manfredonia, respectively. Growth rates of both species were estimated by daily increment counts on otoliths (unpublished results from Ph.D. thesis of Panfili M.).

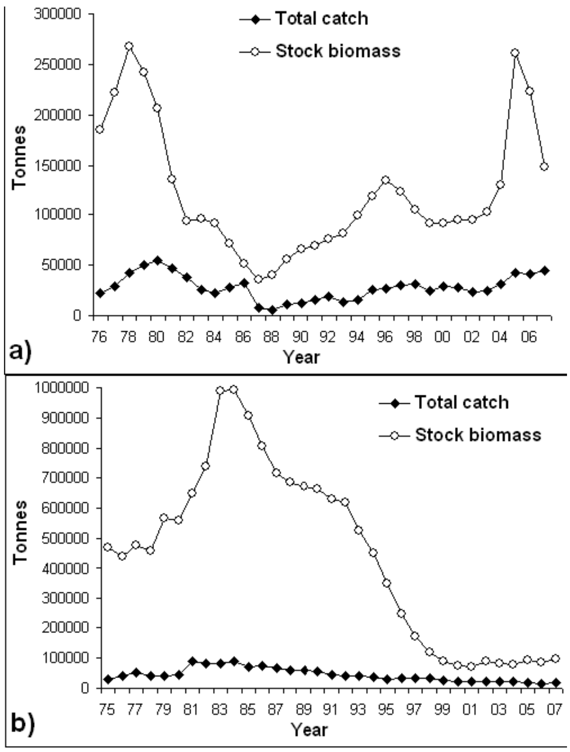

Figure 1: Total catch and stock biomass estimated by VPA of a) anchovy and b) sardine are compared over years; split year was used for anchovy. Redrawn from Cardinale et al., [15].

3 Results and Discussion

The analysis showed a significant correlation between anchovy recruitment and both Po River flow rate (Figure 2 and 3) and autumn SSE and ESE wind stress [16]. The presence of high river flow rates together with moderate wind stress influence the regime of the Western Adriatic Coastal Current (WACC): nutrient rich waters move offshore sustaining an increased zooplankton and phytoplankton biomass. In particular, zooplankton is really important for both the species as far as recent studies indicate that European anchovy feed on zooplankton more than phytoplankton [22]. The influence of the NAO index is less clear: it could have a link with salinity variation in the intermediate layer of the Adriatic Sea, with positive values being associated with higher salinity [23, 1]. On the other hand, Po River flow rate seems to have some positive influence on sardine recruitment, but the effects are not statistically significant as in the case of anchovy (Figure 4). A significant positive correlation between the recruitment of sardine and the autumn NAO index of the previous year was observed [17]. The results relative to the Po River flow rate are consis-

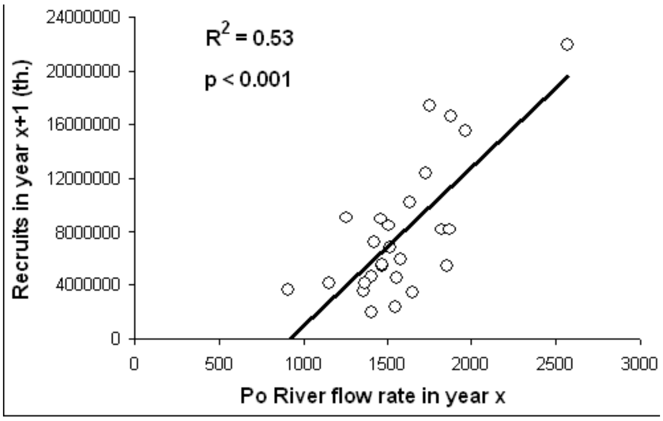

Figure 2: Anchovy recruit numbers (thousands) estimated by VPA in split year x+1 are plotted as a function of Po River flow rate ($m^3 \cdot s^{-1}$) of the calendar year x from 1976 to 2000. The results of simple linear regression analysis are shown.

tent with some features of the distribution of both anchovy and sardine in the Adriatic system. Sardine spawning grounds are in the eastern part of the Adriatic Sea, corresponding to the northern area off the Dugi Otok Island and the southern area around the exterior of the mid-Dalmatian Islands and extending offshore to Palagruza, thus miles away from the Po River. On the other hand, the main spawning areas of anchovy are in the coastal waters of the western Adriatic, with the largest numbers of eggs being observed in the Gulf of Trieste and off the Po River delta [8, 24, 11, 10, 5]. Moreover, in the Adriatic Sea, anchovy spawn at lower values of salinity than sardine and relatively low salinity values are observed just off Po River delta [23, 25]. For all these reasons, the statistically significant influence of Po River flow rate obtained for anchovy recruitment rather than sardine one was expected and, thus, it is a consistent pattern. Finally, insight about the role of Po River runoff was also gained from the mentioned investigations on the growth pattern of young stages of both species. The instantaneous growth rate estimated for sardine late larvae (0.20 mm day-1) resulted to be lower than those estimated for anchovy (0.46 and 0.64 mm$\cdot$day^{-1} in June and November, respectively). In particular, in the case of anchovy, length at age data of individuals caught in November were significantly ($p < 0.001$) higher than those collected in June. These results are not in agreement with the general pattern observed in another Adriatic ground (Ortona), where the instantaneous growth rate showed a higher value in May (0.82 mm$\cdot$day^{-1}) and decreasing in August (0.54 mm$\cdot$day^{-1}) and November (0.55 mm$\cdot$day^{-1}) [26]. Spatial and seasonal changes in environmental conditions largely affected the instantaneous growth rate of anchovy. Positive effects of temperature and food on the growth of fish are well documented [14, 20, 16] and the higher growth rate in November off the Po River delta, when the flow rate displays a seasonal peak [21, 16], could be

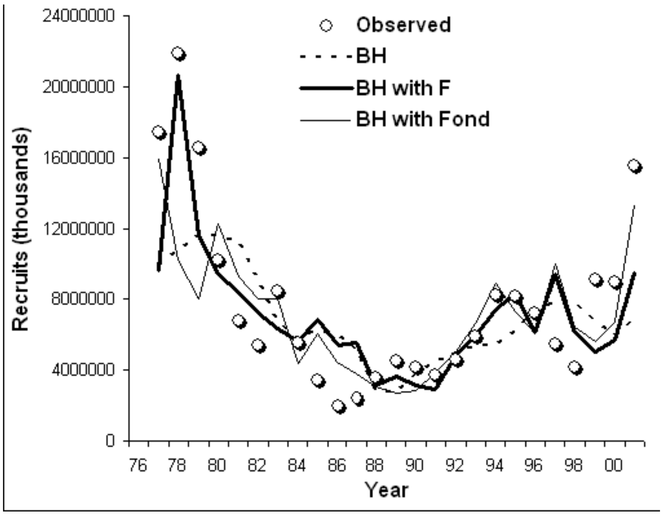

Figure 3: Observed (Rv) and expected (all BHs) values of the anchovy recruit numbers (thousands) over years. Expected values were obtained by fitting the Beverton-Holt model to log values of recruit numbers estimated by split year VPA. The Beverton-Holt was simple or *ad hoc* modified with predictor variable F and Fond, i.e. annual and autumn (ond = October-November-December) Po River flow rate ($m^3 \cdot s^{-1}$) in previous calendar year, respectively. Redrawn from [16].

related to trophic rather than temperature changes.

4 Future perspectives

In order to obtain the most reliable estimates of stock abundance, the use of different methods to estimate some input data as well as different population dynamics methods will be evaluated. In the former case, an example is given by the use of the natural mortality rates M taken as variable (instead of constant) over age class; this feature was taken into account in the most recent assessments of the two Adriatic stocks of anchovy and sardine (GFCM-SCSA, Malaga, 30 November - 4 December 2009 http://www.gfcm.org/gfcm), where the values of M for the age class 0 (i.e. recruits) were estimated to be quite higher than in previous assessments. About the use of different models, the analysis performed with VPA could be repeated using the Integrated Catch Analysis or ICA. This treats the catch at age and auxiliary information on fish abundance at sea (e.g. derived from echo-surveys) as measured with error in contrast with classic VPA [14]. Therefore, the numbers of both recruits and spawners estimated using variable M at age and ICA could be related again to the mentioned environmental parameters, on the basis of updated time series. In conclusion, further investigations in both stock assessment and biology (e.g. growth patterns) could provide a better understanding of the influence of environment on the recruit-

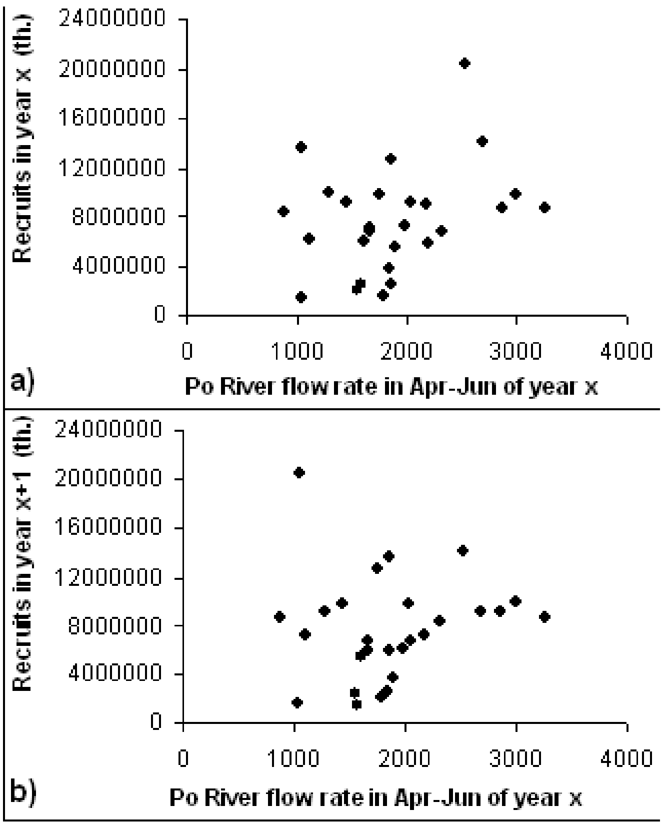

Figure 4: Sardine recruit numbers (thousands) estimated by VPA are plotted as a function of Po River flow rate ($m^3 \cdot s^{-1}$) in April-June of years from 1974 to 2001, a) without and b) with 1 year lag. Simple linear regression analysis did not provide significant ($p > 0.05$) slope. Redrawn from [17].

ment of Adriatic anchovy and sardine, allowing also an improvement in stock management. In particular, some environmental variables could be employed in the predictions of catch and biomass at sea in the future. These can be performed on the basis of population dynamics methods, where usually more scenarios of fishing effort / fishing mortality and strength of recruitment are evaluated. The knowledge of the effects due to environment could be taken into account to select these different scenarios of recruitment at least for short term predictions, reducing, therefore, the uncertainty in the future estimates.

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Population Dynamics of the Clam, *Chamelea gallina*, in the Adriatic Sea (Italy)

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Abstract

In the western Adriatic Sea the fishery for the clam, *Chamelea gallina*, by means of hydraulic dredgers is by far the most important bivalve fishery, having reached landings of over 100000 t in the early 1980s. Owing to this intense fishery, the clam population has undergone profound modifications and substantial reductions in biomass throughout the years. Thus, between 1984 and 2001 the scientific community was given the task of annually assessing the state of this resource. This paper is aimed at presenting the results obtained from the surveys carried out in the Ancona (AN) and S. Benedetto (SB) Maritime Districts between 1984 and 2001. The results are indicative of a resource and a fleet heavily dependent on stochastic substantial recruitment events. A stressed resource was revealed which was unable to cope with the intense exploitation. Large recruitment events followed by significant natural mortality episodes and the paucity of older individuals may suggest a shift in the allocation of energy, from growth to reproduction. Following the collapse of the resource in 2001, in SB especially, part of the vessels were redistributed in the nearby AN district. This caused a considerable increase in fishing effort and a further depletion of the resource as highlighted by the long closure periods. Despite this no further surveys were funded. Its is our hope that this contribution may, somehow, act as a catalyst for the resumption of the assessment of a crucial coastal resource.

1 Introduction

Chamelea gallina is an infaunal clam of the Veneridae family (Bivalvia: Lamellibranchiata: Veneridae), locally known as ‘vongola’ or ‘lupino’. Venerid clams are mostly found in temperate and tropical regions where they inhabit the particulate sands of the infralittoral and circalittoral zones [1]. *Chamelea gallina* is a gonochoristic species with a long spawning period (April to August), during which spawning takes place at intervals, and seems to be followed by a resting stage [2]. Following spawning a trochophore larva is produced which, once the egg membrane is shed, becomes free-swimming and pelagic [3]. The larval shell is then secreted bringing the larva into the veliger stage. The length of such pelagic life is not known, but probably does not extend beyond 20 to 30 days [1], following which the larvae settle in the substratum, becoming benthic. In the central Adriatic the growth rate of *C. gallina* is high in the first three years of life [1] and then progressively decreases, reaching a maximum length of 49-50 mm (≈ 6 years of age [4]). Nevertheless, growth is slow and it takes one year to reach a size of 18 mm and two years to reach the minimum landing size (MLS) of 25 mm [1]. *Chamelea gallina* appears to exhibit density-dependent growth rate and mortality: years of exceptional recruitment may lead, the following year, to very poor stocks of large individuals due to juvenile mortality, or to very large stocks of small individuals due to growth inhibition and resource competition. It has been reported that a proportion of the population reaches maturity by the end of the first year of age (18 - 19 mm) and all individuals are ready to reproduce within the second year [1]. In the western Adriatic Sea the fishery for *C. gallina* is by far the most important bivalve fishery, having reached landings in excess of 100,000 t in the early 1980s [1]. This species is also exploited in Albania, France, Spain and Turkey [5, 1, 6]. Owing to the introduction of the modern hydraulic dredge into the Adriatic, the *C. gallina* population has been subjected, for the past 40 years, to constant and consistent exploitation. The pioneer hydraulic dredge was introduced in the late 1960's and by the 1980s it had been replaced by the modern version used today. With the advent of the modern hydraulic dredge, the *C. gallina* fishery in the western Adriatic Sea escalated progressively, reaching a status of extreme importance for the economy of the fishing community: by 1994 the vessels fishing hydraulic dredges had increased by 115% (808). Whilst the stock was still healthy in 1984, the management procedures adopted were inadequate to protect it from overexploitation: landings increased by approximately 20% between 1974 (annual catch = 80,000 t) and 1984; fishing effort saw a 50% increase [1]. It is estimated that by 1984 the fleet had expanded so much as to be able to cover the whole area of the fishing grounds in one year whilst it takes two years for a clam to reach the MLS of 25 mm. The precarious situation of the *C. gallina* resource in the entire Adriatic Sea was acknowledged by the stakeholders, and from 1984 the scientific community was given the task of assessing the state of the resource in each District with the aim of giving management guidelines for the year following. This contribution summarises the results obtained from the analysis of the long time series of survey data collected from the Ancona (AN) and S. Benedetto (SB) Maritime Districts between 1984 and 2001. These two areas have been in conflict since the beginning of the fishery; conflict which escalated in 2001 when the resource collapsed and the fishery in the SB District was closed prompting a redistribution of the SB vessels into the adjacent AN District and the creation of a new "Fishing District" in the area of overlap (Civitanova Marche).

2 Methods

The annual surveys aimed at analysing and quantifying the clam population in the AN and SB Districts were carried out from 1984 to 2001 by ISMAR-CNR Ancona, with an interruption in 1988-1990. For this purpose, the entire AN District was divided into 21, 2 mile-wide transects perpendicular to the coast, between the mouth of the river Cesano and the mouth of the river Chienti (Figure 1). Similarly, the SB District was divided into a further 13 transects, between the mouth of the river Chienti and the mouth of the river Tronto (Figure 1). Within each transect, a sample was taken at every metre depth between 3 and 12 m. For

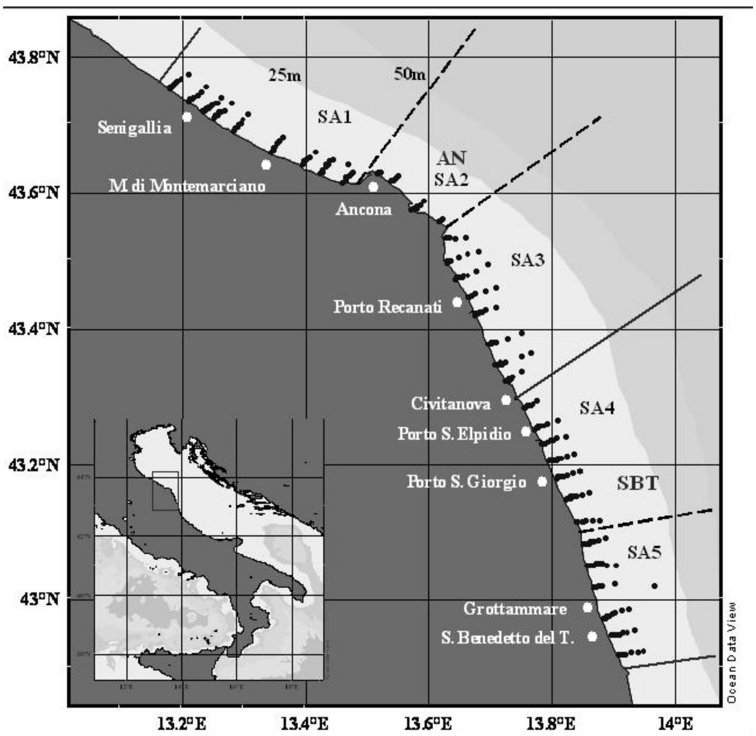

Figure 1: Map of sampled stations (black dots) within the Ancona (AN) and S. Benedetto (SB) Maritime Districts during the annual *C. gallina* stock assessment surveys showing District boundaries (solid line) and sub-area (SA) boundaries (dashed lines).

statistical analysis purposes, and for their intrinsic differences, the entire area comprising the two Districts was divided into 5 sub-areas (SA), three of which in the AN District and two in the SB District (Figure 1):

- SA 1: 'North of Ancona': between Senigallia and Ancona harbour;
- SA 2: 'Conero': between Ancona harbour and Sirolo;
- SA 3: 'South of Conero': between Sirolo and Civitanova;
- SA 4: 'North Aso': between Civitanova and Altidona;
- SA 5: 'South Aso': between Altidona and S. Benedetto del Tronto.

The sampling was carried out using chartered commercial vessels mounted with experimental hydraulic dredges. The experimental gear was divided into 3 sections each 80 cm wide, the lateral sections having a grid-spacing of 12 mm (as required by law) and the central one of 6 mm (this latter grid size allows the dredge to retain all individuals above 14 mm and a proportion of smaller ones). The vessel steamed to the chosen transect and depth and deployed the dredge which was towed, parallel to the coast, using the anchor technique, for a distance of 50 +/- 5 m (for details see [7]). The catch retained by the central portion of the dredge represented the benthic community present on 40 m^2, the total area covered by the dredge in one tow being 120 m^2. On each tow, a bag of catch collected in the central section was weighed and retained and the remainder was weighed and returned to sea. The retained material was frozen at -18°C and subsequently examined in the laboratory where each sample was sorted. All organisms were identified, counted and weighed. *Chamelea gallina* individuals were measured to the lowest millimetre along the major axis (termed length hereafter). The data were treated using the formulae proposed for the application of stratified random sampling by Cochran (1963, in Russell [8]). The area of each stratum (strata were defined by depth intervals of 2-4 m, 5-6 m, 7-8 m, 9-10 m and 11-12 m for SAs 1, 3, 4 and 5, and depth intervals of 3-9 m and 10-12 m for SA2) was calculated, and the sampling units and the individual tows were estimated in areal terms, allowing to express the area of a stratum in terms of the total possible number of sampling units contained within it [8]. This enabled the calculation of mean catch per stratum (both in terms of biomass and abundance), its variance and ultimately total biomass, abundance and their variances (Cochran, 1963, in [8]). Both biomass and abundance were calculated using these equations. To estimate biomass a mean weight was assigned to each size class based on the following length-weight relationship (Froglia, unpublished data):

$$Log_{10}Wt = 2.7554 * Log_{10}L - 3.1787,$$

where: Wt = weight (g); L = length (mm). When necessary the identification of statistically significant trends in time was carried out using Kendall's coefficient of rank correlation test applied to trend lines calculated using Kendall's robust line-fit method for nonparametric regression [9]. The significance criterion for all tests was set at alpha = 0.05.

3 Results

Overall, both Maritime Districts have undergone considerable year-to-year fluctuations in total biomass (Figure 2a). Minimum total biomass estimates were reached in 1991 (1063 t) in AN and in 1994 (468 t) in SB, whilst maximum values were recorded in 1999 (97270 t) and 1998 (96160 t) in the two Districts, respectively. No significant decreasing trend in biomass through time was discernible for either District, but each District experienced collapses of the resource that resulted in temporary closures of the fishery (AN in 1991, SB in 1991, 1994-1995, 2001). Similarly to total biomass, commercial biomass (Figure 2b) fluctuated widely with estimates for AN ranging between 490 t (1991) and 12351 t (2000) and for SB between 309 t (1994) and 8955 t (1985). Low estimates of commercial biomass appear to have been the rule for the SB with the exception of 1984 and 1985. In 1986 the commercial stock fell in both Districts, reaching extremely low values in the early 1990s when they were comparable. 1994 saw a recovery of the AN commercial stock that was not paralleled by a similar recovery in SB, where the relatively high estimates recorded in 1985 were never to be reached again. Since 1994 commercial biomass estimates have been considerably higher in AN consistently across the years and this was especially evident in 1999 and 2000 when commercial clam estimates were approximately five times greater in AN. A marked decline of the exploitable fraction of the resource occurred in 2001 in both Districts, when estimates fell to 3127 t in AN and 671 t in SB. Inter-annual fluctuations were even more marked upon consideration of the younger portion of the examined population ($\leq$ 18 mm) with years of extremely low biomass being followed by very high biomass estimates in the successive year (e.g. SB 1997 *cf* 1998), or with several subsequent years in which recruitment was virtually absent (e.g. AN 1984-1987) (Figure 2c). Figure 2c appears to indicate recruitment events as sporadic events. Successful recruitment in one year will result in high estimates of individuals smaller than 19 mm in the successive year. Upon comparison of the two Districts, SB appears to have had the most successful recruitment events since 1984 and this was especially so in 1998 when the juvenile fraction reached 78597 t. The results show that years of intense recruitment (e.g. 1996 and 1998 in SB) were not coupled with proportional increases in biomass of the commercial fraction in subsequent years. On the other hand, a clear relationship emerged between the juvenile ($\leq$ 18 mm) and 'medium'-sized ($\geq$19 $\leq$ 25 mm) fractions of the population. The attempt made at evaluating whether a relationship existed between stock and recruitment in terms of abundance highlighted the fact that recruitment is largely independent of stock size having consistently low values across the range of stock abundance considered, with few exceptions (Morello, unpublished data). In order to gain information on the profitability of the resource from an economic point of view, the surface area corresponding to increasing commercial clam densities was calculated for each year and District (Figure 3) and considerable differences were revealed between Districts in this respect. The percentage area supporting no clams was consistently higher in SB (Figure 3b) compared with AN (Figure 3a), whilst the converse was true for the percentage surface area supporting $\geq$12.5 kg$\cdot 1000 \cdot \mathrm{m}^{-2}$. The higher percentage of highly productive grounds in AN was further confirmed upon comparison of surface areas supporting densities greater than 75 kg$\cdot 1000\mathrm{m}^{-2}$, which, in AN have been variable but with peaks around 45 km^2 in 1999 and 2000, whilst in SB they have been very close to zero since 1986 (Figure 3b). The lower overall exploitable area available in SB is likely to play only a partial role in this difference. The results illustrated in Figure 4 show that there has been a progressive decrease in the mean length of the commercial ($\geq$ 25 mm) fraction of the *C. gallina* population examined from 1984 to 2001 in both Maritime Districts. Kendall's coefficient of rank correlation test revealed that this 'progressive decrease' could be attributed to statistically significant decreasing trends with time in both Districts. Figure 5 illustrates the length-frequency distributions (LFD) of *C. gallina* in the AN Maritime District between 1984 and 2001. Overall the time series of LFDs indicates a drastic change in the size composition of the population across the years which is likely to be strictly connected with the fishery. In the early years (1984 and 1985; Figure 5a, 5b) the population was healthy, skewed towards the larger sizes (maximum size $\approx$ 45 mm) with bi- or tri-modal LFDs having cohorts at modal sizes around 22 mm and 32 mm. From 1987 onwards there appears to have been a progressive depletion of the larger individuals, a decrease in the modal sizes (e.g. 15 mm in 1994, Fig 5h; 19 mm in 1999, Figure 5m) and left-skewed LFDs. Any recruitment event recorded (e.g. 1991, Figure 5e) appears to have grown slowly disappearing promptly as soon as it reached 22-25 mm. The LFDs are not shown here for lack of space, but the situation of the SB District clam population is very similar though highlighting an even more precarious situation than that described for AN. The paucity of adult commercial-sized individuals, which became particularly evident in AN from 1994, appears to be an intrinsic characteristic of the SB *C. gallina* population and was evident from the first year scientific sampling took place. The LFDs for this area are thus characterised by an alarming scarcity of large clams, left-skewness and small modal sizes of the main mode ($\approx$19 mm), most likely due to a fishery that has not allowed the population to grow much larger than 23 mm. A study carried out on the discards of the hydraulic dredge fishery in the two Districts, in fact, strongly emphasized the differences in gear selectivity between vessels from the AN fishing fleet and those from SB. In AN, the commercial portion of the catch was never smaller than 21 mm and the percentage representation of the 21-22 mm size category was always very low. In contrast, in SB, the appearance of individuals at 19 mm in commercial catches was very frequent and the percentage representation of the 21-22 mm size class was conspicuous (Morello, unpublished).

4 Discussion

Overall, the results obtained from the annual surveys to assess the *Chamelea gallina* stock in the Maritime Districts of Ancona (AN) and S. Benedetto (SB) revealed considerable year-to-year fluctuations in the total population, as well as in the commercial ($\geq$ 25 mm) and juvenile ($\leq$ 18 mm) fractions of the population, and no significant decreasing trends with time were evident. The high commercial biomass estimates obtained for both Districts in 1984 and 1985 drastically decreased in 1986. The decrease can most probably be attributed to concurring factors, amongst which the fishery but also adverse environmental conditions (e.g. sediment resuspension consequent of storms and increased freshwater inputs in the autumn of 1985 were the likely cause of a mass mortality event in SA5 [10]). No scientific surveys took place in the period 1988-1990 and when they resumed in 1991 an extremely depleted commercial resource was revealed, which in both Districts reached the historic minima. Very low biomass estimates were reported for the same year in virtually all Maritime Districts of the Adriatic Sea [11]. The summers of 1988 and 1989 coincided with the occurrence of abundant floating gelatinous aggregates in the coastal areas of the western Adriatic Sea [12] which likely reduced newly settled *C. gallina* through suffocation, having direct consequences on the commercial stock ($\geq$ 25 mm) two years later, in 1991 [11]. The high biomass of juvenile clams in 1991 in the SB District may have been the result of a recruitment event which occurred in 1990 as a reaction to the stress induced by the presence of marine snow in the previous year. Mass mortality of older cohorts has been reported to be one of the preconditions triggering unusually successful settlement in sedentary contagiously distributed populations [13]. The years following 1994 saw a recovery of the *C. gallina* stock in the AN District, which increased to attain very high commercial biomass estimates in 1999 and 2000. The same was not true for SB where the commercial fraction of the population never equalled the high production levels recorded in the mid-1980s. The overall surface area covered by the clam grounds is very different in the two Districts (304 km^2 in AN; 216 km^2 in SB), but not enough to account for the differences in commercial biomass estimates. In a similar manner, the percentage surface area devoid of *C. gallina* was consistently greater in SB, and that supporting over 5 kg$\cdot 1000 \cdot \mathrm{m}^{-2}$ was higher in AN across the years, especially from 1996. A density of 5 kg$\cdot 1000 \cdot \mathrm{m}^{-2}$ is considered to be the lowest economically viable density of the resource in depleted conditions [11]. These results further confirm the precarious situation of the clam stock in SB where most of the surface area supported clam densities below this lower-limit value. A drastic reduction in biomass was observed between 2000 and 2001 in both Maritime Districts. In SB, the biomass was reduced to such an extent as to prompt a call for the complete cessation in commercial fishing activity (starting September 2001). A reason, additional to high exploitation, can be given for the crash of both populations in 2001 and this is the very high freshwater input and particle suspension which was recorded in November 2000, when the Po river flooded as a result of very heavy rainfall attaining discharge values around 12100 $\mathrm{m}^3 \cdot \mathrm{s}^{-1}$ (Pecora & Frollo, www.arpa.emr.it) compared with a mean discharge of 1560 $\mathrm{m}^3 \cdot \mathrm{s}^{-1}$ (www.ilfiumepo.net). The interannual variability in juvenile biomass estimates was even greater than that recorded for commercial biomass and this was true for both Districts. The results point to the fact that high recruitment events are a sporadic event. *Chamelea gallina* is a rather long-lived species, reported to reach eight years of age [4]. Consequently, under ideal conditions (i.e. no fishing) the species has no need to produce excessive numbers of recruits every year, especially in light of the fact that growth, and natural mortality, are reported to be density-dependent [1, 11]. Overall, the results obtained allow no general considerations to be made on the relationship between stock size and recruitment; recruitment being largely independent of stock size. This is a situation widely reported for many infaunal bivalve stocks [13]. It may well be that this absence of relationship has saved the *C. gallina* resource in the area from commercial extinction under this regime of intensive exploitation. The results indicated statistically significant decreasing trends in the mean size of commercial estimates between 1984 and 2001 in both Districts. It is well known that fishing, by targeting large economically profitable individuals, has the overall effect of removing the older cohorts from the harvested population and the direct consequence of this is a decrease in the mean size of the population, particularly of the commercial fraction [14, 15, 16]. For this reason, the mean, or median, size of a harvested population is considered as a biological reference point, i.e. a yardstick against which the status of a population can be gauged [17, 16]. Furthemore, Trenkel and Rochet [16] in an analysis of the performance of several biological reference points, highlighted the fact that mean length of catch was one of the most precisely estimated and most powerful indicators of fishing impact. The significant decreasing trend in the mean size of the commercial *C. gallina* fraction of the population is, thus, a good indication of the negative effects of the hydraulic dredging fleets have had across the years on the clam resource in the study area. The length-frequency distributions obtained from both Districts revealed that the medium-sized fraction of the resource has been heavily fished throughout the years. The fishery appears to have been responsible for maintaining the population in a stable state centred around medium-sized individuals. Indeed, Marrs et al. [18] reported that the fleet seeks areas supporting high clam densities and not areas where the size of individuals is large; once the densities drop the fleet changes area (rotation of areas). This inevitably results in the medium-sized (undersized) fraction of the population being harvested to yield high quantities, rather than seeking large individuals that would yield proportionately lower catches per unit time. The size frequency distributions, the biomass estimates and the lack of a relationship between stock size and recruitment, as well as the removal strategy adopted by the fleet, highlight the fact that the resource and the entire fleet, are heavily dependent on the stochastic event of substantial clam recruitment events; and only such events will yield a cohort so conspicuous as to withstand and survive the fishing pressure to which it is subjected. In the past several years, large recruitments have occurred and significant natural mortality events, mostly of adult clams ($\geq$19 mm in size), have been observed towards the end of the spawning season. The origin of these mortality events is still unclear as these phenomena were recorded under normoxic conditions both in areas that were heavily exploited and in areas closed to dredging for several months. Early and protracted spawning, significant and protracted recruitment events followed by mass mortalities, and the scarcity of clams over 30 mm, may all be indications of a shift in energy-allocation strategy from one favouring growth to one favouring reproduction. The adoption of a more *r*-oriented life strategy inevitably generates a series of micro-cohorts which will further increase variability and further impede the ability to follow a specific cohort through time. The repercussions on the life cycle may be multiple; a progressive reduction, through harvesting, in the older fraction of Atlantic cod, *Gadus morhua*, for example, has been reported to have led to a reduction in mean age at first maturity and to a modification of the temporal succession of spawning events within one season [15]. Little is known about annual fluctuations in recruitment success in *Chamelea gallina*, as for many other inshore bivalves (e.g. *Spisula substruncata*, [19]). Adopting a precautionary approach, the fishing effort (e.g. in terms of number of licences) allotted to an area (be it an entire Maritime District or a single sub-area) should be based on resource estimates obtained for years of low recruitment in the case of species with a more *r*-orientated strategy such as *Chamelea gallina*. Buchanan [20] has stressed that biomass is no guide to productivity in infaunal echinoderms and this is equally true of bivalves. Just because a resource is plentiful now does not mean that it can sustain heavy and prolonged exploitation. *Chamelea gallina* in the Adriatic has been widely reported to reproduce in its first year of benthic life, around 18 mm in length [1], well below the MLS of 25 mm set by law. The high intensity of fishing to which the stock is subjected in the study area is such as to ensure the capture of the majority of an annual cohort as soon as it reaches at least 22 mm shell length on average. This may create serious problems for the resource in years of low recruitment. Despite this, in light of the biology of the species in the area, an MLS of 25 mm can be considered a precautionary size and this may be one other reason why the resource has not become commercially extinct. In light of these results, an effective control of fishing effort and active enforcement of existing rules regarding the characteristics of *C. gallina* dredges and sieving equipment are deemed fundamental to maintain this 'high-tech' fishery; but over and above this it is especially important that the stock be assessed, via surveys, at least yearly. The rotational nature of the fishery and the occurrence of mass mortalities, make it very difficult to follow a cohort through time, especially on an annual basis. This could be remedied by carrying out more surveys in one year, Marrs et al. [18] suggest two, so as to gain a more realistic quantification of the two sources of both fishing and natural mortality.

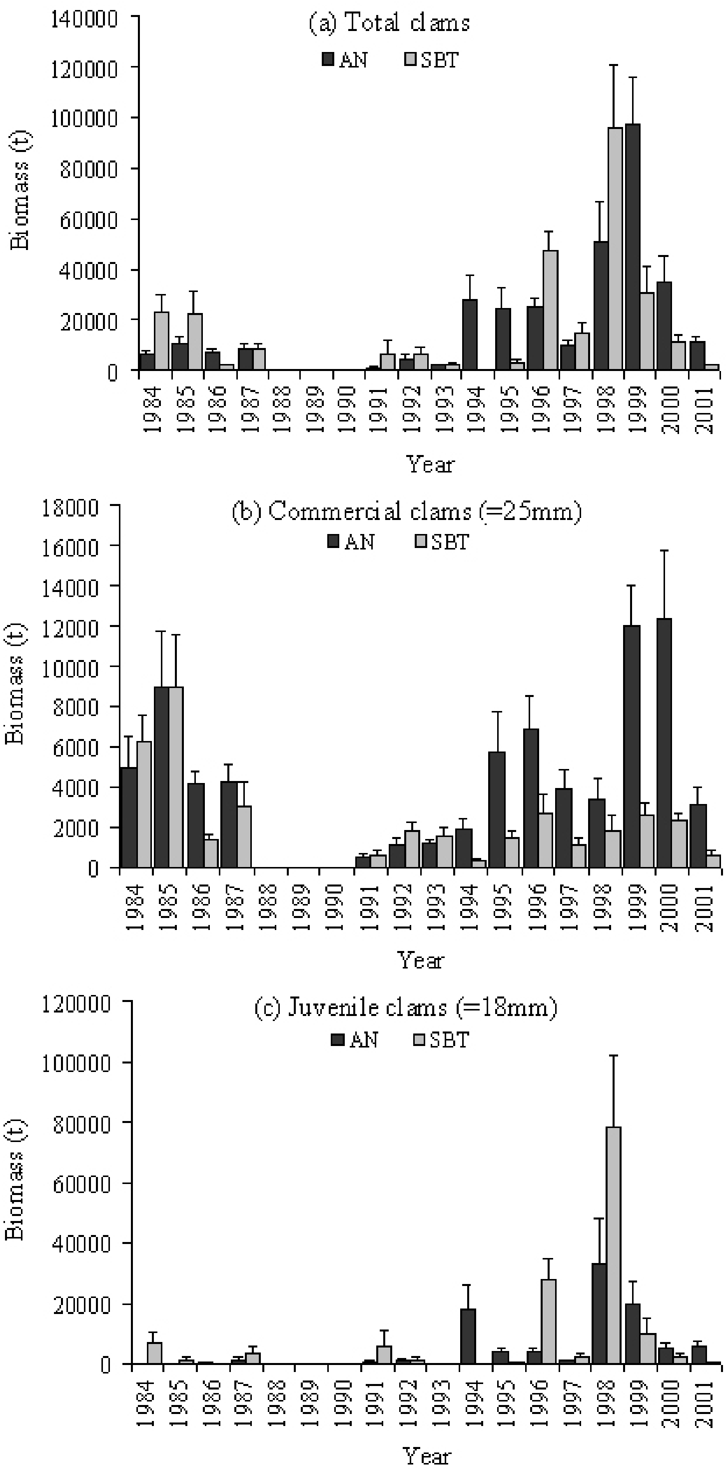

Figure 2: Estimates of (a) total, (b) commercial (≥25mm) and (c) juvenile (≤18mm) *Chamelea gallina* biomass (+ 95% C.I.) in the Ancona (AN) and S. Benedetto (SBT) Districts for 1984-2001.

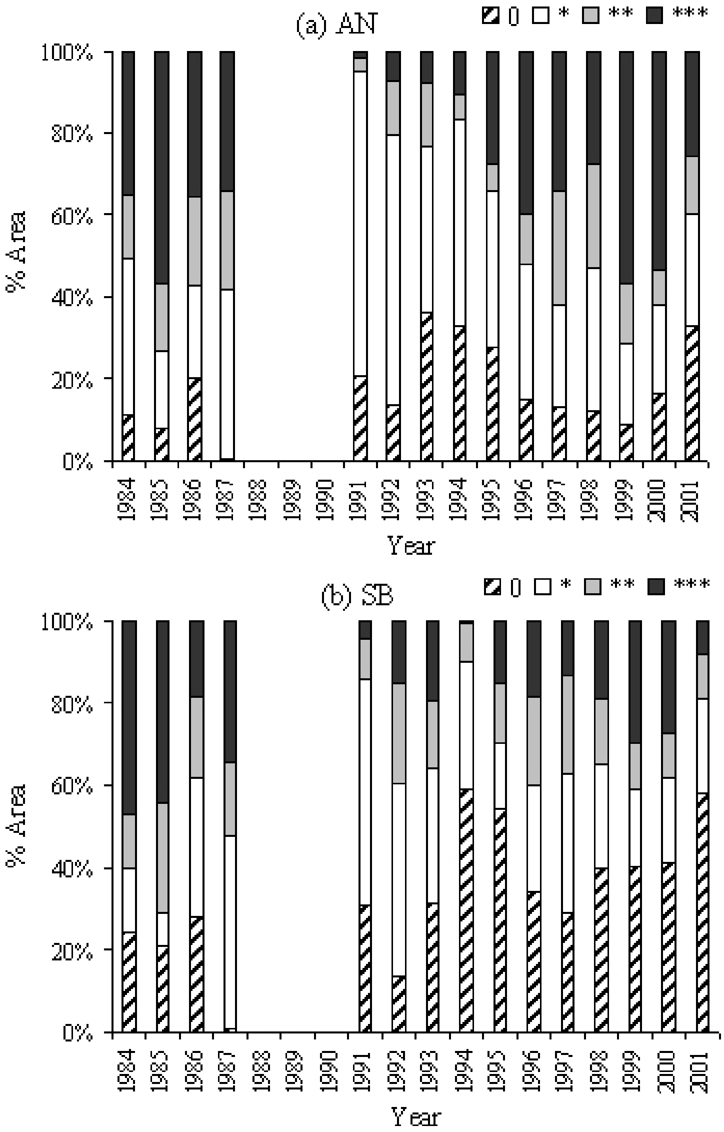

Figure 3: Surface area (%) corresponding to increasing commercial *Chamelea gallina* densities (0 = 0 kg$\cdot 1000\mathrm{m}^{-2}$. * = 0-5 kg$\cdot 1000\mathrm{m}^{-2}$; ** = 5-12.5 kg$\cdot 1000\mathrm{m}^{-2}$; *** = $>$ 12.5 kg$\cdot 1000 \cdot \mathrm{m}^{-2}$) in the (a) Ancona (AN) and (b) S. Benedetto (SB) Districts between 1984 and 2001.

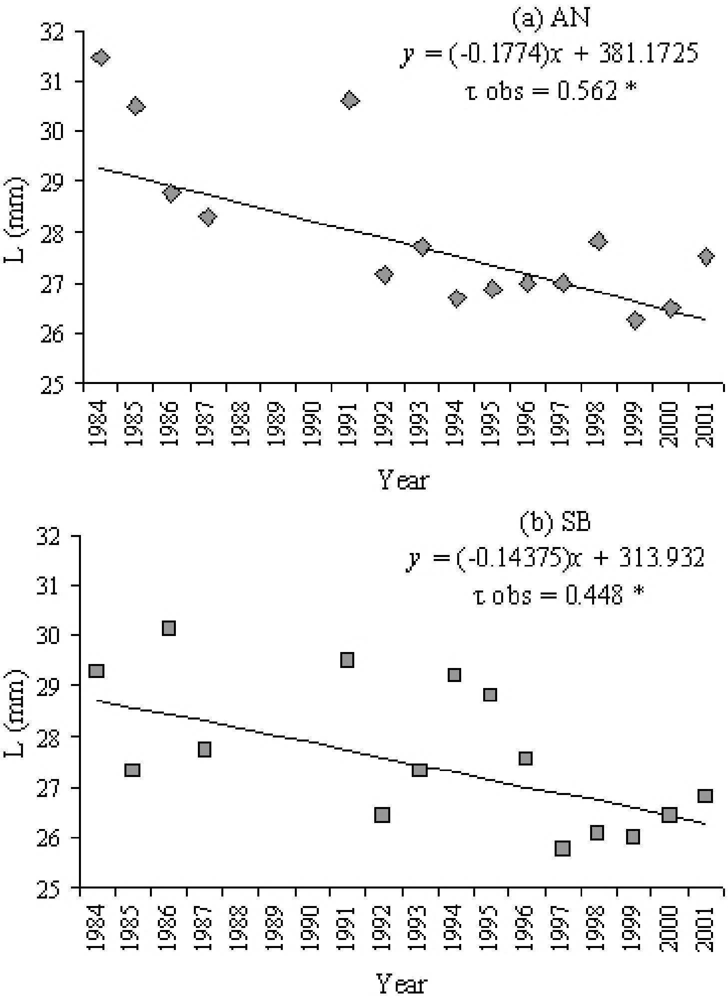

Figure 4: Variation in mean length of commercial *Chamelea gallina* ($\geq$ 25 mm) between 1984 and 2001 in the (a) Ancona (AN) and (b) S. Benedetto (SB) Districts with the trend line and non-parametric regression equation calculated according to Kendall's robust line-fit method [9], indicating the results of the Kendall's coefficient of rank correlation (tau) used to establish the significance of such trends (* = significant at alpha = 0.05).

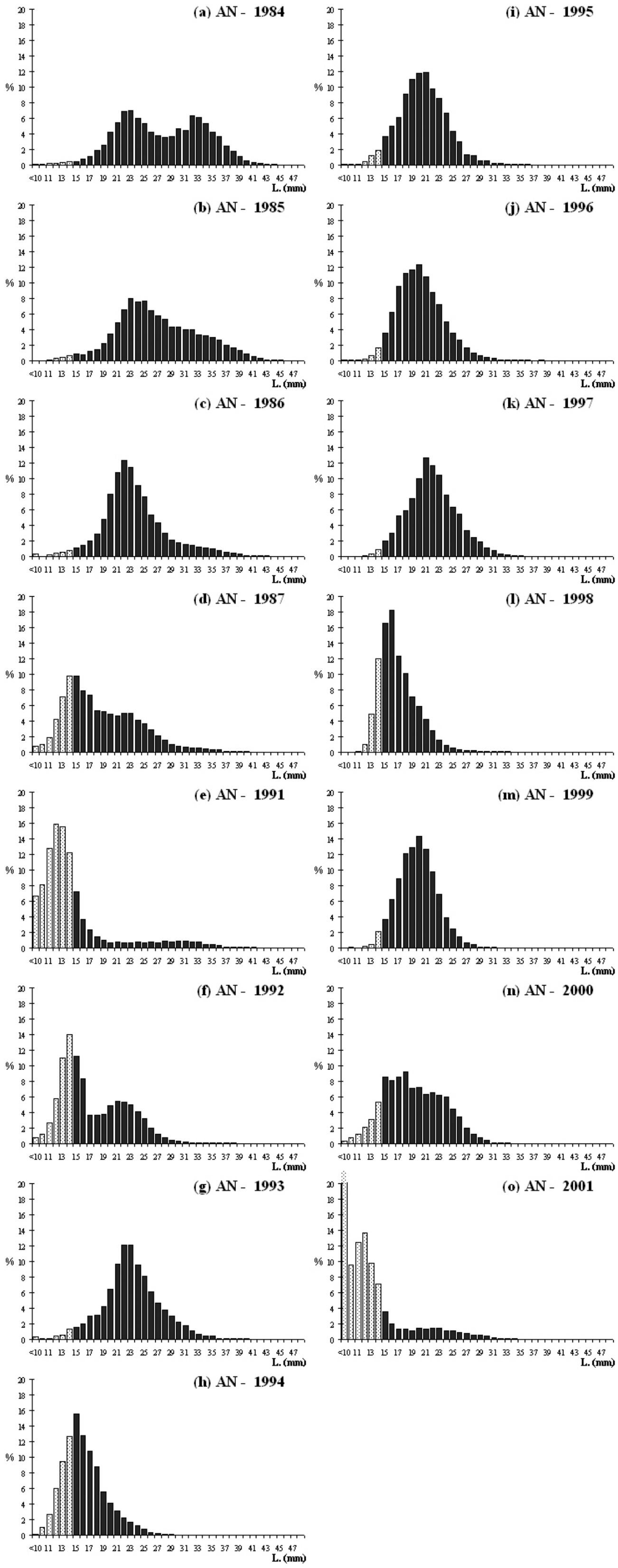

Figure 5: Length-frequency distributions of the sampled *Chamelea gallina* population in the Ancona Maritime District (AN) from 1984 to 2001. Dotted bars represent the underestimated fraction of the population.

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The Potential Importance of Shipwrecks for the Fisheries, the Environment and the Touristic Fruition

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Abstract

This paper examines different “opportunities” when dealing with “natural” and deliberately scuttled shipwrecks, and what has been done on the subject by a CNR’s research team in the marine waters of Sicily: their impact, positive and negative, on the fisheries; their effect on the environment when employed as artificial reefs; and their utilization as an attractor for sustainable touristic activities. An inventory of the existing gear graspings in the Strait of Sicily has been conducted, mapping the results and assessing the risk of damaging the nets thru an integrated roughness index. Underwater visual censuses have been carried out in and near some shipwrecks around the Strait of Messina, quali quantitatively evaluating the associated ichthyofauna and some ecological and biodiversity parameters. Preliminary feasibility studies have been advanced for a holistic approach to the sea going tourism, proposing interventions on the surface (an artificial “atoll” made around submersible cages) and on the bottoms (sinking de commissioned vessels). Finally, the paper presents some future research and activities, trying to overcome the existing bureaucratic obstacles, and offering reasons for establishing more artificial barriers with one (or more) shipwrecks as core, even suggesting to build ready to sink hulls. In conclusion, sunken vessels may be employed, alone or with other structures, to produce a positive, synergic effect on the marine resources, on the environment and on the touristic fruition of the coastal sea.

1 Fisheries

Fishermen have two opposing feelings regarding ship wrecks and other man originated sunken structures present on the fishing grounds: one is negative, because they may damage the fishing gear; on the other hand, as a general rule, the waters surrounding these submerged artifacts yield better and larger catches.

It is still controversial whether this increase in catches is due to an enhanced production of biomass, or just to herding thigmotropic effects, or both; actually, whichever is true, there is more valuable fish near the structures. In fact, commercial fishermen are often willing to risk their gear closing to the potential grasping for the reward of a catch bounty.

The positive effects are particularly evident when the artifacts are situated on flat, muddy bottoms, which are ipso facto quan-

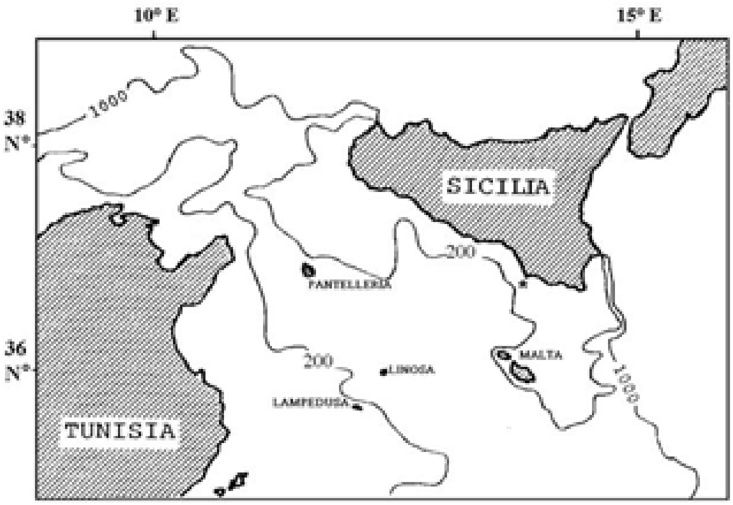

Figure 1: Map of the Strait of Sicily.

Reason for invalidation	**Number of hauls**
grasping ("afferratura")	113
net tearing and/or gear damage	77
unsuitable bottoms	36

Table 1: Not valid hauls recorded during Grund and Medits surveys carried out in the Sicilian Strait (1985-2002).

titatively and qualitatively less rich than rocky substrata. Conversely, while the structures may still be exploited by low impact, artisanal, small scale fishing gear (traps, lines, trammel nets, etc.), the more destructive trawling gear must avoid the core of the interested area to escape damages.

The Strait of Sicily (Figure 1) is home of the largest trawl fishing fleet of the Mediterranean; the vessels stay at sea for trips of several days, and loosing a gear is a very serious economical accident. Therefore, a precise knowledge of how many "grasping sites" exist in the fishing bottoms, and of what kind, and where they are, is of primary importance, especially when exploring "new" grounds.

With this in mind, an assessment of the known has been carried out, integrating historical records of war and peacetime naval sinkings, chart markings, fishermen's information on graspings ("afferrature"), and data collected during twenty years of experimental trawl surveys carried out in Mazara del Vallo by the CNR local institution (now, Ist. per l'Ambiente Marino Costiero – IAMC, formerly, Ist. Ricerche sulle Risorse Marine e l'Ambiente – IRMA, historically, Ist. Tecnologia Pesca e

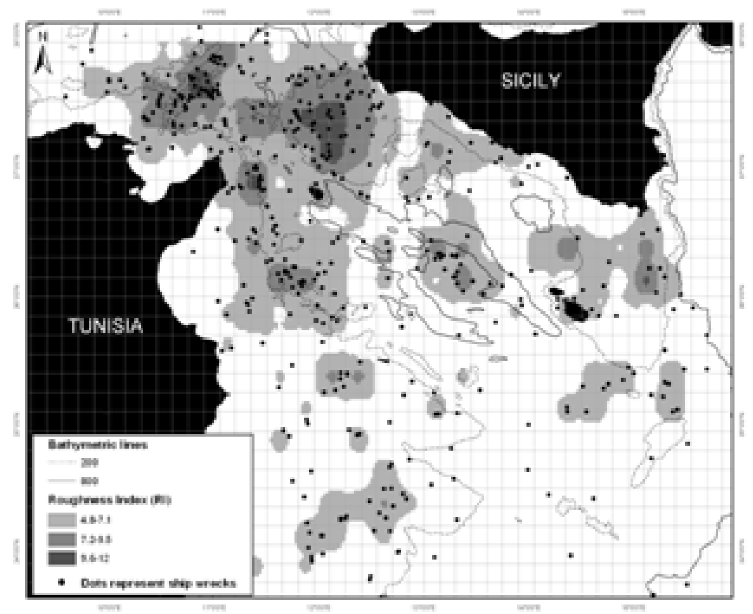

Figure 2: Integrated "surface map" of the roughness index derived from fastenings and ship wrecks in the Strait of Sicily; ship wrecks are superimposed (black dots).

Pescato – ITPP). In fact, IAMC Mazara has carried out, almost without interruption, two trawl surveys (for a total of over 4000 hauls), one in late spring (international survey Medits, since 1994) and the second in autumn (national survey Grund, since 1985); the experimental design is random depth stratified (10,880 m), in the GFCM's area GSA 16, broadly corresponding to the Italian half of the Strait of Sicily, and has a framework agreement with the Maltese MCFS; altogether the regularly monitored area is 57,500 km^2 (over a total of 257,500 km^2 for the entire Strait).

First, rough, hard to trawl or untrawlable patches has been recovered (Table 1), based on the occurrence of not valid hauls (interrupted before scheduled time or with evidence of gear malfunctioning), caused by both natural (cliffs, rocky outcrops, boulders and coralligenous habitats) or man induced (shipwrecks, "mazzare", etc.) graspings, which make fishing dangerous for the gear, during the Medits and Grund scientific surveys [1].

Thereafter, data on all the known ship wrecks occurring in the Strait of Sicily have been gathered from different sources, both published [2] and unpublished; altogether, about 1300 records were collected, allowing the identification of 457 different ship wrecks, which were imported in a GIS database (pixels of 1x1 nm). Since naturally untrawlable areas could have a synergic effect with the man made obstacles to trawling, a joint "roughness index" has been calculated,

$$RI = \frac{\#of\ fastenings\ in\ the\ pixel}{total\ graspings} \cdot 100 + 1.5 \cdot \frac{\#of\ wrecks\ in\ the\ pixel}{total\ wrecks} \cdot 100$$

and an integrated map (Figure 2) of ship wrecks and rough bottoms has been produced for the investigated area [3, 4]. A similar approach as been attempted also in the southern Tyrrhenian, in a small area just north of the Strait of Messina (off shore of Palmi), integrating the historical records (both from literature and from experimental trawl surveys carried out by IAMC Messina) with a campaign of remote sensing using MultiBeam and lateral scan eco survey (Figure 3) with the CNR's RV "Urania" [5].

Incidentally, another man made impediment for trawling, besides the ship wrecks, is the presence of fields of large stone blocks (called "mazzare"), used as anchor of FAD devices, and left on the spot at the end of each fishing season [6]; the fishery that creates the "mazzare" fields is most typical of the eastern Sicilian Strait [7].

2 Environment

"There are undoubtedly many potential benefits that might be derived from the creation of protected areas in the marine environment. Nature conservation calls for them, scientific research desperately needs them and even fisheries might benefit from them" (page 299, in [8]). Although the concept of "Marine Protected Area" (MPA) encompasses a wide range of definitions and applications [3], [9], fishery reserves or no take zones (NTZs) are usually distinguished from the MPAs, the latter should be more oriented towards enhancement of marine environment and conservation of biodiversity [10], whereas the former are generally designed within fishery scenarios [11]. Shipwrecks or other steel debris prevent trawling and therefore represent an important cause of not vulnerability of the bottoms to trawls; moreover, the presence of already untrawlable patches should make the establishment of regulated areas around them more acceptable for fishermen who use the most impacting gear. Since the early '80s there has been ample literature[12, 13, 14] on whether ship wrecks may present a redeeming ecological value. The ships often become artificial reefs (Figure 4) and habitats providing shelter for many highly vulnerable, low mobility and seriously depleted species [15, 16], with effects that spread on the surroundings [17]; in fact, fishermen keep fishing on "risky" bottoms because they balance the chance of damaging the gear with that of greater and better catches.

The effects of sunken vessels was preliminarily assessed on a single wreck, south of Messina, comparing by underwater visual census [18] the fish fauna associated with the artifact vs. the neighboring sandy bottoms; it resulted that the wreck was richer in biomass and species diversity [19], similarly to what observed on classical concrete barriers and other submerged artificial structures [20]. It appears also that using a more silent, non bubble breathing apparatus (e.g., a closed Inspiration Rebreather), fish behave differently than to the normal SCUBA diver [21]; this is of importance for the validity of the visual census, because some species are more frightened than others and may hide from observation when disturbed.

To examine the ichthyofauna associated

Figure 3: The shipwreck “Viminale”, north of Messina, detected from the surface.

with shipwrecks, 4 wrecks laying at different depths near the Strait of Messina, whose characteristics are reported in Table 2, were investigated by underwater visual census [22], based also on some ecological indexes; d: species richness or Margaleff index; H’: species diversity or Shannon index; J’: equitability or Pielou index), all wrecks show a better situation in comparison with the surrounding homogeneous, sandy or muddy bottoms, not only presenting higher abundances but a variety of species (Table 2), and larger sizes.
It is evident that shipwrecks, whether originated by accident or purposely ad hoc sunken, have in any event a beneficial effect on the coastal ecosystem, and could be of use as a tool for managing the both the protection (MPAs) and the exploitation (NTZs) of the marine environment [23]. In fact, at least they:

- physically deter bottom trawling;
- offering protection to broodstock and juveniles, have a role as settling nucleus, which spread its effect on the surrounding bottoms;
- may create occasions for sustainable uses, particularly when associated with “surface” interventions;
- last but not least, are useful for scientific experimentation.

Moreover, their presence offer an alternative for diving and small scale fisheries, therefore helping to lower the fishers’ and tourist pressure from adjacent natural reefs and sites [24]. Of course, a complete assessment of the pros and contras of a specific artificial reef requires a complex study [25]; their effect on the surrounding bottoms could be limited to the barrier itself [26], or could extend over a very large neighboring surface [27].

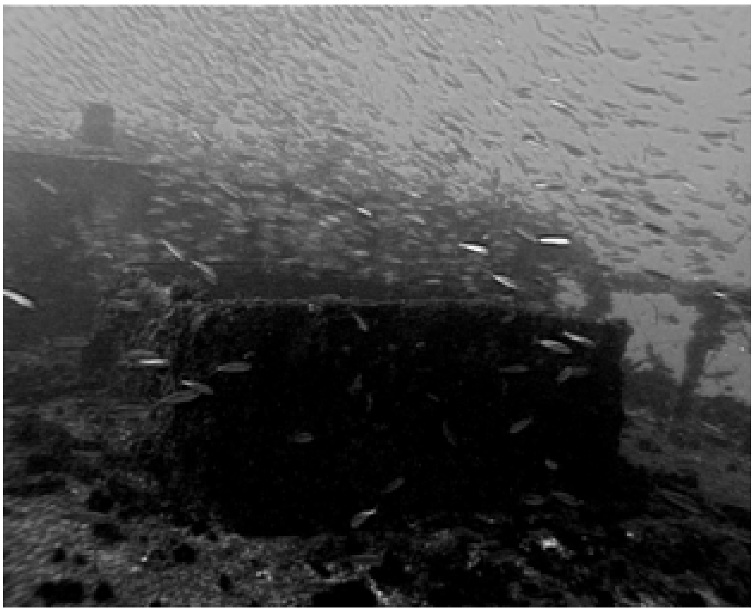

Figure 4: Schools of fish around the “Nave di Faro”.

3 Compatible tourism uses

The “natural” shipwrecks situated in shallow to medium depths are among the preferred spots for the SCUBA divers (Figure 5); since in general their exploration requires some local expertise, diving professionals are often accompanying the underwater tourists, and checking, besides their safety, that they do not damage the artifacts which offer them a source of living; therefore the “problem of the commons” is normally avoided when referred to the shipwrecks. Obviously, the same attraction for divers is exerted by any shipwreck, involuntarily or deliberately sunken, thus making an otherwise “dull” bottom a source of eco compatible and sustainable revenues [28].

Nevertheless, underwater recreation is generally limited to active users, leaving out too many average tourists. Thinking at that, a floating “atoll ” (Figure 6) , assembled near the wreckage, shall act as a starting basis and allow different activities also on the surface, such as mooring for catering, rod fishing inside a stocked pen, safe swimming “in the blue”, education and experimentation, besides being a commercial “kiosk in the middle of the sea”; its thigmotropic effects (i.e., the search of peculiar spatial conformations for orienting themselves and “settling” activities) should be particularly efficacious for coastal boating (up to 3 NM off shore) in areas with flat bottoms and coastlines [29].

The floating isle is made of 4 submersible net cages, a technology regularly employed for off shore aquaculture [30], which will be sunk to avoid heavy storms and for in loco winter storage, with a total surface

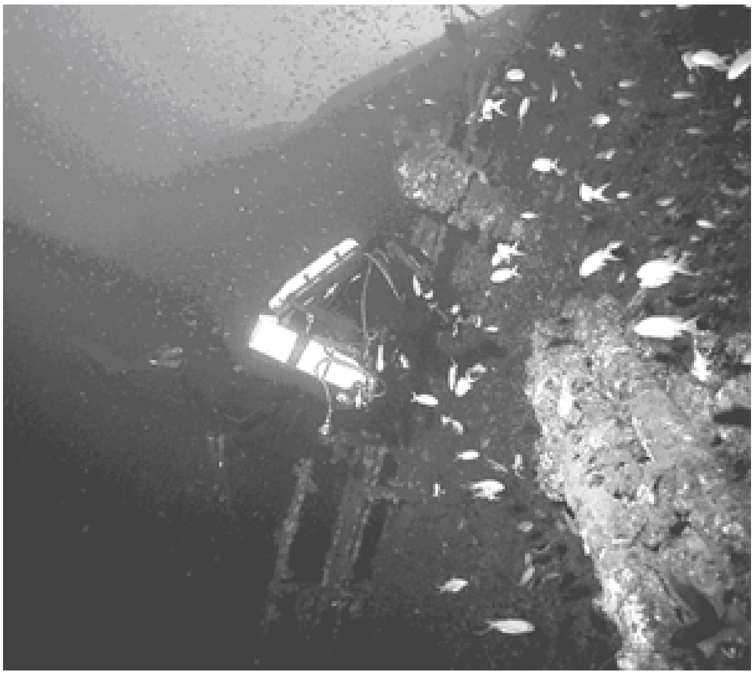

Figure 5: Divers and fishes near a shipwreck.

around 2500 m^2. A pontoon, also submersible and of low profile, serves as supporting space for catering; the electric appliances, the first aid station, the communications center, a small "country store" for bait and for nautical bits and pieces are arranged in an autonomous servicing boat [31].
A feasibility study suggested that the initiative could be economically viable [23]. Besides the induced advantages - environment, artisanal fishery, fish stocking [32], fishing-*cum*-tourism [33], attraction of tourists to the area, etc. -, the break even point in an hypothetic scenario off the Roman coastline may be reached in the event of 20 pleasure boats (each with 4 5 customers using its services) docking daily at the structure during the week days and 80,100 boats during the week end, just in a 2 month peak season [34]. Moreover, the modular design may permit to easily expand the structure and to adapt the initiative to other suitable sites.

4 Future developments

Matter of factly, the Mediterranean, a sea highly trafficked and theatre of many wars, contains many shipwrecks [2] lying on coastal and shallow bottoms, of which a large amount has the potential of becoming an integrated fishery reserve cum touristic attraction. It is envisageable that many of the censed shipwrecks will have a potential for multiple uses, with environmental, fisheries and touristic aims. Still, the dis-

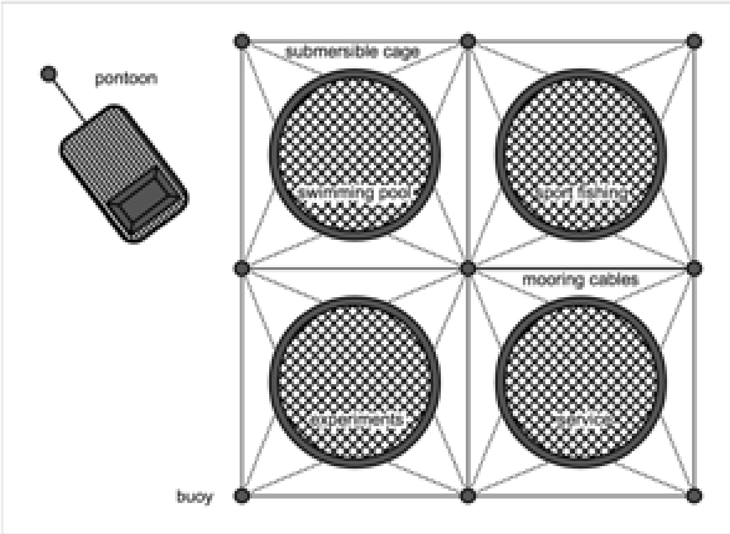

Figure 6: Top view of the artificial "atoll" module.

tribution of the shipwrecks seems contagious, with concentration in some locations and lack of wrecks in others; therefore, besides putting at work the existent, it may be envisaged to scuttling decommissioned ships in areas with low numbers of shipwrecks, in particular where the customer affluence is greatest, e.g. near touristic resorts and large cities. An example is the region near Rome: 5 million residents, 100 km of homogeneous, flat coastline, almost no wreck; the Italian side of the Adriatic, with plenty of seaside resorts, is another emblematic case.

Also the southern coasts of Sicily lack "natural" wrecks with suitable characteristics as trawling deterrent and diving spots [31]. The creation of artificial reefs with decommissioned (and cleared) fishing boats was first attempted in the early '90s, when one 30 m steel and 9 wooden (20-33 m) bottom trawler were deliberately sunken off Capo Granitola, east of Mazara, at a depth of about 30 m [35]. Now, only the steel ship remains, but the distance from the Mazara harbour, the lack of surveillance, the illegal fishing, and the frequent water turbidity have hampered the biological goals and the promotion of leisure diving.

The present situation is even more suitable than before for such kind of intervention, in Italy in general and very specifically in southern Sicily; in fact, there is:

- a diffused discomfort of the artisanal fisheries, also because of trawling too close to shores ;
- a limited development of tourism associated with the fishing activities ("pesca turismo"), and with underwater explorations ;
- a large availability of steel hulled vessels, which are already out of work and officially decommissioned, awaiting to be scraped since many years, rusting on dock and polluting the harbours.

Figure 7: "Artistic" rendering of an integrated approach.

The future should focus in promoting the establishment of a network of locations with ad hoc scuttled/sunken vessels [36], notwithstanding the Italian legislation, which in any case must be adapted and conformed to the EU directives. In fact, at present the article 4 of the Barcelona Convention of 1995 (ratified by the EU States, e.g. the Italian law 175/99) prohibits dumping vessels at sea, but the term "dumping", as stately defined in article 3 "… does not include … (b) Placement of matter for a purpose other than the mere disposal thereof, provided that such placement is not contrary to the aims of this Protocol". The European Commission ruled in favor of the establishment of artificial barriers made by scuttling decommissioned fishing boats (EU Regulation 1198/2006, in particular art. 23/1c), stating that ".… The permanent cessation of fishing activities of a fishing vessel may be achieved only by: … (c) its reassignment for the purpose of the creation of artificial reefs …". Moreover, it has financed specific research programs (MoSS, Monitoring, safeguarding and visualizing North European shipwreck sites) toward the protection and conservation of "historical" shipwrecks [37]. The Italian situation is confused, because from one side it allows (Ministerial rule actuating DL 114/2008, article 1c) for scrapping hundreds of out of work, decommissioned boats laying idle in port thru sinking with the scope of creating artificial barriers, while on the other it still considers them as "special waste" (DPR 915/1982, article 2, comma 4), a priori forbidding dumping at sea. Besides the bureaucratic and administrative hassle, even the scientific community has diverging attitudes toward the effects of shipwrecks, with some in favor [38, 39] and some against it [40], because of a precautionary approach. The major reasons which are opposed against sinking decommissioned vessels and fishing boats could easily be refuted [41], using scientific results and plain common sense (contrasting the widespread "Petrarch' principle", [42]):

a) Source of pollution: a real issue for the ship that sunk by accident [43], is a no starter for deliberately sunken vessels, which undergo extensive clean up following internationally agreed procedures [44], taking out painting, oil, plastic material, insulation, batteries and any other polluting stuff.
b) Dangerous for diving: of course, diving in a swimming pool could be safer ... but wrecks remain the most sought destination for underwater exploration [45]; the same international procedures require numerous intervention for the safety of SCUBA divers (doors are welded, passages are ample, holes are blocked, spikes and cutting edges are fixed or removed, and so on).
c) Incompatible with MPAs: on the contrary, vessels have already be positioned on MPAs bottoms, because they deter illegal fishing ("silent policemen"), offer refuge to sensible species, permit an eco sustainable fruition of the area.
d) Risky for navigation: the preliminary "sector meeting" encompasses all the official stakeholders, among whom the Coast Guard; with them, suitable sites outside the commercial sea lanes are selected, and a minimum clearance of 15 m is guaranteed.
e) Hindering the legal fishing too: it is impossible to damage the gear involuntarily, because the shipwreck location is known, fixed in time and space, signaled locally with buoys and drawn on charts, thus easily avoided.
f) Causing ghost fishing: apart from the fact that nets and gear should not be employed to close to any grasping site, the fishing power of lost nets does not last long, since they become self entangled on the metallic structures [46]; moreover, diving centers working on the wreck are often voluntarily removing the gear snarled on it [47].
g) Helping poaching: shipwrecks are effectively richer in fish than the surrounding area, a tempting occasion for poachers. The question whether the wrecks function via increased production or just because they attract neighboring specimens is debatable [48], [49], [50]); in fact, if that is just a "fish concentrate", poaching will deplete existing resources, but recent research seems to corroborate the thesis of new fish biomass generation [51], [52], [17], and therefore illegal fishing on scuttled wrecks does not worsen the status quo ante.
h) Altering environment and biocoenoses: it has been shown, even on very extended and complex artificial barriers, that geomorphological alterations are limited to the very proximity of the manufacts, and that surrounding biological communities are unscathered [53].
i) Just too "ordinary": in fact, even before sinking, vessels are better than classical concrete barriers: cost less (or marginally nothing), are easy to transport, can be readied without disturbing the neighboring coast; once in water,

they last comparable times, and attract (or produce) not only fish, but divers too.

j) Hiding toxic wastes: all the procedure for scuttling is transparent, and supervised by environmental and security agencies; moreover, the regular diving activity acts as a deterrent against criminal uses [54].

Still, purposely sinking old boats has some disadvantages, since the hull must be completely cleared from toxic oil and paints, electrical parts, heavy metals, asbestos, and so on; it must be secured also for diving safety (doorways, metal spikes and sheets, etc.); in some instances, scraping could be more convenient than a complete, state of the art clean up. With this in mind, a possible alternative solution [23] is to build boats specifically designed to be sunken and fulfill both the pleasure expectancies of the diving parties and the biological requirements of an artificial barrier [20]. The hull, built ex novo in a shipyard near the sinking ground, should be 35 50 m long, and be refurbished in every significant structure as a small cargo (propeller, portholes, cabins, antennas, etc.), but without paint, engines, electrical cablage, insulation and so on. The proposed project, the deliberate sinking of decommissioned fishing vessels, is multi focused (Figure 7), being aimed at: i) the environmental protection, ii) an increase of resources for the artisanal fishery and iii) an eco compatible touristic exploitation [55]. Other environment friendly innovative interventions, such as the "Exfol" (anti climax, self exfoliating concrete slabs, supposed to maintain a high diversity of the benthos) and the "pseudo kelp" (ropes imitating plants, making a sort of submerged prairie) could be associated to the sunken vessels, to enhance their value as fish oasis [56] and tourist attraction. Another possibility to increase the size of the artificial reef and to augment the substrate diversity is to associate the wrecks with sections of "classical" concrete barriers, and with other decommissioned structures, such as old oil rings and platforms [57]. This effect will be even greater if the sinking is integrated with other interventions, such as the creation of artificial "atolls", to establish a real "marine protected area" (MPA), where activities, both commercial and touristic, are regulated in a broader conservation strategy. In conclusion, common, already available techniques may be integrated to produce a positive, synergic effect on the marine resources, on the environment and on the touristic fruition of the coastal sea.

VALFIORITA[1]: cargo ship (vehicles), 6200 tsl
sunken 08/07/43, depth: 45-61 m on muddy sand
commercial species: *D. sargus; E. costae; E. marginatus*
most common species: *A. anthias; B. boops; C. chromis*
n° of censed species: 14   n° of censed families: 5
fish density/m^3: 1.84   d: 1.97   H': 1.49   J': 0.57

FARO'S WRECK[2]: tramper ship, about 3000 tsl
sunken around 1910, depth: 33-68 m on gravel
commercial species: *S. dumerilii; M. rubra; E. costae*
most common species: *A. anthias; B. boops; C. chromis*
n° of censed species: 18   n° of censed families: 9
fish density/m^3: 0.78   d: 2.96   H': 1.67   J': 0.58

RIGOLETTO[3]: merchant ship, about 2000 tsl
sunken in the '70s, depth: 0-39 m on sand and gravel
commercial species: *D. sargus; M. barbatus; E. marginatus*
most common species: *C. chromis*; *B. boops; C. julis*
n° of censed species: 36   n° of censed families: 14
fish density/m^3: 1.22   d: 5.66   H': 1.69   J': 0.47

VIMINALE[4]: oceanic passenger liner, 8650 tsl
sunken 25/07/43, depth: 100 m on sand and mud
commercial species: *Epinephelus* sp., *Scorpaena* sp.
most common species: *Anthias anthias*
n° of censed species: 14   n° of censed families: 8

* * *

List of the species recorded near the shipwrecks:
Anthias anthias[1, 2, 3, 4]; *Apogon imberbis*[3]; *Boops boops*[1, 2, 3]; *Chromis chromis*[1, 2, 3]; *Conger conger*[2, 4]; *Coris julis*[1, 2, 3]; *Dentex dentex*[4]; *Dicentrarchus labrax*[3]; *Diplodus annularis*[3]; *Diplodus sargus*[1, 2, 3, 4]; *Diplodus vulgaris*[1, 2, 3]; *Epinephelus aeneus*[4]; *Epinephelus caninus*[4]; *Epinephelus costae*[1, 2]; *Epinephelus marginatus*[1, 3, 4]; *Labrus viridis*[3]; *Lichia amia*[4]; *Lithognathus mormyrus*[3]; *Liza aurata*[3]; *Lophius piscatorius*[4]; *Mullus barbatus*[3]; *Mullus surmuletus*[3]; *Muraena helena*[3, 4]; *Mycteroperca rubra*[2]; *Oblada melanura*[2, 3]; *Pagrus pagrus*[1, 4]; *Parablennius gattorugine*[2, 3]; *Sarpa salpa*[3]; *Scorpaena notata*[3]; *Scorpaena porcus*[3,4]; *Scorpaena scrofa*[3, 4]; *Seriola dumerilii*[2]; *Serranus cabrilla*[2, 3]; *Serranus scriba*[1, 2, 3]; *Sparisoma cretense*[2, 3]; *Spicara flexuosa*[3]; *Spicara maena*[1, 2, 3]; *Spicara smaris*[1, 2, 3]; *Spondyliosoma cantharus*[1, 3]; *Symphodus doderleini*[3]; *Symphodus mediterraneus*[3]; *Symphodus roissalii*[3]; *Symphodus tinca*[3]; *Thalassoma pavo*[1, 2, 3]; *Tripterygion delaisi*[3]; *Tripterygion tripteronotus*[3]; *Uranoscopus scaber*[4].

Table 2: Characteristics of the 4 shipwrecks examined near the Strait of Messina and summary of visual census. d: species richness (Margaleff index); H’: species diversity (Shannon index); J’: equitability (Pielou index).

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Use of Eugenol and 2-Phenoxyethanol as Pre-Treatment in the Stunning/Killing Procedure of Farmed European Sea Bass (*Dicentrarchus labrax* L.)

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Abstract

This work aims to compare the behavioural responses during stunning/killing phases using the anaesthesia with Eugenol and 2-Phenoxyethanol as pre-treatment of the immersion in water and ice of farmed European sea bass with the behavioural responses during the traditional direct stunning/killing in water and ice usually adopted in aquaculture to assess the impact of the anaesthetic pre-treatment on well-being of fish destined to human consumption. Moreover, in order to contribute to the body of knowledge regarding the anaesthetic characteristics of Eugenol, the only anaesthetic that could be used on fish destined for human consumption, we compared it with 2-Phenoxyethanol, which performs excellently as anaesthetic on fish. Behavioural observations at the time of mortality showed notable differences between the fish stunned and killed in water and ice and those pre-treated with anaesthesia. The subjects in the first group died while thrashing around violently, while the fish which had been anaesthetized died without violent reactions or evident muscular spasms, allow us to considerer the anaesthetic pre-treatment a valid tool for the well-being of fish during the stunning/killing. The anesthetic responses showed the notable anaesthetic power of Eugenol; the induction phase was significantly faster compared to 2-Phenoxyethanol. Moreover the subjects anaesthetized with Eugenol tended to recover very slowly in comparison with the fish treated with 2-Phenoxyethanol.

1 Introduction

For many years fish-farming has years played a central role in the world trade. Total marine and brackishwater aquaculture production on Mediterranean area, including all categories and species, has increased from 89 959 tonnes in 1985 to 234 446 tonnes in 1995 and thereafter to 377 984 tonnes in 2006 [1]. The production of the two species European sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) was 92.4 % (respectively the 46.2% and the 46.0%) of finfish production in 2006 [1]. In Italy, only the sea bass (*Dicentrarchus labrax*) has an annual production of about 9.200 tonnes [2]. Recently, the increasing exploitation of these animals in intensive farming conditions have raised the level of attention by the general public and the scientific community, that expressed the need to avoid unnecessary suffering during the routine fish-farming practices (measuring or weighing fish, administration of vaccines, sampling for blood or gonad biopsies, transport and stunning/killing for human consumption). In a similar context, the stunning/killing methods used on farmed fish, destined to become food for humans, play a focal role. On this regard, International Organizations such as the EFSA (European Food Safety Authority) and the DEFRA (Department for Environment, Food and Rural Affairs), have expressed their opinions about the humane aspect when stunning or killing farmed fish [3, 4]. These Organizations identify the stressful methods of killing fish as being asphyxiation, whether in water and ice or only in ice, narcosis using carbon dioxide, decapitation and bleeding to death removing of the gills. On the contrary, the Organizations considerer like "humane" the blows to the head, the sedation/anaesthesia and, above all, the electronarcosis. Both in Italy and abroad, on fish farms, especially for sea-water fish of great commercial interest (e.g sea bass and sea bream), is common to adopt the technique of stunning/killing in water and ice. This practice, considered stressful by the above mentioned International Organizations, is permitted by the UE legislation. However, the methods considered to be humane, such as blows to the head and electronarcosis, present many limitations in application on a wide scale [5]. In particular, electronarcosis is considered inadequate for carrying out stunning properly, because it is not able to make the fish unconscious for a period of time long enough to ensure death. In addition, the difficulties of its use on marine fish-farms, where sea-water has raised conductive properties, should not be under-evaluated. Blows to the head are used principally on salmon, trout and other big fish [6]. Finally, among the stunning/killing methods, there are also the techniques of sedation and anaesthesia, about which unanimous favorable opinions have been expressed by the International Organizations. Anaesthetics are used routinely to produce moderate sedation, to reduce stress and to immobilize fish for periodic observations, artificial reproduction, measurements or marking, transport and others. However, at present it is considered that these techniques cannot be applied to use for the stunning/killing of subjects destined for human consumption for the toxicity of the substances and withdrawal periods required. One option to anaesthetize fish is Eugenol, which is relatively new as fish anaesthetic. Eugenol is the major component of clove oil (70–90% by weight) and is obtained by distillation of the flowers, stems and leaves of the clove tree (*Eugenia aromatica* or *caryophyllata*). In addition, besides meeting the necessary anaesthetic requirements [7, 8, 9, 10, 11], it does not seem to pose any threat to public health if used in aquaculture, as it has also been employed for centuries as a topical analgesic in dentistry [12, 13]. Moreover, Clove oil is cheaper and more potent than other anaesthetics used in fish and the U.S. Food and Drug Administration classified it as a 'generally considered as safe' Q (GRAS) compound [14]. The aim of the present work was to evaluate the behavioural responses and the mortality times during stunning/killing phases using the anaesthesia with Eugenol and 2-Phenoxyethanol as pre-treatment of the stunning/killing in water and ice of European sea bass (*Dicentrarchus labrax*) and comparing this methods with the traditional direct immersion in water and ice usually adopted in aquaculture to assess the impact of this pre-treatment on well-being of fish. Moreover, in order to contribute to the body of knowledge regarding the anaesthetic characteristics of Eugenol, the only anaesthetic that could be used on fish destined for human consumption, we compared it with 2-Phenoxyethanol, which is considered an excellent anaesthetic on fish, having a short biological half-life and very fast induction and recovery times as reported by the available bibliographical data [15, 16, 17, 18, 19, 20, 10, 21]. In particular, to evaluate the anaesthetic characteristics of 2-Phenoxyethanol and Eugenol during induction and recovery, these substances were administrated in increasing concentrations to farmed European sea bass and then making a direct comparison between them.

2 Materials and Methods

2.1 Fish

A total of 240 farmed European sea bass (*Dicentrarchus labrax*) aged around 2 years, weighing 223.3 $\pm$ 95.1 g (Mean $\pm$ SD) and measuring 24.2 $\pm$ 3.1 cm (Mean $\pm$ SD), were examined in this study. The fish came from the off-shore fish farm "Hippocampus" (Villafranca Tirrena - Messina, Italy).

2.2 Experimental design

The experiments required the use of Eugenol (ALDRICH, St. Louis, MO, USA) and 2-Phenoxyethanol (MERCK, Whitehouse Station, NJ, USA). Eugenol and 2-Phenoxyethanol were used in the form of 99% (v/v). From the initial solution the required amounts were mixed with water from the tanks in order to obtain the desired final concentrations. The study was designed to include eight experimental trials (from I to VIII), one for each concentration of the anaesthetics chosen. Four increasing concentrations of 2-Phenoxyethanol were used in trials I to IV (0.2, 0.3, 0.4 and 0.5 $ml \cdot l^{-1}$ respectively) and four increasing concentrations of Eugenol were used in trials V to VIII (0.03, 0.06, 0.1 and 0.14 $ml \cdot l^{-1}$ respectively). These concentrations were selected after reference to other studies [23, 17, 19, 24, 25]. The entire study was performed during the month of July 2007. A diagram with a schematic representation of the experimental design is presented in Figure 1. Each trial was carried out on the boat owned by the fish-farm "Hippocampus" and used for farming operations (feeding, fishing, maintenance of the fish cages). All the trials were carried out during the routinely fishing operations on the farm. For each trial were used 30 fish. During each trial, a pen containing seabass was approached with the boat and 30 fish were caught with a net. Ten of the thirty fish caught were randomly selected and immersed in a tank (on the boat) containing 400 l of water and ice, in a ratio of 1:2. Following this, fish behaviour was observed and the times of death were recorded. In the meantime, the remaining twenty sea bass were placed in another 400 l tank, provided with a flow-through supply of aerated seawater at a temperature of 24.3 $\pm$ 0.8°C and salinity of 36.3 $\pm$ 0.78 ppt and were then anaesthetized. The sea bass undergoing this study were carefully observed in order to note the characteristics of anaesthesia induction; the data collected are presented in a table on the anaesthetic stages based on the physiological responses of the fish following administration of the anaesthetics (Table 1). After having reached the maximum state of anaesthesia, ten of the twenty fish were placed in the tank with water and ice which

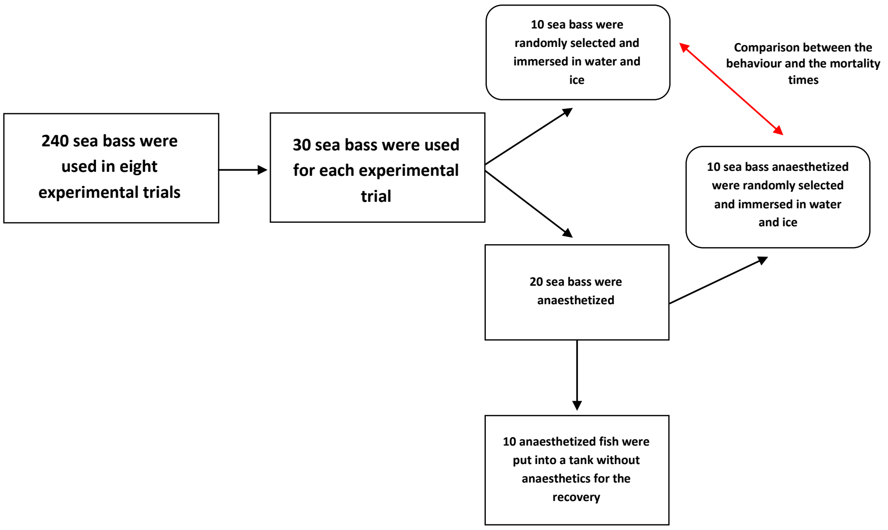

Figure 1: The experimental design of the study. The diagram shows the schematic representation of a single experimental trial.

had previously been used for the stunning of the first 10 fish, and left there until their death. Their behaviour and times of death were also recorded. The remaining 10 fish anaesthetized were put into a 400 l tank provided with a flow-through supply of aerated seawater at a temperature of 24.4 ± 0.7°C and salinity of 36.4 ± 0.8 ppt without anaesthetic, where they were able to recover gradually. The characteristics of the recovery from anaesthesia were collected in a table on the anaesthetic stages based on the physiological responses of the fish following administration of the anaesthetics (Table 1). The criteria employed by Trzebiatowski et al. [22], and modified by the authors of this study, were used for the classification of the stages during anaesthesia induction and recovery. The evaluations of the anaesthetics were based on a comparison of the substances during the phases of induction and recovery, with a direct comparison between the concentrations of 0.2 and 0.03, 0.3 and 0.06, 0.4 and 0.1, 0.5 and 0.14 ml·l^{-1} of 2-Phenoxyethanol and Eugenol respectively; for both phases, the time to reach the anaesthetic stage (TRAS) expressed in seconds, and opercular movements (OM) expressed in beats per minute, were evaluated for each stage of anaesthesia induction and recovery. The protocols of animal experimentation were reviewed and approved in accordance with the standards recommended by the Guide for the Care and Use of Laboratory Animals and EEC Directive 86/609.

2.3 Statistical Analysis

A T-test for unpaired data was used to determine the differences for times of death between the subjects pre-treated with anaesthetics and the subjects directly immersed in water and ice. The statistical analysis of the results obtained from the TRAS and OM of each stage of induction and recovery using 2-Phenoxyethanol and Eugenol was carried out using the Mann-Whitney U test for non-parametric values. For all the statistical tests $p<0.05$ was considered statistically significant. All statistical analyses were performed using the STATISTICA 7.0 software package.

3 Results

3.1 Behavioural observations during stunning/killing phases and mortality times

Behavioural observations showed notable differences between the fish stunned and killed in water and ice and those pre-treated with anaesthesia. The subjects in the first group died while thrashing around violently, in comparison with the fish which had been anaesthetized and subsequently placed in water and ice. These fish died without violent reactions or evident muscular spasms. The mean $\pm$ standard error of the mean (SEM) for the times of death of the two groups are showed in Table 2. The statistical analysis of the times of death revealed a highly significant difference ($p<0.0001$) between the pre-treated fish and the fish directly immersed in water and ice in all the experiments. In fact, the latter took much longer to die than the fish which had been anaesthetized and placed in water and ice.

3.2 Mortality following anaesthesia

The mortality rate for the fish undergoing anaesthesia was 3.2% of the total. Amongst these, there was only one subject treated with 2-Phenoxyethanol, at a concentration of 0.2 ml$\cdot$l^{-1} and three with Eugenol (two at a concentration of 0.1 ml$\cdot$l^{-1} and one at a concentration of 0.14 ml$\cdot$l^{-1}).

3.3 Comparison between the anaesthetics

The median time to reach the anaesthetic stage (TRAS) and the opercular movements (OM) during each stage of induction and recovery with 2-Phenoxyethanol and Eugenol are reported in Table 3 and 4. Comparison between the substances showed higher values of TRAS after administration of Eugenol at the first three concentrations and higher values of TRAS after administration of 2-Phenoxyethanol at the highest concentration. Moreover, higher values of OM were recorded with 2-Phenoxyethanol administration at all concentrations. In the recovery phase, comparison between the substances revealed higher values of TRAS at all stages at all concentrations after administration of Eugenol; OM showed higher values in subjects treated with 2-Phenoxyethanol.

	stage	characteristic
Induction	**0**	physiological position; normal locomotor activity, normal opercular rate
	1	physiological position, decreased locomotor activity, slight increased in opercular rate
	2	physiological position, increased locomotor activity, very high and erratic opercular rate
	3	slight tilting on the flank, uncoordinated locomotion, decreased locomotor activity, high and erratic opercular rate
	4	flank position, immobilization, low opercular rate
	5	back position, immobilization, breathing stopped
Recovery	**0**	flank position, immobilization, low opercular rate
	1	slight tilting on the flank, uncoordinated locomotion, low locomotor activity, slight increased in opercular rate
	2	physiological position, increased locomotor activity, increased opercular rate
	3	physiological position; normal locomotor activity, normal opercular rate

Table 1: Stage and phases of physiological responses caused by the anaesthetics, during the anaesthesia induction and recovery. Data from Trzebiatowski et al. [22] modified by the authors.

Trial	Treatment	Mortality times in seconds (Mean ±SEM)
I	A	47.2 ± 4.76
	WI	644.4 ± 40.6
II	A	57.2 ± 6.72
	WI	622.1 ± 47.23
III	A	63.7 ± 6.15
	WI	728.6 ± 47.04
IV	A	57.3 ± 5.41
	WI	647.3 ± 44.18
V	A	54.2 ± 4.21
	WI	663.5 ± 34.21
VI	A	68.1 ± 8.13
	WI	588.8 ± 51.82
VII	A	65.1 ± 4.19
	WI	655.6 ± 58.35
VIII	A	67.5 ± 6.4
	WI	620.5 ± 50.54

Table 2: Mean ± standard error of the mean (SEM) (n = 160) of mortality times in seconds. In all experiments, the values of mortality times between immersed in water and ice and pre-treated with anesthesia fish are statistically different ($p<0.0001$) (T test). A, anaesthesia pre-treatment; WI, direct immersion in water and ice.

TRAS (in seconds)								
	Stage 1		Stage 2		Stage 3		Stage 4	
2-Phe - Eug concentrations (ml L^{-1})	**anaesthesia induction**							
	2-Phe	**Eug**	**2-Phe**	**Eug**	**2-Phe**	**Eug**	**2-Phe**	**Eug**
0.2-0.03	143.5±23[a]	145±13.1[a]	224.6±27.8[a]	180±14.5[b]	371.8±29.1[a]	301±9.1[b]	466±12.3[a]	562.5±23.8[b]
0.3-0.06	59.7±6.3 [a]	15.9±1.4[b]	80±10.2 [a]	20±8.2[b]	122.5±7.8[a]	24.4±24.2[b]	181.5±10.8[a]	27.8±0.8[b]
0.4-0.1	30.75±2.9[a]	5.9±1.3[b]	42.9 ±2.3[a]	12.05±2[b]	80.5±8.8[a]	16.7±1.6[b]	107±11.6[a]	20.25±1.9[b]
0.5-0.14	8.25±11.5[a]	4.65±0.4[b]	8.7±0.9[a]	9.65±0.4[b]	12.8±1.1[a]	12.4±0.5[a]	19.75±2.1[a]	17.15±0.9[b]
	recovery from anaesthesia							
0.2-0.03	14.44±1.6[a]	17.3±2.4[a]	20±0[a]	41±2.1[b]	30±0[a]	89±4.5[b]	---	---
0.3-0.06	21.1±1.6[a]	222±15.4[b]	41.7±2.3[a]	274±5.1[b]	68±6.3[a]	306±8.4[b]	---	---
0.4-0.1	17.5±2.6[a]	435±42.4[b]	37±4.6[a]	467.5±41[b]	59±9.6[a]	503.7±39[b]	---	---
0.5-0.14	85±5.2[a]	304.4±7.2[b]	126±5.1[a]	335.5±4.8[b]	140±8.1[a]	372.2±9.7[b]	---	---

Table 3: Mean ± standard error of the time to reach the anaesthetic stage (TRAS) expressed in seconds during the anaesthesia induction of seabass (n = 160) and recovery of seabass (n = 80) with 2-Phenoxyethanol and Eugenol at four increasing concentrations. For comparison of 2-Phenoxyethanol and Eugenol concentrations at each stage, values with different alphabetic superscripts differ significantly at $p < 0.05$ (Mann-Whitney U test).

OM per minute								
	Stage 1		Stage 2		Stage 3		Stage 4	
2-Phe - Eug concentrations (ml L^{-1})	**anaesthesia induction**							
	2-Phe	**Eug**	**2-Phe**	**Eug**	**2-Phe**	**Eug**	**2-Phe**	**Eug**
0.2-0.03	81.55±2.2[a]	63±0.8[b]	95±0[a]	64.5±0.7[b]	93±0 [a]	65.6±1.04[b]	51.8±0.6[a]	48.35±1.04[b]
0.3-0.06	73.05±1.6[a]	69.5±2[b]	77.8±1.7[a]	76.6±1.3[b]	87.3±2.2[a]	72.7±1.7[b]	66.4±1.7[a]	64.8±1.1[a]
0.4-0.1	71.7±0.9[a]	72.2±1.6[a]	75.1±2.1[a]	70.7±1.1[b]	82.5±1.8[a]	64.6±1.7[b]	65.5±1.3[a]	59.7±1.9[b]
0.5-0.14	72.4±1.1[a]	73.1±1.2[a]	87.3±3.7[a]	68±0[b]	83.25±0.8[a]	67.6±0.5[b]	59.6±1.7[a]	64.3±4.5[a]
	recovery from anaesthesia							
0.2-0.03	61.88±.4[a]	51.4 ±0.9[b]	75.66±1.3[a]	64.2±1[b]	83.67±1[a]	70.8±1[b]	---	---
0.3-0.06	68.4±0.5[a]	47.1±1.4[b]	74.3±1.1[a]	58.2±1.7[b]	73.2±1.2[a]	68.6±0.9[b]	---	---
0.4-0.1	64.8±1.4[a]	58.87±1.2[b]	75.2±1.7[a]	65.25±1.6[b]	71.1±1.1[a]	70.5±0.9[a]	---	---
0.5-0.14	59.6±1[a]	55.1±1.3[b]	63±0.8[a]	65.6±2.3[a]	72.9±0.8[a]	73±0.8[a]	---	---

Table 4: Mean ± standard error of opercular movements (OM) per minute during the anaesthesia induction of seabass (n = 160) and recovery of seabass (n = 80) with 2-Phenoxyethanol and Eugenol at four increasing concentrations. For comparison of 2-Phenoxyethanol and Eugenol concentrations at each stage, values with different alphabetic superscripts differ significantly at $p < 0.05$ (Mann-Whitney U test).

4 Discussion

We believe that this study has contributed to knowledge both regarding the impact of the anaesthetic pre-treatment on the well-being of farmed fish during the stunning/killing phases and the anaesthetic properties of the substances employed.

4.1 Comparison between the anaesthetics

Our results showed that higher anaesthetic concentrations resulted in shorter induction times both with Eugenol and 2-Phenoxyethanol administration, as reported in other studies [26, 18, 19, 10, 24]. Comparison of the substances revealed the notable anaesthetic power of Eugenol as reported by other authors [7, 26, 8, 9, 27, 19, 10, 28, 11]. In fact, after administration of Eugenol, the induction phase was rapid and subjects reached deep anaesthesia in significantly shorter times compared to 2-Phenoxyethanol, at all concentrations except the lowest. The frequency of opercular movements also assumed values that were generally lower after administration of Eugenol, revealing once again its notable anaesthetic power and its capacity to induce very deep and long-lasting anaesthesia even at intermediate concentrations. In general, and in agreement with other authors [29, 26, 18, 24], the recovery times tended to increase with increasing concentrations of the two anaesthetics, except for the highest concentration of Eugenol which, in contrast to 2-Phenoxyethanol, showed a faster recovery time than at the lower concentrations. This is consistent with the study of Mylonas et al. [10], in which European sea bass and gilthead sea bream treated with Clove Oil and 2-Phenoxyethanol showed similar or shorter recovery times at higher anaesthetic doses. They suggested that this may be explained by the fact that higher doses induced anaesthesia more rapidly, and thus the fish were removed from the anaesthetic bath and placed in clear water earlier than fish exposed to lower doses. The shorter exposure time to the anaesthetic bath, the smaller amount of anaesthetic absorbed by the body, and the faster its removal from the blood of the fish could explain the reduction in the recovery times. A similar phenomenon has also been reported in red pacu (*Piaractus brachypomus*) [30], and in largemouth bass (*Micropterus salmoides*) using low concentrations of clove oil [27]. The comparison of substances during the recovery phase showed particularly significant differences in the characteristics of the two anaesthetics, confirming the capacity of Eugenol to induce very deep anaesthesia in fish [8, 18, 9, 27, 19, 10]. In fact, the subjects anaesthetized with Eugenol tended to recover very slowly, even at low concentrations, in comparison with the fish treated with 2-Phenoxyethanol, which always recovered rapidly, even at higher concentrations. The frequency of opercular movements in the fish at recovery, however, did not show any differences. The singles specimen of sea bass died as a consequence of anaesthesia with 2-Phenoxyethanol, at the lowest concentration, allow us to ascribe this event to a predisposition phenomenon. The condition was different for Eugenol, because the three subjects that died at the two highest concentrations confirm its capacity to induce markedly deep anaesthesia, which in some cases could cause death.

4.2 Impact on animal well-being of the anaesthetic pre-treatment

An ideal method of stunning/killing should determine the conditions to induce permanent unconsciousness and insensibility of animals until death [31]. Many studies have demonstrated that, even if less well developed than in other farmed animals, fish also have sense organs able to detect painful stimuli, sensory substrate for processing stimuli and brain pathways that process this information able to generate behavioural responses [32, 33, 34]. As stated initially, our study analyzed the possibility of using anaesthetics as a pre-treatment method for subjects intended to be immersed in water and ice, in order to evaluate a possible contribution to the protection of animal well-being. In this context, as already described in our results, the behaviour of the fish which were subsequently immersed in water and ice after being anaesthetized was reassuring. In fact, the fish pre-treated died without violent reactions or evident muscular spasms and in shorter times. The most important issue for the assessment at time of slaughter is animal suffering, which is also difficult to measure. The results of this work evidence that the behaviour represent a good noninvasive indicator of fish welfare as it often responds rapidly to multiple stimuli from the environmental changes included the stunning/killing phases, in agreement with other studies [35, 36, 37]. However, in order to evaluate the impact of anaesthetic techniques on the well-being of farmed fish using enable objective and more measurable assessments of the fish conditions, it would be desirable in future to perform further studies with a multidisciplinary approach that, together with the study of fish behaviour, takes into account the different biochemical and physiological processes (brain function, haematological and endocrine responses) involved. In conclusion, the behavioural evidence and mortality times of the fish recorded in the present survey allow us to identify the anaesthetic pre-treatment as a valid tool for the protection of the well-being of fish during the stunning/killing phases. In fact, both Eugenol and 2-Phenoxyethanol could potentially represent, for their anaesthetic characteristics, an important addition to the classic stunning/killing method of immersion in water and ice. Moreover, the comparison between the two anaesthetics showed that the Eugenol, the only anesthetic that does not pose any threat for the public health if used in aquaculture, provides better induction performance with significantly shorter times of deep anaesthesia compared to 2-Phenoxyethanol. In consideration of these excellent inductive characteristics of Eugenol and the equally valid characteristics of 2-Phenoxyethanol at recovery, we can consider the former more suitable for pre-treatment of famed fish during stunning/killing practice and the latter more suitable for short manipulation, surgery or transport of fish.

5 Acknowledgments

We would like to thank the team at the Department of Experimental Sciences and Applied Biotechnologies of the Faculty of Veterinary Medicine, University of Messina for their technical and logistic support.

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Analysis of Fuel Cost/Catch Prices Ratio in Artisanal Fisheries Gillnet in the Egadi Arcipelago (Siciliy)

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Abstract

Sustainable development of fisheries requires methods and tools to measure and compare the impact of human activities on the environment. The markets of the agroalimentary sector requires products that respect the principles of environmental and economic sustainability. The large industrial fisheries are easily adapted to new market conditions but the artisanal industries are finding many difficulties to follow the new trends. The present work aims to evaluate the consumption and fuel costs for 1 kg of fish product of a boat along the coast of Favignana Island. From May to June 2006, a 9 meters of lenght boat with Fiat Aifo inboard engine (130 KW) was used. During the experiment, the routes and the fishing time were recorded. In 29 fishing trips 601.7 kg of product were catched, comprising 26 different fish species. The boat engine worked for 5.03 per trip hours with a fuel consumption of 22 $kg{\cdot}h^{-1}$ on average. The fisheries product was sold on the local market at an average price of € 10.74 / kg. The results show that the average fuel consumption per kg of fish was 4.46 kg, with a great incidence of about 30% on the cost of product. Ours results indicate the great importance of the additional factors to the traditional parameters of fish catch. Moreover, studies like this are laying the basis knowledge for analyzing the Life Cycle Assessment (LCA) of fisheries products.

1 Introduction

Sustainable development requires methods and tools to measure and compare the impacts of human activities, both goods and services, on the environment [1]. Managing products life cycle (Life Cycle Assessment) is a framework methodology for the estimation and evaluation of environmental impacts that can be attributed to goods and services. The Life Cycle Assessment (LCA) can be used in several impact assessments that include: climatic changes, reduction of the stratospheric ozone layer [2, 3], the so-called ”smog” accumulating in the troposphere, eutrophication [4, 5], acidification [6, 7], toxicological stress on ecosystems and / or human health, exploitation of renewable and non-renewable resources [8, 9], water and soil, noise, and many others. The markets of the food industry are increasingly requiring food products in accordance with the principles of environmental and economic sustainability, in order to meet two conditions: the growing demand for low environmental impact-products and the improved energy efficiency of the production cycle [10]. The production of the Italian fishing fleet was, in 2003 [11], 312.000 tons per year (€ 1.466 million Euros). Small-scale fisheries accounted for to 51.333 tons per year with an average price of production of to € 6.68/kg, i.e. the highest income-yielding fishing system in Italy. Despite product's high quality, the small coastal fishing is undergoing a major crisis in the recent years, partly because of the policies aimed at to reducing fishing effort, as well as the increased production costs (fuel consumption per kilogram of catch). Seafood globally occupies a major share of the proteins consumed by the world population; then, the demand for fish products by highly industrialized countries is growing steadily [12]. Within this context, the rules for the production and regulation of trade are playing a key role in many sectors of the fishing industry [13]. The evolution of the Italian and European legislation increasingly leading towards specific production standards is pushing the fishing industries to improve industrial eco-efficiency. Markets for marine environment-friendly products are quantitatively increasing: as a result, further studies and tests on fishery products LCA are needed. Today, commercial fishing is characterized by an almost predominant use of diesel-cycle engines and many studies on fishing trawlers have already discussed the impact of fuel prices on fisheries incomes (kg fuel/kg fish) [10].

2 Materials and Methods

2.1 Study area

The study was conducted in the Egadi Archipelago, Sicily. The Egadi are a group of three islands: Favignana (33 km^2), Marittimo (12 km^2) and Levanzo (10 km^2) and some smaller islets and reefs including the island of Formica and the rock of Maraone. The waters surrounding the islands are part of a Marine Protected Area established in 1991 and now managed by the Trapani Coastal Guard (Figure 1). Sea bottoms are characterized by sandy and rocky substrates covered with extensive of oceanic Posidonia meadow, hosting a wide variety of flora and fauna. Artisanal fisheries is an important sector of local economy and it is practiced throughout the years in the waters surrounding the islands [15]. The studies on the production cycle were performed from March to May 2006 close to the island of Favignana (Figure 1).

2.2 LCA general aspects

The analysis on the LCA management is characterized by a precise and detailed classification of emissions, resource consumption, as well as the emission trading being relevant at each stage of production [16]. This study is referred to as the "from cradle to grave" product analysis, including raw materials, energy acquisition, materials production, manufacture, use, recycling, final disposal, etc (Figure 2). The life cycle, which includes the associated materials and energy flows, is called product system [17]. Each LCA to be properly implemented should identify in advance: an objective, its scope and an associated functional unit. In this study, the main points of criticism of small-scale fisheries are identi-

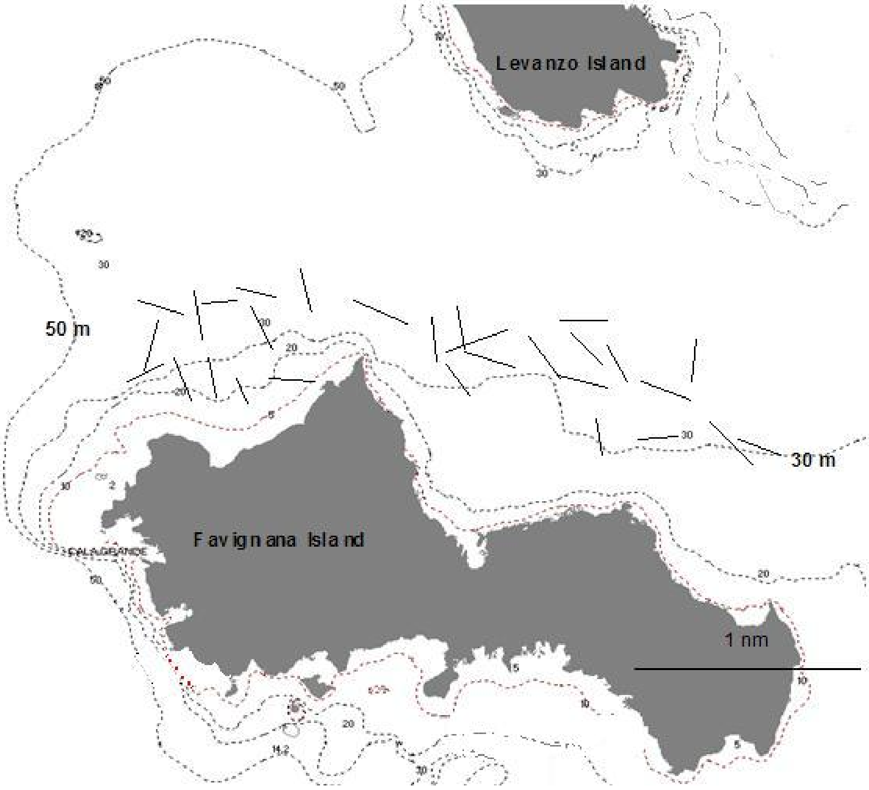

Figure 1: Map of the study area. The black lines represent the nets position

fied and assessed, in order to highlight any Key Success Factor (KSF) in comparison to other types of fishing and find out feasible management strategies different from those undertaken to date. The definition of the objective and scope for the LCA provides a description of the product in terms of system, boundaries and functional unit. The functional unit is the base enabling goods or services to be compared and analyzed. One of the main opportunities provided by the LCA is the ability to compare different types of production, through the same functional unit. The knowledge of build and environmental costs of a building, of a boat, of one kg of salmon, are typical applications of this method. Such a methodological choice allows to compare products, even very different among themselves, through the use of a single unit in order to identify opportunities and production-related weaknesses [18]. The main objective of the inventory of a product life cycle (Life Cycle Inventory) is to estimate what kind of and how many resources are commonly used, what waste is produced and what emissions are caused or may be associated to this particular type of fishing. LCI shows how the production of waste or emissions can be very complex.

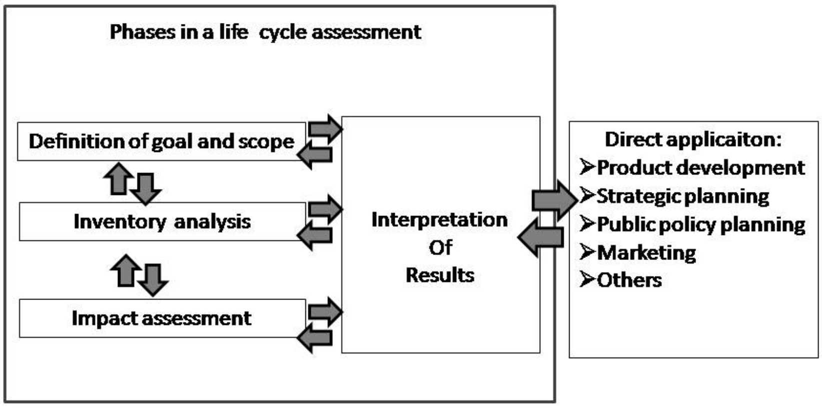

Figure 2: Phases and applications of an LCA (based on ISO 14040, [14]).

This survey can be analyzed geographically, by identifying several areas where waste or emissions are produced. The approaches are basically of two types:

- splitting the amount of emissions for each stage of production (fishing, processing, disposal, transportation, sales);
- comparing the stress produced at different times or in multiple generations of contemporary production.

The life cycle-associated processes and the related material and energy flows into and out of the system enable to represent the production system and its relationship with the natural system. The network of these relations among the production system, the inventory of used materials and the exchanges with the environment, can be linked to the so-called "boundaries of a system".

2.3 System Boundary

The boundary of the system is represented by the whole set of intermediate products' process flows, which carry one or more defined functions [17]. The boundary of the system is divided into a number of process units; these are in turn linked by flows of intermediate products. The boundaries of the system in this preliminary study include every phase of the fishing process, until the sale/delivery of the product on the docks. The study of the system starts with an inventory of materials needed for the production, i.e. fuel and fishing equipment. In the

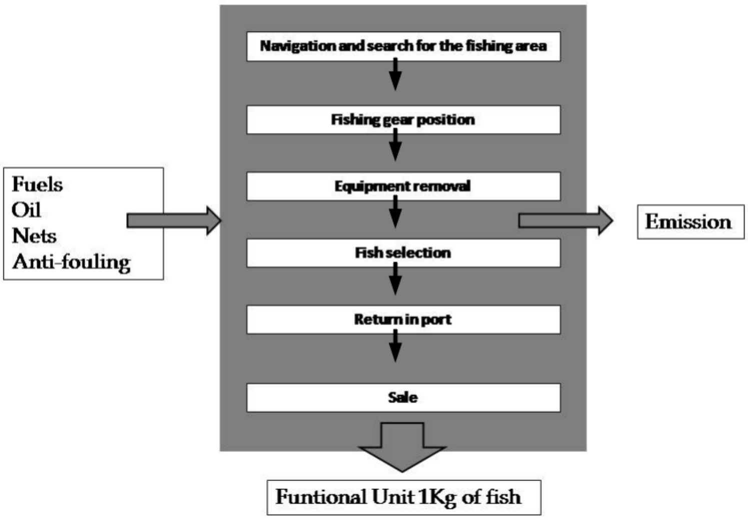

Figure 3: A simplified system boundary of artisanal fisheries.

case of artisanal fishing practiced in Favignana, the product can be directly sold on the docks, or delivered to other operators who sell seafood in the local market. Currently, the majority of fish and seafood is sold to wholesalers in Favignana who resell it in Trapani. The Favignana local market is able to almost entirely absorb the production only in the tourist season (from June to August). In Figure 3, the boundaries of the systems are divided into 6 process units, which are described below:

1. The identification and choice of the fishing area is crucial for the success of the trip and it can be affected by many variables: weather conditions, presence of other fishing gears, shipping, seasonality, type of boat, onboard equipment, etc.
2. Fishing gears positioning. Once the fishing area has been chosen and the on board fishing devices (depth sounder, GPS) have been operated, the net is hauled in accordance with the sea bottom features, (morphobathymetry), coastal streams, tides, etc.
3. Equipment removal. After a variable time, usually 6 hours, the nets are hauled in by an electro-hydraulic winch powered by the engine board.
4. Fish selection. The fishery product is selected by species and size, stowed in special boxes and covered by ice.
5. Return to port. The fishing vessel returns to port during the fish selection and preparation activities.

6. Sale. The fishery catches are directly sold to end-users in a local market and/or sold to traders and destined to other markets or restaurants.

2.4 Functional Unit

It seems clear that the complexity of LCA does not allow a direct approach, but systemic and gradual. Preliminary studies relating to the analysis of product life cycle need an LCI, as described above. The result will lead to the compilation of a ”road inventory”: this will comprise the use of resources, functional unit- associated emissions, including substances and chemical compounds. The functional unit enables to highlight the energy performance of the product/service for which an LCA is to be carried out [19, 20]. In this case, the functional unit is represented by kg of diesel fuel needed to produce one kg of food fish: this choice will allow to compare different LCA developed for other types of fishing. In addition, the same LCI will permit to compare functional units specifically addressed to a resource target (red mullet, cod, anchovy, etc.) [21].

3 Results

3.1 Inventory Results

The results of the LCI analysis (Table 1) include either data associated to a traditional LCA approach, or the data characterizing the sole fishing activity. The quantitative uncertainties on the materials involved in the process do not prevent to identify the most important activities for improving environmental performance.

3.2 Fishing

The data on catches and consumption were obtained using a 9-m long boat practicing traditional fishing along the coast of the island of Favignana, equipped with an inboard 130 KW Fiat Aifo engine. During the experiment, the marine-weather conditions and sampling effort were recorded, and the trip as well. The net was hauled daily and it fished for some hours after the dawn. The geographical coordinates of the starting and ending points of the net, and the intermediate points as well, were recorded for each minute of positioning. Also, the time spent for positioning and launching the net, as well as the net immersion time, were calculated. At the end of the fishing activity, after the return to port, the weighing and measuring of the species, and the economic assessment of species caught, were carried out. These data allowed to calculate the costs and kg of fuel consumed per kilogram of fish produced. 27 fish species were caught along 29 days. The catch was characterized mainly by two species, i.e. Boops boops and Sardinella aurita, representing respectively 31.92% and 19.44% of the total catch weight, and 28.14% and 17.14% in terms of economic value. Fish discards were represented by: undersized and non-commercial species, and partially looted species of commercial interest. In all three cases, the weight of non-tradable products was considered irrelevant for the purposes of life cycle inventory. In fact, both undersized and non-commercial species are caught alive, so that they can be re-introduced into the ecosystem. Furthermore, non-tradable species represent a negligible weight, i.e. less than 1% of the total catch.

Unit process	Specific Data	General Data
Energy Production		
Diesel	✓	
Raw Production		
Gas emission		✓
Lead Sinkers for Fishing Net		✓
Fishing Nets		✓
Fishing Net float		✓
Fisheries		
Fishing Species	✓	
Motor hours	✓	
Oil	✓	

Table 1: Data specifications

3.3 Fuel Consumption

Most fishing boats craft uses an inboard diesel engine. The vessel under study is one of the largest operating in the archipelago, and its average fuel consumption is 22 $\text{l}\cdot\text{h}^{-1}$. On average, for each day of fishing 109.07 liters (92.70 kg) of fuel were consumed and 22.78 kg of fish product were landed and selled (5.25 liters/1 kg fish).

3.4 Conservation

The maximum time the fish spends on board, from capture to the landing, is 2h. The storage conditions in this time frame comprises the fish storage in plastic tanks filled with water and ice, taking care not to cause excessive heating of the catch. This period from catch to processing has to be minimized to avoid loss of organoleptic quality. The ice used for storage is bought in dedicated production facilities, therefore such material is not considered in the LCI.

3.5 Equipment

The use of equipment may also play a significant impact on production costs and environmental concerns. In our experiment, the boat was equipped with two 900 m long monofilament nets, 2.2 m high and provided with jersey No. 11 (i.e. 11 knots in 25 cm of the stretched mesh). It was estimated that the net was almost completely renewed every 2 years. This figure is extremely significant due to the depredation activities and damage caused by dolphins. The nets were provided with a quantity of lead and net floats proportional to their size. The loss of lead-sinkers, replaced floats and nets are not included in this phase of the LCI.

3.6 Anti-Fouling

Antifouling paints covering the hulls of ships and boats contain biocide components, aimed at countering the growth of organisms on immersed surfaces, that reduce the frictional resistance to advancement and would tend to grow over time. The anti-fouling varnish (3 $\text{lt}\cdot\text{year}^{-1}$) was painted annually on the vessel used for the experiment.

4 Conclusions

This preliminary analysis of coastal fisheries around Favignana allowed to get some methodological assumptions as a preliminary base for further and more thorough study on the entire production cycle, through the "from cradle to grave" approach. The incidence of production and environmental costs in the fishery industry is now a critical factor. Lowering the cost of production is a competitive advantage for firms occupying the same niche, but this saving does not always entail a reduction of the enterprise's ecological footprint. This LCA preliminary study in the fishing industry showed that one of the major costs for fishing activities around small islands is represented by the fuel. The low standards in improving the boats energy performance, together with the current situation of fishery markets, reduce the market shares even for high-quality products. The current funding system provided by the EFF should, if properly directed, be enable to promote gradual and structural measures aimed at improving the eco-efficiency of the fishing vessels. In addition, it would be desirable to support these interventions with the most innovative tools of soft-economy in order to strategically reorganize some sectors of fisheries. In fact, it is important to invest in on high-quality products and intangible assets such as reduced emissions, discards reduction, preservation of endangered species (dolphins, turtles, seagrass, etc.), buyers lobby groups, etc.

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Echinoderms: Model Organisms for Marine Environmental Monitoring and Development of New Emerging Technologies

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Abstract

Traditionally, our research group has been mainly involved in basic research, studying the molecular mechanisms underlying sea urchin embryo development, with particular interest in cell adhesion. Recently, the group interest moved towards laboratory and field studies for the assessment of cellular responses to physical and chemical stresses and for the validation of molecular markers using the sea urchin as a suitable model system. In addition, during the last 10 years we learnt how to produce sea urchin juveniles, thus beginning our exciting experience in aquaculture. Such interest has many implications for studies on: the biology of larval growth and metamorphosis, the use of larvae in ecotoxicology and the development of suitable conditions for the commercial exploitation of the Mediterranean sea urchin species *Paracentrotus lividus*. Current research lines include: the understanding of basic mechanisms involved in biomineralization processes of sea urchin embryos and adults, stem cells in marine invertebrates, identification/purification of biologically active molecules from echinoderms. In the following, we will describe the “state-of-the-art” of our research projects aimed to test the cellular and molecular effects of different environmental hazards on echinoderms as model systems and to the establishment of sea urchin aquaculture technology. For brevity and appropriateness we will omit basic research studies which still attract our interest.

1 Introduction

In recent years growing attention has been given to climate changes and environmental pollution and, in particular, to the marine environment, which is subjected to increasing and significant impacts, including physical and chemical transformation, habitat destruction and changes in biodiversity [1]. Thus, numerous studies have been undertaken to assess the health of the marine environment using multidisciplinary approaches. In fact, from the environmental viewpoint, experts emphasize that integrated methodologies can provide more significant and realistic profiles of polluted habitats [2].

2 Choice of a model system for marine environment

One of the first steps to approach studies on environmental monitoring is to identify an appropriate model organism. This should

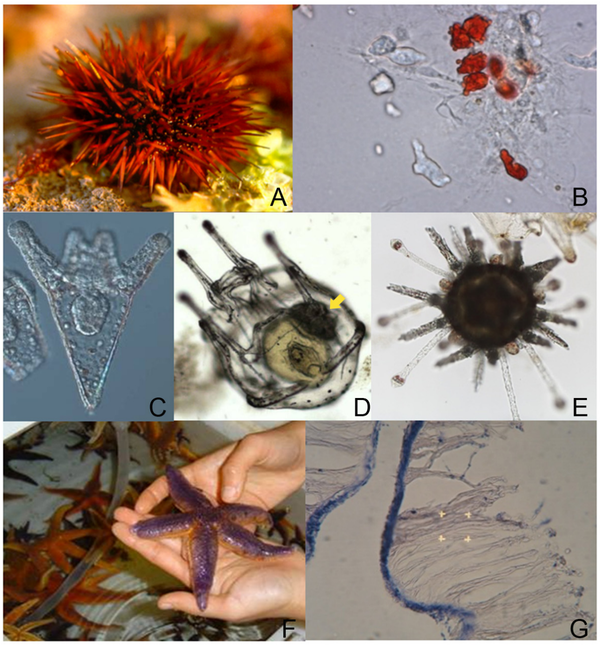

Figure 1: The sea urchin *Paracentrotus lividus*: adult sea urchin (A); adult immune cells (coelomocytes), in focus red and white amoebocytes (B); embryo at the pluteus stage (C); 8-arms larva with rudiment (D) indicated by the yellow arrow, and the intestine as a spherical shaped structure of a yellowish color, consequence of the brown algae ingested; juvenile (E). The sea star *Asteria rubens*: adult sea star (F); section of amputated arm (G), in blue coelomic epithelium.

satisfy the following conditions: 1) plentiful in number in the wild; 2) broadly available in the marine environment; 3) ecologically relevant; 4) easily manageable in laboratory experiments. The ideal model organism can then be used for a number of purposes: 1) to analyze toxicity of environmental hazards and chemical pollutants; 2) to elucidate the mechanism of their toxic action at various biological levels (i.e. population, whole organisms, cellular and molecular); 3) to understand how marine organisms cope with toxicants and respond to them at the physiological or biochemical levels. Furthermore, an ideal model organism is also of great importance for the development of standardized alternative biomolecular ecotoxicological tests. Among marine organisms, echinoderms are highly sensitive to physical and/or chemical environmental changes occurring in the sea water ecosystems where they live. They are among the most familiar marine invertebrates, characterised by a great morphological variety of their members, some of them being very popular and fascinating for their shapes (sea urchins) and colours (sea stars). Conditions for successful culture/maintainance of echinoderms in the laboratory have been established since many years, a necessary prerequisite for any organisms to be adopted as model for bioassays. Thus, also by virtue of their commercial importance and considering their abundance and wide distribution, echinoderms are emerging as new potential sentinel organisms able to perceive environmental hazards. Figure 1 shows the most commonly used among echinoderms, namely sea urchin and sea star adults, embryos, larvae and adult immune cells (coelomocytes). A good number of studies have focused on effects of chemicals on sea urchin embryos or larvae and on development of molecular biomarkers for the detection of the risk to metal exposure [3, 4, 5, 6, 7, 8]. In the following we will review our research activities related to the use of the model organism of choice in ecotoxicological and biomolecular tests, both in field and laboratory studies.

3 Field studies

3.1 Oceanographic campaigns

It is well known that some heavy metals occur naturally in the ecosystem with large variations in concentration and yet, in some cases, they have vital or beneficial effects on living organisms. In modern times, large amounts of heavy metals have been introduced in the ecosystem by anthropogenic sources and, since they are non-biodegradable and persist in the environment for long periods, can have serious ecotoxicological impacts. This poses a main challenge in the assessment of potential remediation procedures which are under study.

In 1995, we began to be interested in assessing marine pollution, when we took part to an EU Summer Course on "Monitoring of environmental stress using modern techniques". In that context, *Paracentrotus lividus* sea urchin coelomocytes were used for the first time for monitoring environmental stress. In those pioneering studies, we analysed and validated a few cellular and molecular markers of stress, opening the way to other field studies and introducing sea urchin coelomocytes as biosensors of environmental stress [9]. Coelomocytes are a population of different cells types that freely circulate in the body fluid of the adult coelom. They represent the echinoderm defence system, which reacts to injuries, host invasion and adverse conditions [10, 11, 12, 13, 14, 15]. Variations in the usual number of cells within each subpopulation were considered as a first indication of cellular stress [9]. During the oceanographic campaigns held in 1995, coelomocytes were collected from animals of three different Croatian coastal sites, some identified as urban or industrial contaminated areas, others referred to as unpolluted controls. Significant differences were observed in coelomocytes from polluted sea waters as it was found a consistent greater number of red amoebocytes (Figure 1B), a subpopulation of the immune cells which is usually poorly represented [9]. At the molecular level, the marker analysed was the heat shock protein 70 kDa (hsp70). The protein belongs to an evolutionary conserved family of proteins, virtually found in all living organisms, from bacteria to humans [16]. Hsps are constitutively expressed in cells under normal conditions where they act as chaperones, controlling protein synthesis and folding [17]. In response to a variety of different stressors, their expression is rapidly up-regulated at the post-

transcriptional level, playing a central role in cellular homeostasis as one of the protective mechanisms against stressors [18]. In coelomocytes obtained from polluted areas, either urban or industrial, a two-fold increase in hsp70 levels was observed [9].
Sea urchin coelomocytes have been later used for monitoring environmental stress in campaigns that took place in June 2003 and July 2004, in the frame of the EU 5th FP Project "Research on Environmental Damage caused by Chemical Ordnance Dumped at sea", leaded by Istituto Centrale per la Ricerca scientifica e tecnologica Applicata al Mare. The project aimed to evaluate potential damage caused by the 2,4,6-trinitrotoluene (TNT) and its degradation/hydrolysis products from corroded bombs dumped at sea. The site was the Protected Marine Area of the Tremiti archipelago in the Southern Adriatic Sea, Italy. Specimens of *P. lividus* sea urchin were collected along the Pianosa Island seabed, full of conventional ordnance remains of the World War II, and were compared with specimens collected in a not impacted site of the same archipelago (Caprara Island). We found a higher number of red amoebocytes and high hsp70 levels in those specimens collected around Pianosa [19].
Confirming what obtained with sea urchins, interesting findings came from studies on coelomocytes of the sea star *Asteria rubens* collected in a Norwegian fjord during a campaign held in May–June 2000, by Drs. Coteur and Dubois (University of Belgium). The sampling site was extremely interesting because it housed some zinc and titanium/iron smelters located at the head of the fjord, allowing the formation of a natural heavy metal gradient. The environmental contamination correlated with the accumulation of Cd, Pb, Zn and Cu in tissues of sea stars living along the metal gradient [20]. In agreement, we later found that the increase of hsp70 levels in sea star coelomocytes were directly related to the heavy metal levels measured in the animal tissues.

3.2 Stratospheric campaigns

UV-B radiation (280–320 nm), the most detrimental to biological systems, can penetrate clear sea water up to 20 meters or more, depending on constituents which are dissolved in sea water [21]. Therefore, in addition to the well known damages caused to humans, deleterious effects of exposure to UV-B have been also observed in marine organisms, especially those that do not possess specialized protective tissues. In general, the major concern after exposure to ionizing radiation is the genomic damage they can cause, although cells have developed a complex defence system able to cope with harmful radiation [22]. Our interest in studying effects of ionising radiation originated thanks to our involvement in two campaigns co-sponsored by the CNR and the Italian Space Agency (ASI), which took place in 2000-2001, aimed to analyse the effects of BIological Radiation on BAllon flights (BIRBA and BIRBA 1 campaigns). Stratospheric balloons represent an alternative and/or a significant match to Satellites or International Space Stations; balloon launches were operated from the ASI Base located in Trapani-Milo and reached the INTAS Base of Huelva. The aim of our project was to study the effects of UV-B on sea urchin embryos in field experiments carried out on a special device loaded on a payload fixed to balloons which reached the stratosphere (over 40.000 meters above the Earth), thus exposing sea urchin embryos directly to UV-

B light for about 16 hours. Flying shielded specimens were also prepared as well as ground controls. After the flight, we determined the differentiation/location of territorial markers by indirect immunofluorescence in whole mount embryos using antibodies to ectoderm-, endoderm- and mesoderm-specific antigens. In parallel, we measured the hsp70 levels by Western blotting. Results showed a modest increase in the hsp70 levels measured in flying specimens in comparison to ground controls, whereas no appreciable differences were found in the differentiation of embryonic territories (unpublished results).

3.3 Other field studies

Recently, we started a collaboration with the Istituto per l'Ambiente Marino Costiero (IAMC - Messina), in the frame of a PON project (2007-2008) titled: "Developoment of advanced systems for management and environmental monitoring of off-shore cages and performances verification". The project included among its aims the development and testing of a farming system for the sea bass *Dicentrarchus labrax* in submerged cages. This required the analysis of health state and growth of fish reared in the experimental facilities, through the study of various parameters. The research involved a comparison between two different experimental conditions, i.e. fishes reared in submerged versus surface cages (see also [23]). Among a specific panel of various physiological indicators, we studied the levels of hsp70, as a molecular marker of stress. We were able to develop conditions for the sampling procedure and the extraction of total proteins from blood and liver cells of *D. labrax*, which can be used for the evaluation of stress proteins levels. Experiments are in progress to determine a relationship between the health state of fish, farmed in submersible and surface cages, and hsp70 levels in blood and liver cells.

4 Laboratory studies

As important as field studies, laboratory experiments can provide important information for the evaluation of impacts of chemical or physical hazards, by reproducing polluted environments "in vitro". One of the advantages is that all the parameters are under control and it becomes easier to correlate the observed effects with the stressor used and its mechanism of action. On the other hand, it can be difficult to translate data obtained in the laboratory into accurate predictions of possible effects in the field. For this reason both field and laboratory approaches are needed and the data obtained should be integrated.

In our laboratory in the past 15 years, sea urchin and sea stars have been used to investigate the toxic effects of different pollutants by means of analyses at morphological and molecular levels. Usually, the preliminary analyses take into consideration any morphological variation in treated samples compared to controls, i. e. differences in the number of cell morphotypes in a mixed population of adult immune cells (coelomocyte) or defects in the canonical development of embryos or larvae (Figure 2). Significant effects are usually dose-dependent, i.e. more severe defects are observed with higher doses of the stressor. However, this kind of tests has some limitations, since morphological defects are often detectable long time after exposure to the pollutant, i.e. hours or even days. Then, for a rapid evaluation of major environmental hazards, it is crucial the

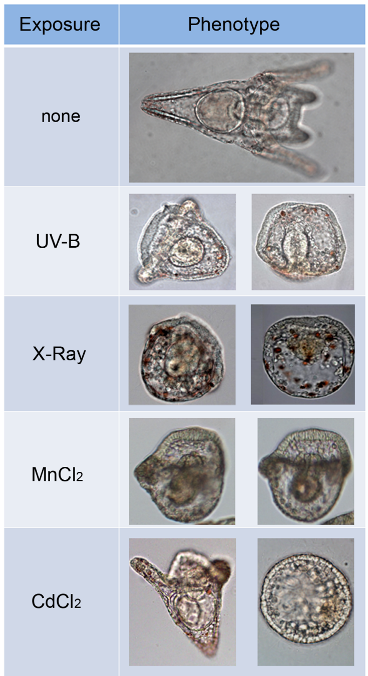

Figure 2: Morphologies of *P. lividus* sea urchin embryos observed after 48 hours of exposure to different pollutants.

use of sensitive stress markers whose responses are detectable in very short periods of time. Preference goes to genes/proteins whose expression is rapidly altered as a consequence of external insults, as those belonging to a variety of cellular protection pathways, including specific stress induction mechanisms (activated by a range of insults), intracellular sequestration mechanisms (playing a role in the detoxification from heavy metals) and elimination of irreversibly damaged cells by apoptosis. Recent advances in cellular and molecular biology have made it possible the identification of numerous specific genes/proteins both in echinoderm adult immune cells and embryos. Several methodologies are used in our laboratory, all sensitive enough to detect variations in proteins and genes levels. These include Western blotting and 2D gels for the analysis of proteins and Northern blot, Q-PCR and "in situ" hybridization (ISH) for the analysis of genes. The diagram presented in Figure 3 summarizes all the hazard factors tested on embryos and adult immune cells of both sea urchin and sea star and the molecular markers analysed in our laboratory so far.

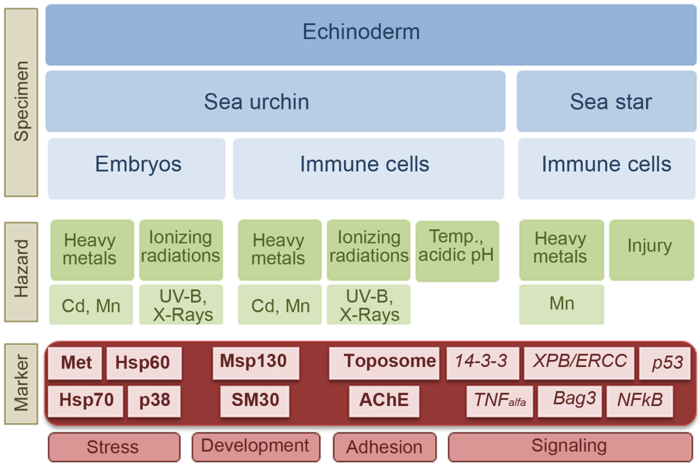

Figure 3: Scheme showing all the hazards taken into consideration for studies on sea urchin and/or sea star (embryos or adult immune cells) and markers evaluated. In bold published data; in italic text, unpublished data. Met, metallothionein; SM30, spicule matrix; Msp130, matrix spicule protein; NFkB, nuclear factor kappa-light-chain-enhancer of activated B cells; XPB/ERCC, xeroderma pigmentosum B/excision repair cross-complementing; TNF alfa, tumor necrosis factor α; AChE, acetylcholinesterase.

4.1 Effects of UV-B and cadmium exposure on sea urchin cells, embryos and larvae

From 2000 to 2003 we participated as partner in an EU 5th FP Project named UV-TOX whose main goal was: "to introduce novel cellular biosensor (marine invertebrate cell culture) and molecular biosensor (optical grating-coupler sensor chips) techniques for the estimate of the health state of marine invertebrates in their natural habits (sponges and sea urchins) or in aquaculture (bivalves)." Our task was to determine the effects of ionising radiations (solar and UV-B) and heavy metals (Cd) exposure, and their combined effects, on sea urchin cells and embryos. In particular, we validated sea urchin coelomocytes as cellular biosensors by exposing them to the stressors in the laboratory and by monitoring the expression of stress genes and proteins (Nothern blot/RT-PCR/Western blotting) or analysing DNA damage (Fast Micromethod). In parallel, we studied the impact on the development of the sea urchin embryos at the morphological and molecular levels, including statistical analyses, detection of stress proteins and induction of stress genes. Results obtained were manifold. First, we developed a new method for maintaining coelomocytes in short term cultures. Under proper conditions, cells attached to the substrate and survived for few hours-days, thus allowing monitoring and

quantification of toxic effects [24, 14]. In addition, we found a dose-dependent increase in DNA damage of coelomocytes, whether the cells were irradiated with UV-B or exposed to low concentrations of cadmium. Unexpectedly, the combination of the two treatments, irradiation with UV-B and subsequent exposure to cadmium or the reverse, rather than cause an additive effect, resulted in lower levels of DNA damages [25]. On the contrary, we found increased levels of hsp70 in both cells exposed to UV-B or cadmium.
Second, experiments on embryos showed that early developmental stages are more sensitive to UV-B radiation than later ones. In fact, we found that: 1) irradiating 16-cell embryos caused more aberrant morphologies than irradiating blastulae; 2) the first UV-B dose harmful to 16-cell embryos was lower than the one effective on blastulae; 3) defective embryos obtained irradiating 16-cell embryos with high doses were not able to overcome the dangerous UV-B effects after 48 h cultures [26, 27]. In general, we observed that the dramatic impairment of skeleton development was directly related to the UV-B doses used (Figure 2). Moreover, the morphological effects were correlated with the dose-dependent increase in the level/activation of some stress markers, namely hsp70 and the p38 mitogen-activated protein kinase (p38 MAPk).
Third, we confirmed metallothionein (Met) as an early marker of cadmium exposure [4]. Embryos continuously cultured in the presence of sublethal cadmium concentrations, and harvested at different developmental stages, showed a time- and dose-dependent increase in the messenger coding for the Met gene, which was recorded long before the observation of morphological abnormalities (Figure 2).
We extended our studies to *P. lividus* larvae (from 4-arms pluteus to metamorphosis) in order to monitor the effects of environmentally relevant cadmium concentrations for longer exposure times. We found time- and dose- dependent delays and abnormalities in larval growth, as well as enhanced apoptotic events. Major defects consisted in the reduction and/or lack of arms and skeleton elongation. Supernumerary apoptotic cells were found in arm buds, ciliary band and apex [6].

4.2 Effects of cosmic radiation (X-ray) on the expression of stress markers on sea urchin embryo

An example of ionising radiations is X-ray, an highly impacting radioactive emission which, although it does not penetrate into sea water, can reach it as a consequence of unexpected accidents at nuclear power stations, negligent dumping of their leftovers and spills from storage sites [28]. X-rays are more penetrating than UV-B and their exposure can cause serious health hazard; the effects on living tissues/organisms are related to the amount of energy deposited, namely the absorbed dose. From 2006 to 2009, we had the opportunity to use the sea urchin embryo as a model in the frame of the ASI project “From Molecule to Man (MoMa): Space Research Applied to the Improvement of the Quality of Life of the Ageing Population on Earth”. Our work-package was devoted to the assessment of the effects of X-rays on embryos exposed at early developmental stages in the laboratory. The project was carried out in collaboration with the IASF (Istituto di Astrofisica Spaziale e Fisica Cosmica of Palermo) and irradiations were performed at the “Livio Scarsi” Laboratory (LAX), where an X-rays beam facility was available. Appropriate anode currents and irradiation times were studied to obtain doses comprised between 0.1 and 5 Gy, which were selected on the basis of X-rays ranges used on different cell types [29]. Dose-dependent effects were observed at morphological and molecular levels, including delay in the developmental schedule as well as major abnormalities in specific embryonic territories (Figure 2) and variations in the expression levels of stress, anti-apoptotic and aging proteins (Bonaventura et al., in preparation).

4.3 Water quality tests and human health

Since year 2000 we are part of the working group ”Biological methods”-”Saltwater/brackish water and sediments” of the UNICHIM ”Water Quality” Commission. Our laboratory is one of the 9 in Italy performing intercalibration tests for standardization of new protocols and methodological procedures on some biological assays (embryology test-Echinoderms). We contributed to the production of a final document on battery tests to be normed by the Italian Minister for the Environment. The final goal is the development and validation of test methods, as well as the promotion and enhancement of the water quality culture. In this contest, we participated in the CNR-PIAS project (Progetto Interdipartimentale Ambiente e Salute), jointly launched by the Earth & Environmental and Medicine Departments, aimed at understanding of the links between pollution sources and toxic effects on human health. Within the working groups GL2 (Monitoring systems for soil and water) and GL4 (Human Biomonitoring), we proposed our biological models for basic studies on the effects of heavy metals and/or nanoparticles on the endocrine, reproductive and immune systems. Biomarkers of exposure were proposed for the evaluation of early damage and the analysis of relationship between epidemiological investigations and “in vitro” - “in vivo” toxicological research.

5 Sea urchin aquaculture technology

Sea urchin gonads are a luxury food product consumed worldwide; their demand in the European and international markets is greatly increasing. Thus, the establishment of a sea urchin aquaculture technology for commercial exploitation is a topic of great interest. We have been recently involved in the assessment of the best laboratory conditions for the development, growth and farming of *P. lividus* sea urchins. Originally acquired in the late nineties, we refined our expertise on the occasion of a EU granted project (POR Sicilia 2000/2006) carried out in Spring 2005. It is worthwhile to mention here that, as many Echinoderms, most sea urchins are characterised by an indirect development which involves the transformation of a planktonic larva (pluteus) into a benthonic adult (juvenile), through a process called metamorphosis (Figures 1C-1E). In *P. lividus* this process occurs in about 28-30 days: each developmental stage has been already carefully illustrated in an atlas by Matranga and Bonaventura [30]. Thus, we developed appropriate protocols for the laboratory small-scale production, including sea urchin larval rearing, and the identification of the best natural diets, such as diatom/benthic algae and biofilms substrates able to induce metamorphosis and promote juvenile growth. The whole process, from the fertilization of gametes to juveniles (10mm test diameter) lasted about 5-6 months. The pilot project for the production of a few thousands of sea urchin juveniles ended with their release at sea, along the coast near Palermo, in a legally recognised farming area. Since 2007, a brochure describing the "Protocol for optimal production of larvae and juveniles of the Mediterranean sea urchin species *Paracentrotus lividus*" has been posted at the Technological Transfers Section of the AgroFood area of application, within the website of the CNR Life Science Department (http://life.cnr.it).

6 Conclusions

Taken all together, results described so far encourage the use of echinoderm adult immune cells and embryos for ecotoxicological studies and development of biomarkers. The biological models used will be instrumental for future comparative genomic and proteomic approaches and may introduce new emerging technologies to be used in protection and remediation countermeasures of sea waters. Studies are currently underway on these topics in our laboratory.

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Recruitment Areas of Demersal Species in the Strait of Sicily (Central Mediterranean)

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Abstract

Locating nursery grounds of exploited demersal species is essential for implementing management measures aimed at reducing the fishing pressure on vulnerable stages of species life cycle and their habitat. The aim of this study was to identify the nursery areas of seven important commercial species of the northern sector of the Strait of Sicily (central Mediterranean): red mullet, European hake, horned octopus, deep-water rose shrimp, greater forkbeard, Norway lobster and giant red shrimp. The data were collected during experimental trawl surveys carried out routinely in the Strait of Sicily from 1994 to 2004 during spring and autumn season. The analysis was performed using the following three-step approach. Firstly the juvenile fraction (recruits) of a species was identified and the relative density indices by haul were estimated. In a second step, yearly distribution maps of recruit density indices were generated through geostatistical analysis. Hence the areas encompassing the highest concentrations of recruits each year were delineated (density hot-spots). Finally, the persistence in time of the density hot-spots was evaluated to identify the stable nursery areas. Results showed that most of the species have nursery grounds well defined and very stable in the long term. The protection of these areas, through limitations of fishing pressure throughout the year or during selected months, may be an important management measure for ensuring the long-term sustainability of fisheries.

1 Introduction

The spatial ecology of fishery target species is a major issue of current fisheries research [1]. The spatial structure of populations, the location of nurseries and spawning grounds, the ontogenetic movements and migration patterns, are investigated in order to better understand the reproductive ecology of fish species and implement effective fishery management. Indeed, the reproductive success and subsequent recruitment of young fish to their parent stocks is one of the most important factors regulating the annual abundance of populations [2]. Accordingly, fishery managers should strive to reduce fishing mortality of the critical fractions of the stocks (e.g. adult/spawners and juveniles/recruits). Most recent research suggests that understanding the spatial distribution of stock fractions and their dynamics, and implementing consistent spatially explicit management measures, such as

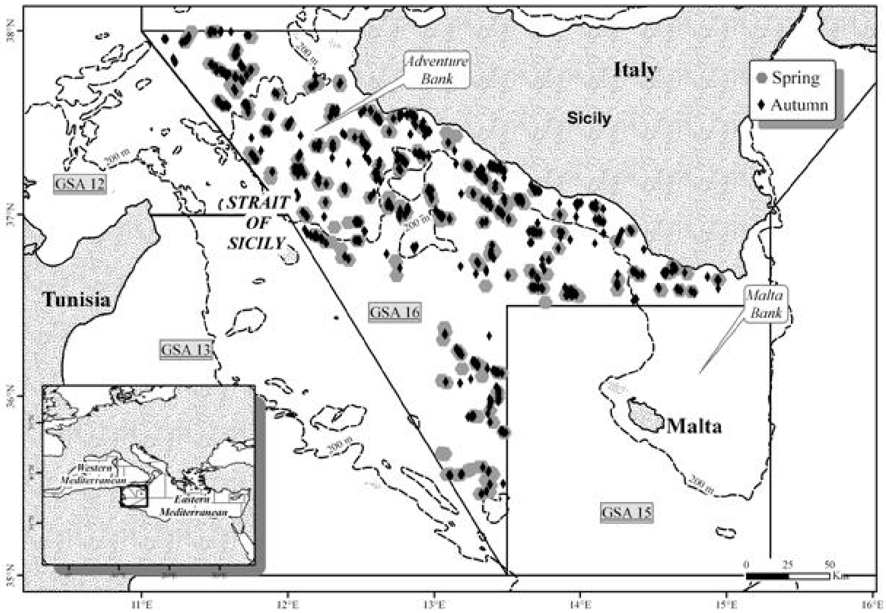

Figure 1: Strait of Sicily survey area (GFCM-Geographical Sub-Area 16) and location of the trawl stations carried out during spring/early summer for the period 1994-2004 and autumn for the periods 1994-1998 and 2000-2004. The dashed line (200 m depth isobath) shows the boundaries of the Italian-Maltese shelf and the North African shelf.

networks of marine protected areas, are the most effective way to ensure long-term sustainability of fishery resources along with their habitats [3, 4, 5]. The temporary or permanent restriction of fishing activity on spawning grounds is believed to be effective for preserving the reproductive potential of populations and for enhancing recruitment [5]. On the other hand, reducing fishing effort on juvenile stages is of utmost importance when juveniles are primary target of unselective fishing gears [3].

Targeting the juvenile fraction of many species, often in violation of laws regarding minimum catch sizes, is still a common practice throughout the Mediterranean due to the traditional high marketability of small fish in many countries [6]. Accordingly, many demersal populations have severely declined and currently are either fully-exploited or overexploited [7]. The over-fishing of demersal resources, which is more or less intense depending on the species, has been a major issue in the Strait of Sicily (central Mediterranean) since the early eighties [8, 9]. In this area, demersal resources are the target of the Mazara del Vallo bottom trawling fishery, one of the most important industrial fleet in the Mediterranean (136 vessels, 19,9527 GT, 54,241 kW in 2009). The regulation of fisheries has

so far been based on limitations of fishing capacity (licenses), minimum landing sizes, net mesh sizes and temporary fishing closures [9], but the establishment of no-fishing zones, particularly within nursery areas, has been increasingly advocated as a further component of the fishery management strategy (European Council Regulation n. 1967/2006).
The present paper is a synthesis of the work carried out to identify the nurseries of the most important fishery target species in the northern sector of the Strait of Sicily. The species studied are: red mullet (*Mullus barbatus*), European hake (*Merluccius Merluccius*), horned octopus (*Eledone cirrhosa*), deep-water rose shrimp (*Parapenaeus longirostris*), greater forkbeard (*Phycis blennoides*), Norway lobster (*Nephrops norvegicus*), giant red shrimp (*Aristaeomorpha foliacea*).
This analysis is based on a definition of "nursery" ground which takes into account the spatial persistence of hotspots of recruit densities over long periods [10, 11, 12]. More specifically, a nursery ground is defined as a discrete area characterized year-by-year by the highest concentrations (density hotspot) of the juvenile fraction of a species. The character of persistence of these areas has clear implications for implementing spatially explicit management options such as the designation of marine protected areas (MPAs) and their inclusion in a conservative network.

2 Study area and sampling scheme

The Strait of Sicily (Figure 1) is a region characterized by complex bottom topography and by important hydrodynamic processes, which determine the water-mass exchanges between the western and eastern Mediterranean basins. Along the southern coast of Sicily, the shelf is characterized by two wide and shallow banks (<100 m depth) on the western (Adventure Bank) and eastern (Malta Bank) sectors respectively, separated by a narrow shelf in the middle. According to the definition by the General Fisheries Commission for the Mediterranean (GFCM) of Geographical Sub-Areas (GSAs) [13], the Strait of Sicily encompasses different fishery management areas. This study concerns the trawlable grounds of GSA 16 (about 34.000 km^2). Data were collected during scientific trawl surveys routinely carried out in the area with the primary aim of monitoring and assessing fishery resources status: the Italian GRUND surveys [14] carried out in autumn and the international MEDITS surveys [15] carried out in spring/early summer. Both programmes employed a random sampling design with a stratification protocol. The prospected depth strata were: 10-50 m, 51-100 m, 101-200 m, 201-500 m, 501-800 m. The time series examined in this study spanned through 11 years, from 1994 to 2004.

3 Data analysis

A total of 523 hauls were analyzed from 11 MEDITS surveys and 611 from 10 GRUND surveys. For each sampling station, all the specimens of commercial species were sorted, counted, measured, weighted and sexed. Density indices were expressed as number of individuals standardized to 1 km^2 (N$\cdot$km^{-2}) assuming a catchability coefficient equal to 1. Recruits, corresponding to individuals in their first year of life, were identified by split-

ting the yearly standardized Length Frequency Distributions (LFDs) into components (combined sexes) and isolating the first modal component. Hence, density index of recruits by haul was calculated as the standardized number of specimens whose length was smaller than the mean length plus one standard deviation of the first modal component of LFD.
The distribution maps of recruit density indices, for each species and survey, were generated by geostatistical approach following two steps. Firstly, the analysis and modeling of the spatial correlation of the raw data was performed (variography). However, the surveys in which the presence of a few positive hauls precluded geostatistical structural analysis were excluded from subsequent analyses. Then a spatial prediction was produced using the fitted model from variography and the ordinary kriging interpolation technique [16].
Nursery areas were identified, by means of GIS techniques, applying criteria based on the persistent presence of high values of recruits, throughout the time series. Specifically, once yearly maps were produced, the area encompassing the highest abundance values up to sum the 75% of the total abundance was outlined over each map to identify the annual nursery. To verify if they were located in the same position consistently throughout time, an index of persistence [10, 17, 11, 12] was calculated. It was obtained by overlapping the maps of the entire seasonal series and counting, on a cell-by-cell basis, the number of times a given area was classified as annual nursery. The index was expressed in percentage term and the level of 60% was taken into consideration to define persistent nurseries.

4 Results

Analysis of LFDs allowed to identify the recruitment period and the mean size of the first modal component of each species for each year (Table 1). The recruitment of red mullet is discrete and occurs in late summer [18], allowing a clear identification of recruits in the LFDs of the autumn surveys. The mean of the cut-off length used to identify the recruits was equal to 89 mm TL (Table 1). Recruits of red mullet occured in shallow coastal waters throughout the southern coast of Sicily (Figure 2a). The spatial pattern was quite variable from year to year with the exception of an area of persistence located close to the southwestern coast of Sicily on the Adventure Bank. The recruitment of European hake occurs in both spring and autumn [18]. The first modal component was well defined and abundant in the spring season with a mean size of 115 mm total length (TL) (Table 1), whereas it partially overlapped the adult component in the autumn season. Recruits were distributed over a wide bathymetric range (50-600 m) with a concentration peak at 250 m depth. The distribution maps showed that recruits were preferentially located over the Adventure Bank and southeast of its border where a large and persistent nursery area is identified in both seasons (Figure 2b). The recruitment signal of horned octopus was detected only in the spring surveys. The average of the cut-off length was equal to 32 mm mantle length (LM) (Table 1). The largest concentrations of recruits were found between 150 and 250 m depth with a peak at 200 m. Data on this species was somewhat sparse in most surveys. As a consequence, it was only possible to perform the spatial analysis for three years. The spatial distribution of the recruits showed

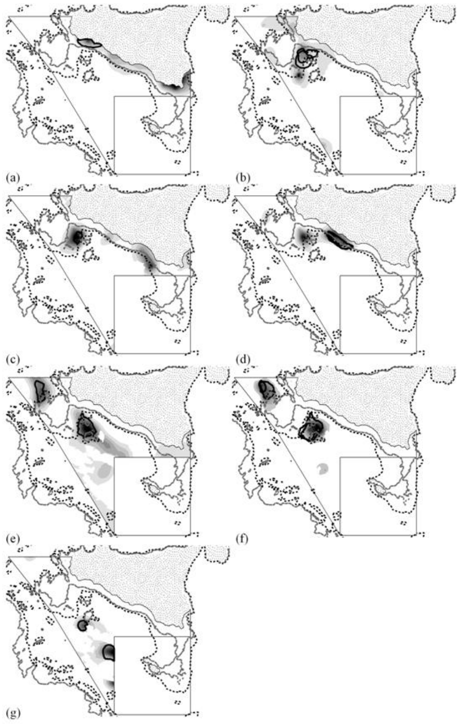

Figure 2: Examples of density maps of recruits of: a) Red Mullet - autumn 2004; b) European hake – autumn 1997; c) Horned octopus – spring 2003; d) Deep-water rose shrimp – spring 1996; e) Greater Forkbeard – spring 1999; f) Norway lobster – spring 1998; g) Giant red shrimp – spring 2002. The stable nurseries identified for each species (in one or two seasons, depending on the species) are outlined.

a variable pattern with a small area of stability (Figure 2c) located along the eastern edge of the Adventure Bank. The recruitment component in the LFDs of the deep-water rose shrimp was always high, especially in spring. The cut-off length varied between 14 and 24 mm carapace length (CL) in spring (average cut-off = 18 mm CL) and between 16 and 20 mm CL in autumn (average cut-off = 17 mm CL) (Table 1). Recruits were mainly present on the outer shelf /upper slope, with the main peak between 150 and 300 m depth. The distribution maps of recruits revealed the existence of several areas of recruit concentrations. The main area was localized along the 200 m isobath in the waters off the central coast of Sicily and it was persistent in both spring and autumn, therefore it is an important nursery of the deep-water rose shrimp in the Strait of Sicily (Figure 2d). Other areas were identified with some degree of annual variability on the eastern and western sides of the Adventure Bank. Recruitment of the greater forkbeard takes place in the spring [18]. The cut-off length

Species	Survey	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	Average
MerlMer	MEDITS	112	137	89	125	114	128	119	89	122	119	113	115
	GRUND	119*	151*	95	140	107		108	137	104	120	131	118
Mullbar	GRUND	97	83	80	81	107		104	85	76	85	90	89
Phyible	MEDITS	156	129	144	141	157	131	148	130	168	153	139	145
Papelon	MEDITS	24	20	17	16	18	14	15	17	20	18	15*	18
	GRUND	11*	17	16	19	19		16	16	18	17	20	17
Arisfol	MEDITS	28	26	27	27	31	28	28	25	30	33*	29	28
Neprnor	MEDITS	27	21	32	26	27	28	24	23	30	33	32	27
	GRUND	29	16	23	26	31		21	21	21	16	24	23
Eledcir	MEDITS	-	31	29	28	28	25	32	29	39	42	40	32

Table 1: Cut-off length (mm) of each species for each year/season. When splitting of the LFD components was difficult (marked by an asterisk), the estimated value was replaced with the average of the cut-off lengths of the corresponding series.

ranged between 129 and 168 mm TL with a mean value equal to 145 mm TL (Table 1). The recruits were present in the epibathial grounds between 200 and 500 m and showed a concentration peak between 400 and 500 m depth. The spatial distribution of greater forkbeard recruits showed a very stable pattern over time. Two persistent nurseries were identified in the western and eastern margins of the Adventure Bank at depths between 200 and 400 m (Figure 2e). The LFDs of the Norway lobster allowed the identification of recruits in both spring and autumn surveys. The mean cut-off length was equal to 27 mm CL in spring and 23 mm CL in autumn (Table 1). Recruits were distributed on the upper slope between 250 and 500 m depth, with a peak of abundance between 400 and 500 m. The density maps of Norway lobster recruits showed a well defined spatial pattern in both seasons with the presence of two stable hot spots . They were located west and east of the Adventure Bank between 200 and 400 m depth (Figure 2f), and they overlapped the nurseries of the greater forkbeard. The recruitment of the giant red shrimp takes place in spring [18], allowing the easy identification of the first modal component in the LFDs of spring surveys. The mean cut-off length was 28 mm CL (Table 1). Recruits were widely distributed over the bathyal grounds (500-700 m) of the central area of the Strait of Sicily. The analysis of persistence, only performed on five years, showed the presence of two areas of stability (Figure 2g).

5 Discussion

The nurseries of a pool of species with significant commercial value in the Strait of Sicily were identified in this paper. The analysis was performed by following three-steps: 1) estimating the juvenile fraction of the population (i.e. recruit density indices); 2) mapping the yearly recruit density index and delineating the areas showing the highest concentrations of juveniles (i.e. recruit

density hot spots); 3) quantifying the persistence over space and time of the recruit density hot spots (i.e. persistent nursery).
Analysis of the decadal time series data from trawl surveys revealed the occurrence of very stable nurseries in the Strait of Sicily for European hake, deep-water rose shrimp, greater forkbeard and Norway lobster. The distribution of red mullet recruits resulted in a degree of spread across space and variable throughout time. Whilst a small area of stability was identified on the south-western coast of Sicily, there were also several sites along the whole southern Sicilian coast where local densities of recruits varied substantially from year to year. Finally, small persistent nurseries were identified for horned octopus and giant red shrimp even if their stability was only evaluated for a few years.
Recently, many studies have highlighted the role of quite stationary mesoscale hydrological features of the Strait of Sicily in controlling abundance, spawning, eggs/larval dispersal and recruitment processes of different demersal and pelagic species [19, 20, 21, 22, 23, 24]. In the Strait of Sicily, cyclonic vortices and thermal fronts were considered responsible for the temporal persistence of nursery grounds of European hake and greater forkbeard [10, 25]. According to [12], young-of-the-year and mature females of deep water rose-shrimp in the Strait of Sicily aggregate where retention and enrichment processes occur. Most of the nurseries identified in this study, are located along the external western and eastern edges of the Adventure Bank. The topography of the Adventure Bank plays an important role in the pattern of currents in the area, particularly of the Atlantic Ionian Stream (AIS), a fresh water surface current of Atlantic origin [26]. It enters the Strait of Sicily west of the Adventure Bank and meanders eastward along the southern coast of Sicily. Its interaction with the complex topography and the dominant northwesterly winds in the area generates a number of semi-permanent features such as upwellings, eddies and fronts [26], which enhance and concentrate marine productivity [27]. This could ultimately favour reproduction and recruitment of marine species and could play a key role in maintaining the spatial structure and the stock unity of local populations. Identifying fish nursery grounds, highlighting the role of oceanographic features in the dynamic and connectivity between spawning and nursery grounds is important to understand the ecology of early life history stages of fish and the processes that determine their survival. The supply of juveniles to the adult stocks forms the basis of the sustainability of a population to commercial exploitation and need to be considered in effective fisheries and habitat management strategies. Marine areas of critical importance to the life-cycle of the commercial species need to be managed from a spatial and temporal perspective with such measures as spatial/seasonal closures or Marine Protected Areas. The spatial planning of marine areas becomes even more important in those regions where fishing resources are shared, as in the Straits of Sicily [28, 29]. Indeed, all of the nurseries identified in this work fall in offshore areas beyond the Italian national jurisdiction except for the red mullet nursery which is located in shallow inshore waters. Accordingly, the enforcement of any management actions could be achieved through enhanced cooperation and coordination between relevant national and international fishery management bodies.

6 Acknowledgements

This study was developed in the framework of the Nursery Project funded by the Italian Ministero per le Politiche Agricole e Forestali (MiPAAF). The authors warmly thank all the technical staff of the IAMC-CNR Mazara del Vallo, who collected data during the trawl surveys.

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Potential Yield and Current Exploitation of Deep Water Pink Shrimp (*Parapenaeus longirostris*), Hake (*Merluccius merluccius*) and Giant Red Shrimp (*Aristaeomorpha foliacea*) in the Strait of Sicily

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Abstract

Deep water pink shrimp (*Parapenaeus longirostris*, Lucas 1846), Hake (*Merluccius merluccius*, Linnaeus, 1758) and Giant red shrimp (*Aristaeomorpha foliacea*, Risso 1827) are the main target species of the bottom trawlers operating in the Strait of Sicily. This paper aims to summarize the results of the studies performed by the IAMC – CNR to evaluate the potential yield and the current exploitation of these main species by using and comparing different assessment approaches. Data used for the analyses derived from both indirect (commercial fisheries) and direct (trawl surveys) monitoring International and National Programs carried out within the frameworks of European Union Common Fisheries Policy. The assessments of *P. longirostris* suggest that the fishery is being exploited the above a level which is believed to be sustainable in the long term. To move the fishing to a more sustainable pattern (F0.1), a decrease of the current fishing mortality ranging between 30% and 45% is recommended. For *M. merluccius*, a long living species sensitive to high fishing pressure, a reduction of current fishing mortality from 45% and 70% is suggested. In the case of *A. foliacea*, the reduction of fishing mortality to reach a more sustainable exploitation ranges from 30% and 65% of the current F.

1 Introduction

The Strait of Sicily appears to be particularly important for demersal fishing, as witnessed by the important fleets operating there, about 450 Italian trawlers in 2010 and the associated yield. The Deep water pink shrimp (*P. longirostris*, herein Pink shrimp), Hake (*M. merluccius*) and Giant red shrimp (*A. foliacea* herein Red shrimp) are main target species of the Sicilian, Tunisian and Maltese trawlers. Considering the fleet based in seven harbours distributed along the southern coast of Sicily, two components can be recognised: small (12<LOA<24m) and large (LOA>24m) size trawlers. The former mainly operates with trips from 1 to 2 days at sea within the Geographical Sub Areas 15 and 16 (according to GFCM classification), yielding

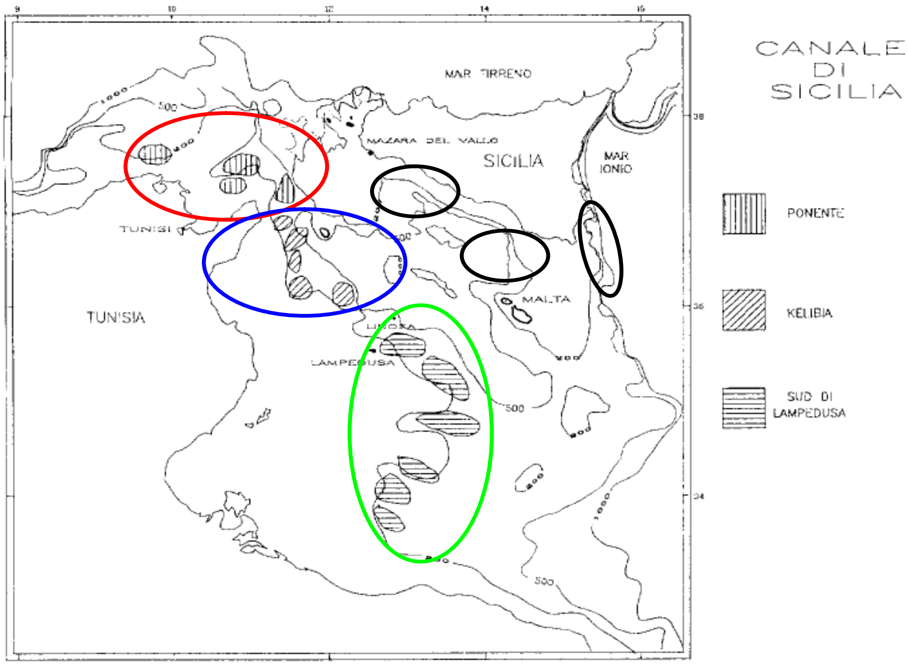

Figure 1: The main fishing areas of pink shrimp in the Strait of Sicily for distant waters (in colour) and coastal (in black) trawlers (modified from Levi et al.,1995).

(IREPA source) about 48% of total Italian landings of the above species (21200 and 17500 tons in 2006 and 2008, respectively). The latter performs longer fishing trips (3 – 4 weeks) in distant waters even outside the Strait of Sicily.
The Pink shrimp is the main target species of the Sicilian trawlers with a yield between 8455 t (2006) and 5941 t (2008) in the last years. This species is caught by bottom trawling both on the continental and upper slope all year round (Figure 1). Hake landing amounts to 1400-1600 t per year. It is caught in a wide depth range (50-700 m) mainly by bottom trawlers (95%) and in lesser extent by small-scale vessels and gill-nets [2].
Red shrimp is fished mainly in summer (1250-1550 t) on the slope of the central – eastern side of the Strait of Sicily (Figure 2). Due to reduction of catch rates since 2004, some trawlers based in Mazara del Vallo have been moving to the eastern Mediterranean (Aegean and Levant Sea) more productive grounds [3].
During the last ten year Sicilian fisheries have shown signs of crisis due, inter alia, to overfishing, fleet overcapacity, heavy dependence to subsidises, low economic resilience, high costs of fishing activities and decline of income of fishermen. This paper presents selected results on stock assessments obtained by IAMC-CNR during the recent meetings of the Mediterranean sub

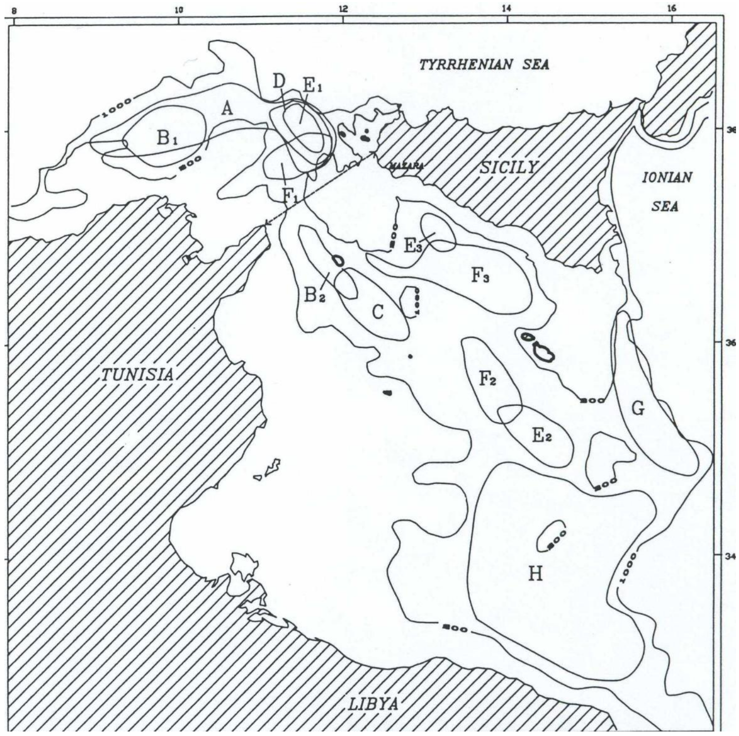

Figure 2: Fishing grounds of red shrimps in the Strait of Sicily (from [1]).

group (SGMED) of the Scientific, Technical and Economic Committee for Fisheries (STECF) of the European Commission, held during the 2008 and 2009 [4, 5]. The presented results mainly deal with the potential yield and the current exploitation of the three main target species of trawl fishery in the Strait of Sicily, derived from yield-per-recruit analyses. The current exploitation is compared with an optimal harvesting scenario within the Biological Reference Points (BRP) framework. We have considered F01, i.e. the fishing mortality rate corresponding to 10% of the slope of the yield-per-recruit curve at its origin as target reference point for optimal sustainable exploitation rate and Fmax, i.e. the fishing mortality rate which maximizes equilibrium yield per recruit as a limit reference point to not be reached in order to reduce the risk of overfishing [6, 7].

2 Material and method

Data and information used in the present paper derives from both indirect (commercial) and direct (experimental surveys) national (GRUND [8]) and international (MEDITS [9]) programs, realised in the Strait of Sicily since 1985 [10]. In particular, the surveys and commercial data gathered from 1994-2008 and 2006-2008, re-

Parameters	*Pink shrimp*	*Red shrimp*	*Hake*
L∞	43	68.9	81.54
k	0.68	0.61	0.15
t0	-0.2	-0.2	-0.08
a	0.0036	0.0013	0.0043
b	2.4423	2.636	3.1525
$L_{50\%}$	21.5	37.2	35.6
g	0.45	0.54	0.29
M	1.04	0.40	0.34
F_t	2.00	0.80	0.75
L'	20.5	n.c.	20

Table 1: Parameters used Legenda: $L\infty$, K and t0 = parameters of the special Von Bertalanfy Growth Function (VBGF), asymptotic (or at infinity) length, Brody (or curvature) coefficient, and the theoretical age at zero length; a and b = coefficient of the allometric length-weight relationship; L50% and g= length at 50% of sexual maturity and the corresponding curvature (steepness) parameter; M and Fter= natural (scalar) and terminal Fishing mortality; L'= length at fully catchability. Lengths are expressed as Carapace Length (CL; mm) and Total Length (TL; cm) in shrimp and hake, respectively. Weight and time units are gram and year, respectively.

spectively, were used to derive the classical curve of yield and spawning stock biomass per recruit (Y/R and SSB/R) in function of fishing mortality (F) by implementing different packages (VIT, [11]; YIELD, [12]). The optimal exploitation was evaluated on the basis of critical values of F, used as biological reference points (BRP).

The current fishing mortality (Fc) was assessed with Virtual Population Analysis (VPA) on length structure of the catches (pseudo-cohort). On the other hand, the MEDITS length frequency distributions (LFD) were used to identify Fc through the total mortality (Z). In the case of Pink shrimp and Hake the estimator of Beverton & Holt and the Length converted catch curve (LCCC routine in the LFDA package [13]) were used, whereas the SURBA 2.20 package [14, 15] was applied for Red shrimp.

For the SURBA application, the LFDs were firstly converted in numbers by age group using the "age slicing" LFDA routine. Then the numbers at age were used to estimate time series of fishing mortality rates, as well as recruitment and SSB (Spawning Stock Biomass) indices. When VIT and SURBA were applied with vectorial natural mortality, the M by size array was obtained using the model PRODBIOM [16].

Since females of all the investigated species show different length structures (largest size), growth-mortality patterns (more sensitivity to fishery pressure) and higher contribute to the landings in weight (60-70 % in Pink and Red shrimps) than males the present analyses only refer to the female fraction of the investigated populations. The main biological parameters used are reported in Table 1.

Exploitation pressure	2006		2007		2008	
	Factor	Y/R	Factor	Y/R	Factor	Y/R
$F_{0.1}$	**0.67**	**2.81**	**0.58**	**2.60**	**0.67**	**2.81**
Fc	1.01	3.04	1.01	2.83	1.01	3.02
F_{max}	**1.80**	**3.14**	**1.32**	**2.86**	**1.58**	**3.09**
F double	2.00	3.14	2.00	2.80	2.00	3.07

Table 2: Main Biological Reference Points in terms of multiplicative factor of the current F (Fc) for pink shrimp according to VIT analyses. The absolute Fc were 1.20 (2006), 1.40 (2007) and 1.11 (2008).

3 Results

3.1 Pink shrimp

The surveys show a fluctuating abundance in time with a decreasing phase (2005 to 2007) (Figure 3), coherent with the corresponding landings data decrease. An example of fishing mortality rates (F) by size and fleet segments of female deep water pink shrimps is shown in Figure 4. It is clear that trawlers operating in coastal water catch shrimp smaller than trawlers fishing in distant waters. An example of estimation of Biomass and Yield per recruit by varying current fishing mortality (Fc) by a multiplicative factor of current F is reported in Figure 5. Assuming no variation in the exploitation pattern, the change in fishing mortalities to reach an exploitation corresponding to F0.1 is reported in Table 2. The current fishing mortality, ranging between 1.11 (2008) and 1.40 (2007) as absolute values, produces about 2.8 – 3.1 g per recruit. A very similar pattern was obtained simulating the likely changes in Y, B and SSB per recruit in function of fishing mortality (F) with the YIELD package. According to the steady state VPA on commercial data, the stock resulted to be overfished. Maintaining the current fishing pattern, a reduction of Fc of 33-42% is advisable to reach F0.1. The light loss in Y/R expected after moving from Fc to F0.1, ranging between 7 and 8% of the current value, is coherent with knowledge of biological parameters (high natural mortality and quick speed to reach maximum length) of the species (Table 2).
Comparing recent Fc (1.34 mean over 2006, 2007 and 2008) based on trawl surveys data (Figure 6) and the F0.1 obtained in Table 3, a decrease of fishing mortality of 38% should be adopted to reach a sustainable exploitation in long term.

3.2 Hake

Biomass indices (sex combined) from trawl surveys by year show an increasing pattern from late nineties to 2005-2006, followed by a slight decrease until 2008 (Figure 7). The pattern of recruitment and SSB estimated by SURBA on MEDITS trawl survey series show a better state of stock abundance in the last years (2005-2007) (Figure 8). Time series of F on females derived from GRUND trawl surveys suggests a quite stable fishing mortality from 1994 to 2008 ranging between 0.66 and 0.90 (mean 0.75 $\pm$0.07) (Figure 9). Fishing

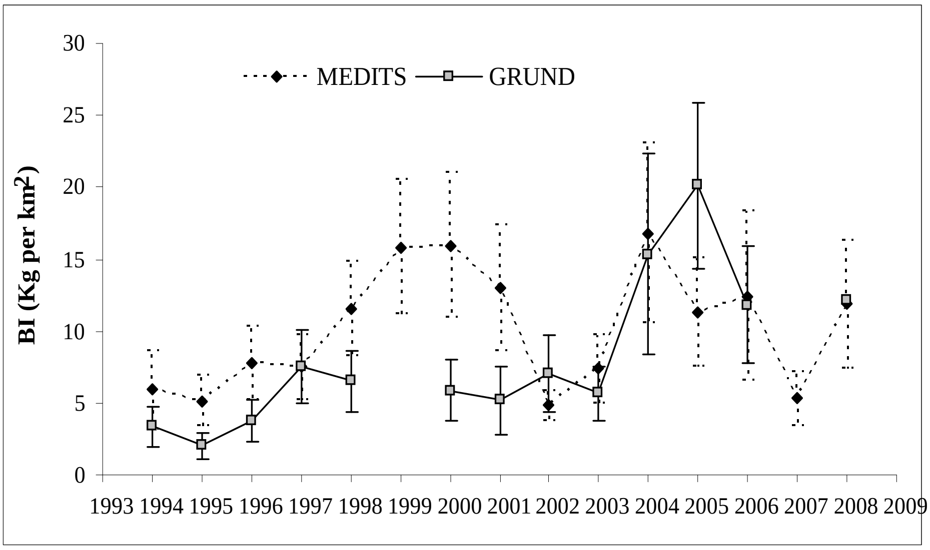

Figure 3: Pink shrimp Biomass Indices (BI; mean ± sd) from surveys in the Strait of Sicily.

mortality rates (F) by size values obtained by VIT on commercial catch show the typical pattern of Hake caught by trawling with the fully exploited size corresponding to size between 20 and 40 cm TL (age group 2-4) (Figure 10). The mean F over 2-4 age class was 0.67, whereas that over the size forming the whole catch was 0.32.

The SSB and YIELD per recruit according to fishing mortality by a multiplicative factor of current F, obtained by VIT (Figure 11), shows a pattern very similar to that obtained simulating the likely changes in Y and SSB per recruit in function of absolute fishing mortality (F) with the YIELD package. According to the steady state VPA on 2006 and 2007 commercial data, an overfishing state is detected. Maintaining the current fishing pattern, a reduction of Fc of 62% is advisable to reach F0.1. The long term gain in Y/R consequent to move from Fc (F= 0.67) to F0.1 (F=0.46) was estimated in 11% of the current value (Table 4). Comparing recent Fc (0.76 mean over 2006-2008) based on trawl surveys data (Figure 11) and the F0.1 (F=0.16) obtained by YIELD package, a more severe decrease of fishing mortality, corresponding to 78%, should be implemented to reach an optimal exploitation in long term (Table 5). The gain in terms of yield should be very high, being about 30% of the current one (from 42 to 57 g per recruit).

3.3 Red shrimp

Biomass indices from trawl surveys show an increasing pattern from 1997 to 2002 (Medits data) and a stable pattern in more recent years (2003-08) (Figure 12). Fishing mortality rates (F) by size obtained by VIT analyses on commercial catches show

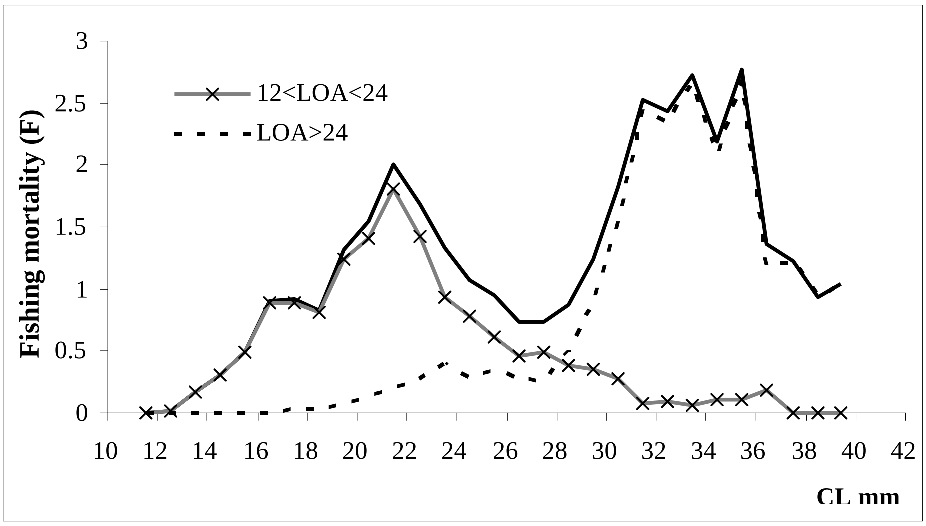

Figure 4: Fishing (F) mortalities rates by size of female pink shrimps in the Strait of Sicily in 2007 according to VIT analyses. F is also distinguished by small trawlers (12<LOA<24m) and trawler (LOA>24m). A vectorial M decreasing with size was used.

that the fully exploited sizes range between 42 and 66 mm CL (Figure 13). The mean F over sizes ranges between 0.73 and 0.81. Assuming no variation in the exploitation pattern, the main results of Y/R analysis in terms of current F (absolute values) and optimal ones are reported in Table 6. Comparing current F with BRP according to that obtained by VIT steady state VPA, an overfishing state was clearly detected.

A very similar pattern was obtained simulating the likely changes in Y and SSB per recruit in function of fishing mortality (F) with the YIELD package. Searching for biological reference points (BRP) through 2000 simulation produced the probability distribution of F0.1 and Fmax (Figure 14). The current fishing mortalities were always above both the limit and the target reference points with the exception of period 1997-1999 (Figure 15). Considering the results of the steady state VPA based on 2006, 2007 and 2008 catches, maintaining the current fishing pattern, a reduction of Fc of 30-65% is advisable to reach F0.1 (Table 6). A minor increase of Y/R moving from Fc to F0.1 was estimated.

Comparing recent Fc (0.75 mean over 2003, 2004 and 2005) based on trawl surveys data and the F0.1=0.40 obtained by YIELD package, a decrease of fishing mortality of 46% should be adopted to reach a more sustainable exploitation in long term. Also in this approach, a little gain in Y/R is expected reducing Fc to F0.1.

4 Discussion

The present results, which summarise some assessments performed by the

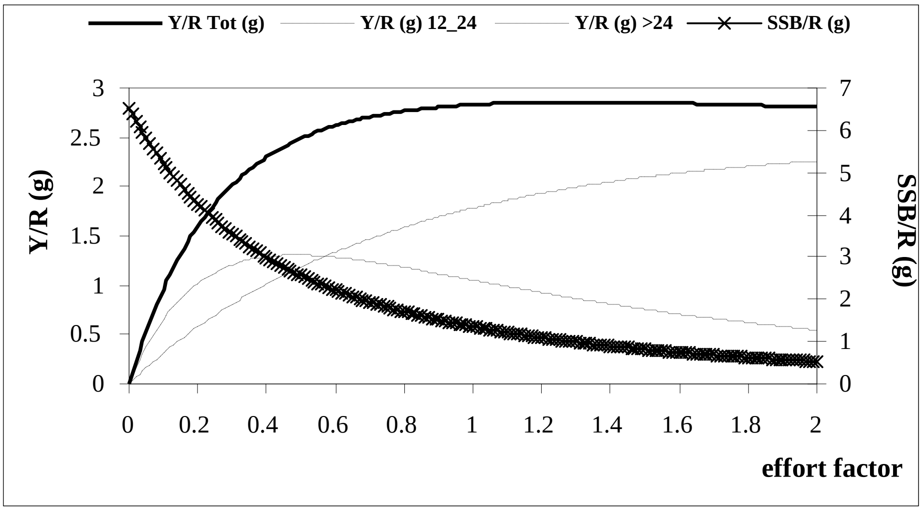

Figure 5: Spawning Stock Biomass (SSB on the left) and Yield (Y on the right) per recruit of pink shrimp varying current fishing mortality (Fc) by a multiplicative effort factor according to VIT analyses. The current fishing mortality corresponds to effort factor being 1. A vectorial M decreasing with size was used.

IAMC-CNR staff of Mazara, highlight an "overexploited status" for three high prised demersal resources of the Strait of Sicily, Awareness of the major importance of seas is growing, as it is the need to preserve and exploit them sustainably. This has been translated into a much stronger emphasis on the ecological sustainability of fisheries worldwide. Although the overexploitation is the more frequent state of fisheries in the developed countries, including the Mediterranean ones [17], it is very difficult to eliminate overfishing given the complexity of interacting economical, social and political constraints raised by the practically open access to the resources. Among the most critical aspects, fleet overcapacity is considered the main factor [18, 19], mainly, because it produces several negative social consequences for fisheries such as a decrease of their economic performance, and stability for industries and fishermen communities, increase of conflicts among fishermen. As a matter of fact, in the recent "Green Paper on Common Fisheries Policy", the European Community has clearly recognised the overcapacity as the main factor at basis of fisheries difficulties [20].

Another source of difficulty in the Mediterranean fisheries context consists in the limited available information concerning the joint biological-environmental response to overexploitation; hampering condition hampers a proper implementation of the so called "Ecosystem Approach to Fisheries", i.e. the use of models that include the ecological processes in the assessment of the whole species assemblage [21, 22].

In such circumstances, the single species

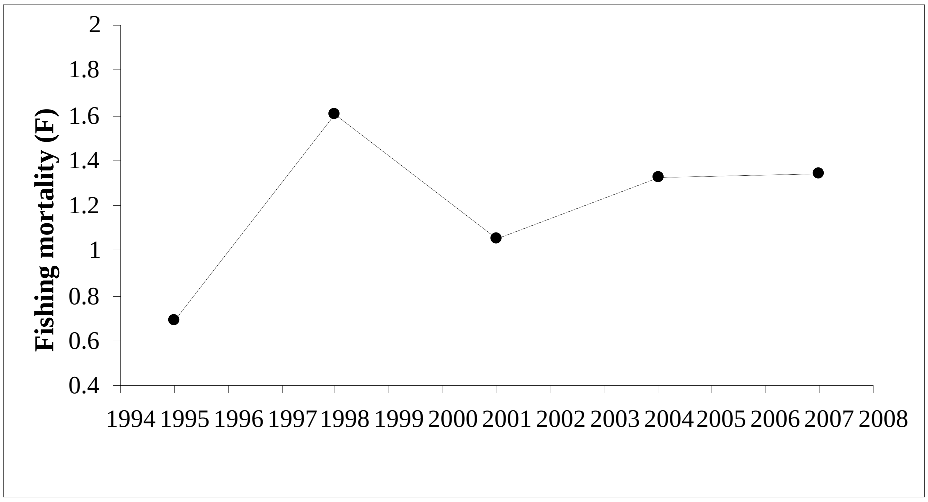

Figure 6: Median of yield and spawning stock biomass per recruit vs. absolute F and corresponding uncertainty of pink shrimp (Yield Package).

assessment, as realised in the present contribute, might represent anyway a suitable path to improve our knowledge about the system and start a recovery plan [23, 24].

Mitigate operationally overfishing on individual target species, for example reducing fishing mortality at F0.1 and increasing the minimum catchable size, could be at least an initial step towards assuring the persistence of exploited stocks.

Contemporarily, it is advisable setting long term management goals. For example, the World Summit on Sustainable Development, held in 2002, identified specific targets for fisheries management, including restoring fish stocks to the Maximum Sustainable Yield (MSY) by 2015. Within a precautionary approach, however, even this objective seems to be not safe enough; in fact, there are strong evidences that both FMSY (the fishing mortality corresponding to MSY) and its proxy Fmax do not necessarily avoid overfishing because the models from which they derive do not account the uncertainty and variability in recruitment [7], which further could also decline even at intermediate spawning stock sizes.

Among the other alternatives considered safer, the F0.1 seems the more attractive as a biologically precautionary target relative to Fmax [23]. It is worth recalling that F0.1 was originally an economic instead of a biological reference point; in fact, it was developed in order to avoid economic difficulties due to overcapacity and overfishing: at F0.1, catch per unit effort is not reduced substantially, but the fishing effort and cost per unit effort are lower than those at Fmax.

It is evident that to balance fishing capacity and mortalities to natural productivity of stock is the main challenge of fisheries in next years. However other aspects must be considered for future sustainable fisheries. Management must involve fishermen and,

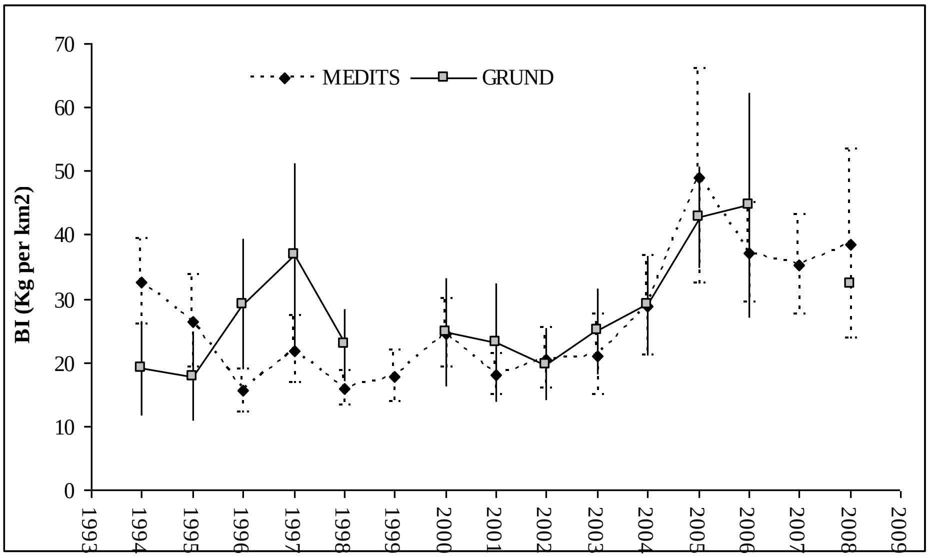

Figure 7: Biomass indices (BI as mean ± sd) of hake from trawl surveys in the Strait of Sicily.

more in general stakeholders. Consumers, processing and retail sectors must require guarantees that the fish they consume and sell originates from well-managed and sustainable fisheries. Fishermen must improve their economic resilience and adapt to changes in the environment and markets. Quality of fishery product, consumer information and the match between supply and demand must be improved in order to increase economic viability for fishermen. Furthermore, technological improvement must not be oriented to increase the fishing power but to raise the gear selectivity and reduce the impact on stocks and marine ecosystems.

Since fish move across national jurisdictions, stocks are often shared among countries. This is the case of shrimps in the Strait of Sicily, where the activity of one fleet has a direct effect on the fishing opportunities of others exploiting the same fish stocks and the same ecosystem. Sustainable fisheries of shared stock, such as Pink and Red shrimps, must be based on international cooperation and on explicit recognition of the absolute need to limit access to resources, under the umbrella of General Fishery Commission for the Mediterranean.

Finally, another crucial aspect to achieve and maintain in the future a sustainable fisheries is the research. Fisheries science is a quite young discipline in Italy, but impressive progress have been reached after the implementation of the first scientific experimental trawl surveys in the middle 80's and CNR was among the pioneers of this new path. It is worth remarking how less than 30 years ago, almost nothing was known about the biological features and state of Mediterranean demersal resources

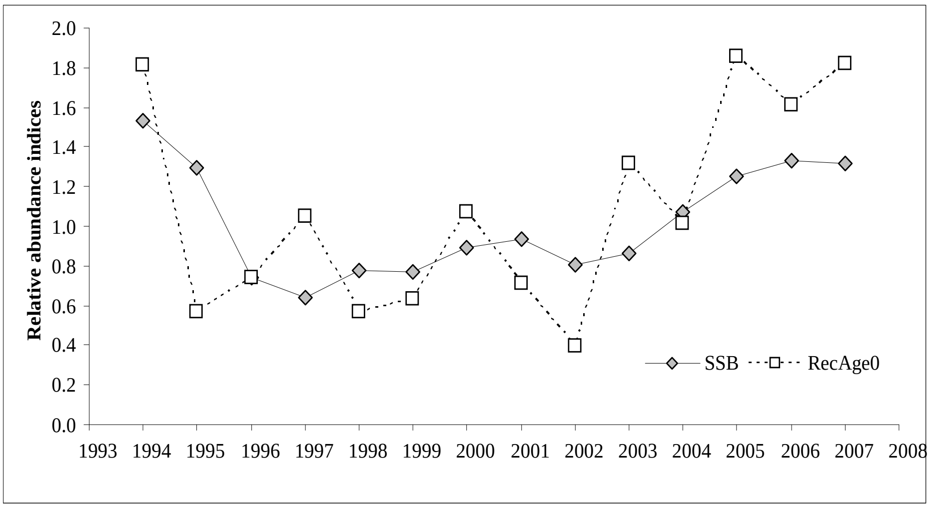

Figure 8: Spawning stock biomass relative indices of hake in the Strait of Sicily (Median values of 1000 bootstrapped runs).

and very few scientists were involved in these studies. Nowadays, there is a ever growing knowledge and more scientists involved in this field, both of them are important pre-requisites to move beyond the single-species paradigm and adopt an ecosystem-based assessment and management approach.

5 Acknowledgements

The authors would like to thank Colleagues involved in stock assessment programs. Data and information of this work derive from national (GRUND) and international programs (MEDITS) and biological sampling of catches now included within the frameworks of EU Data Collection Regulation (DCR).

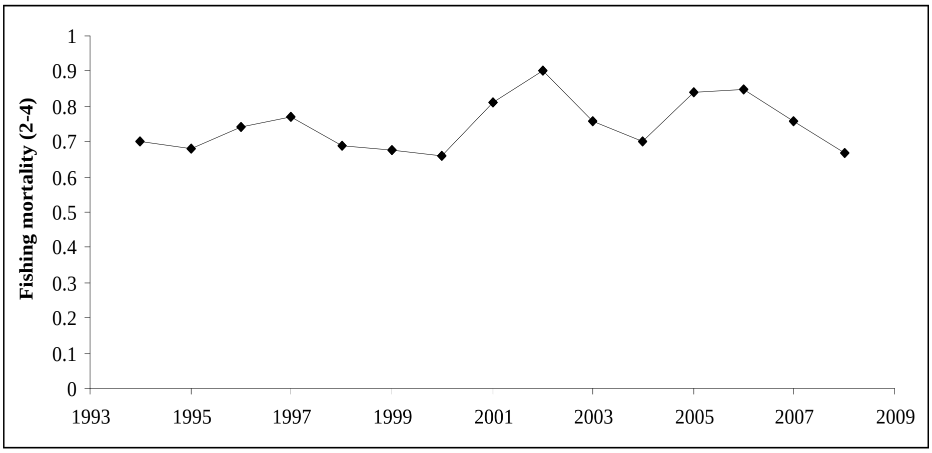

Figure 9: F by time in hake (computed as Z - 0.34). Z was estimated by LCCC. Modified from [2].

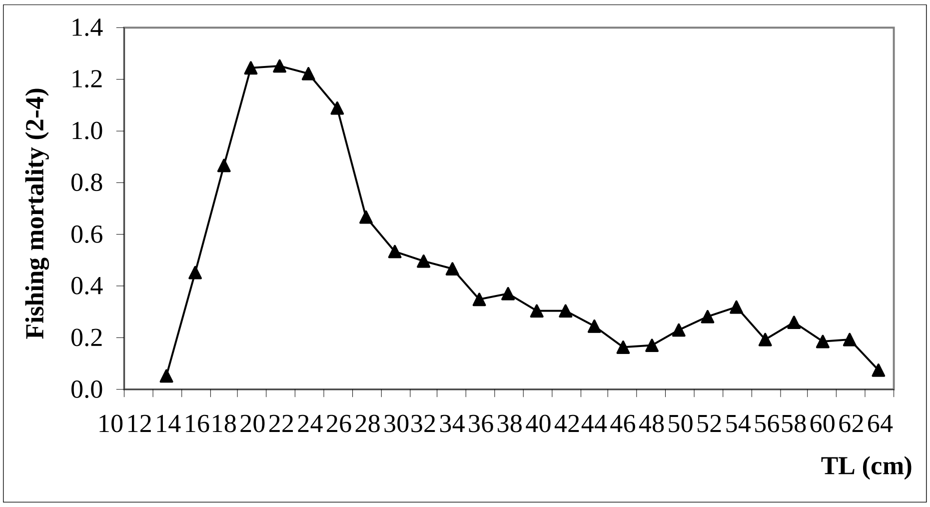

Figure 10: Fishing (F) mortality rates by hake size in the Strait of Sicily in 2006-2007.

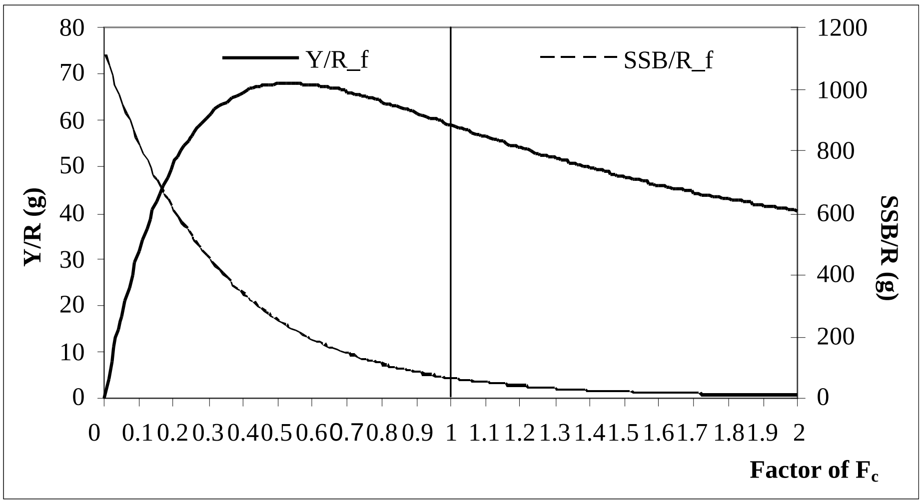

Figure 11: Spawning Stock Biomass (SSB on the left) and Yield (Y on the right) per recruit of hake varying current fishing mortality (Fc) by a multiplicative effort factor according to the VIT package. The current fishing mortality corresponds to effort factor being 1. A vectorial M decreasing with size was used.

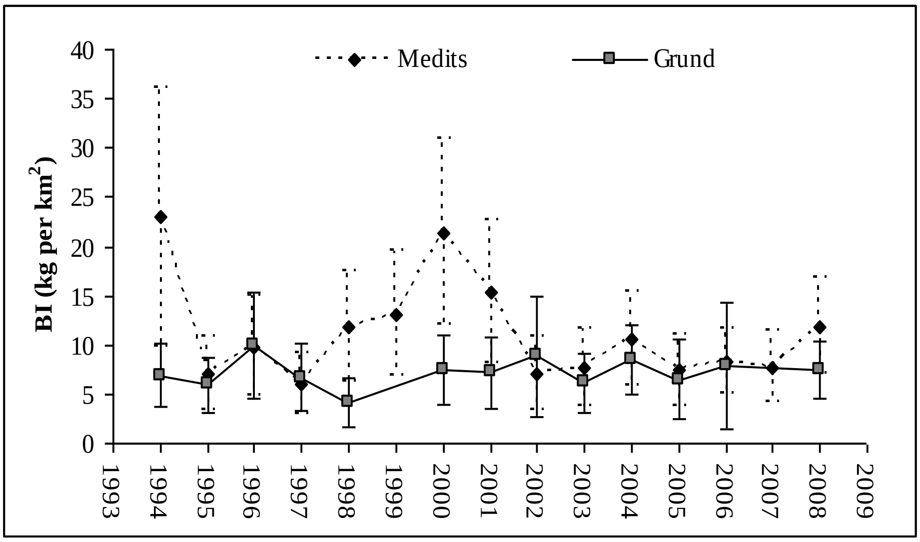

Figure 12: Biomass indices (BI as mean ± sd) of red shrimp from trawl surveys in the Strait of Sicily.

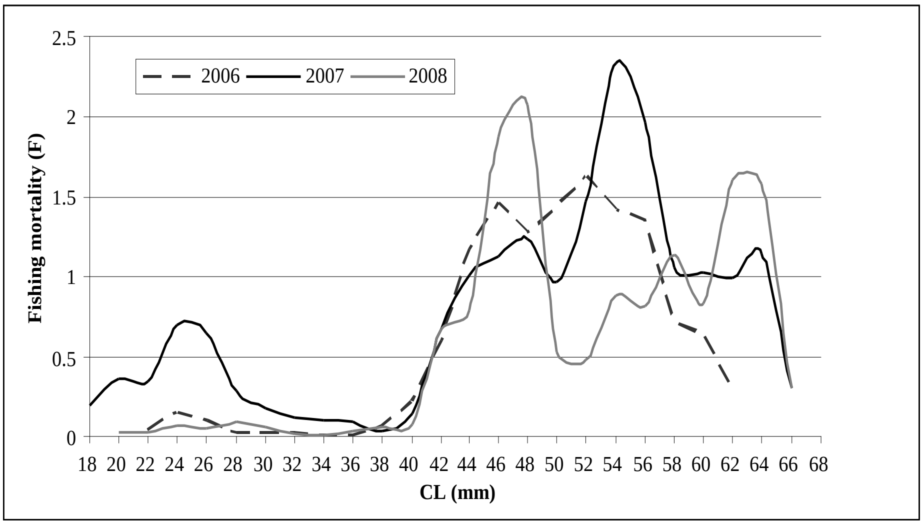

Figure 13: Fishing (F) mortalities rates by size of red shrimp in the Strait of Sicily in 2006, 2007 and 2008 according to VIT analyses. A vectorial M decreasing with size was used.

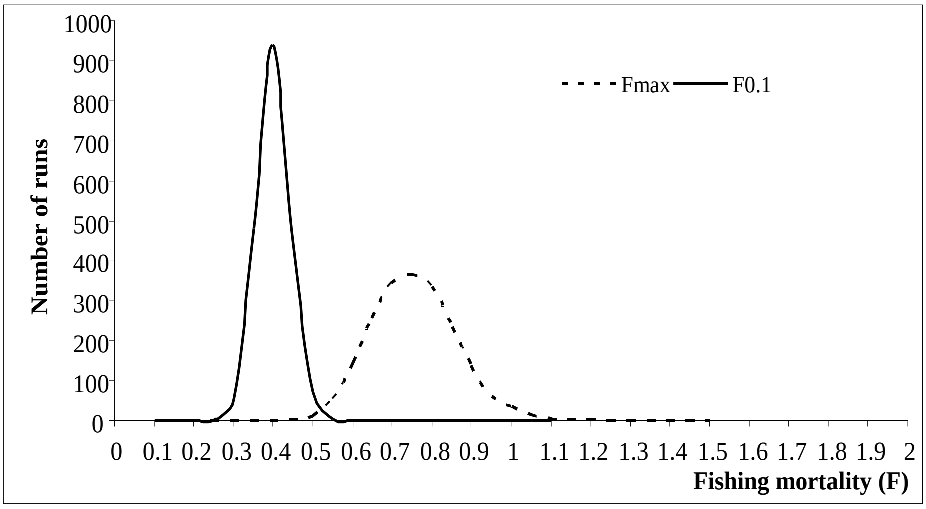

Figure 14: Probability distribution of Fmax and F0.1 of red shrimp according to YIELD package (2000 runs). The median values were F0.1=0.40 and Fmax=0.75.

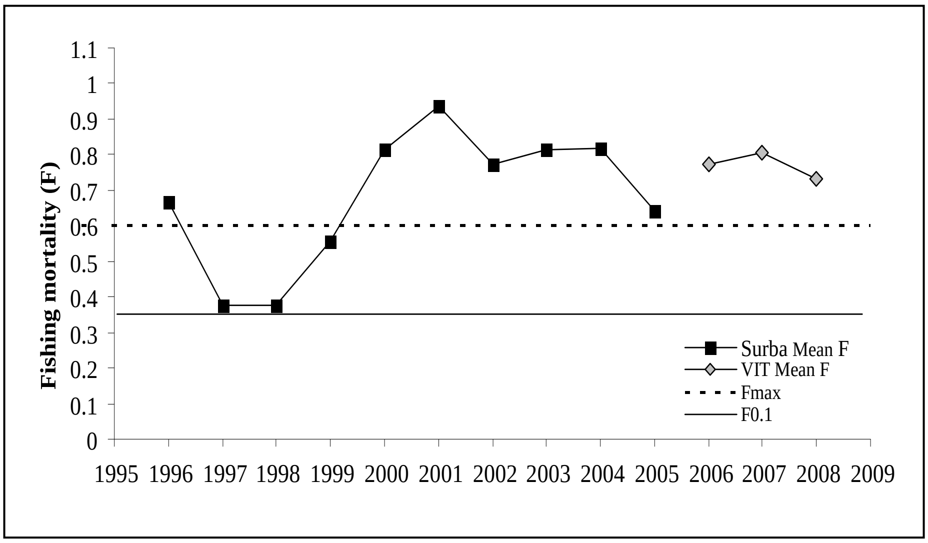

Figure 15: Development of red shrimp F in the Strait of Sicily. The time series was composed by combining SURBA (1996-2005) and VIT (2006-2008) results. The reported BRP were obtained as intermediate values between YIELD and VIT results. Fmax = 0.6 and F0.1 = 0.35 should be considered as the Limit (LRP) and Target (TRP) Reference Point, respectively.

Yield based RP	**values**	**F based RP**	**values**
Y/R_{max}	2.53	F_{max}	1.73
$Y/R_{F0.1}$	2.28	$F_{0.1}$	0.83

Table 3: Median values of yield (g) per recruit and fishing mortality based BRP of pink shrimp in the Strait of Sicily (Yield Package).

Stock state	Factor	Y/R	SSB
Virgin (unexploited)	0	0	1112.60
TRP F0.1	0.38	65.4	353.43
LRP F(Max)	0.54	67.99	228.03
Current exploitation	1.01	58.96	60.64
Doubling effort	2	40.40	4.48

Table 4: Stock state of pink shrimp, as variation from the current effort, in terms of Y and SSB per recruits.

Yield based BRP	female	F based RP	female
Y/R_{max}	59.6	F_{max}	0.244
$Y/R_{F0.1}$	57.4	$F_{0.1}$	0.157

Table 5: Yield (g) per recruit and fishing mortality (absolute values) based BRP of hake in the Strait of Sicily according to the YIELD package. Scalar M was used.

Fishing mortalities	2006	2007	2008
$F_{current}$	**0.774**	0.806	0.733
F_{max}	0.913	0.451	**0.476**
$F_{0.1}$	0.542	0.282	**0.293**

Table 6: Estimation of current F, as Fmean in absolute value, and optimal ones, as Fmax and F0.1, by pseudo cohorts of hake according to VIT package. Median values are reported in bold.

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Fine Structure of Spermatozoa of Three Sparid Fish: Common pandora, *Pagellus erythrinus*, Gilt-head Sea Bream *Sparus aurata* and Blackspot Sea Bream *Pagellus bogaraveo*

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Abstract

This study investigates the fine sperm structure of Sparids *Pagellus erythrinus*, *Sparus aurata* and *Pagellus bogaraveo*. Spermatozoa are differentiated into an acrosome-less head, a short midpiece and a long tail. The head is spherical in *P. erythrinus* and *S. aurata* but ovoidal in *P. bogaraveo*. In all species the nucleus reveals a deep invagination, in which the centriolar complex is located, and a satellite nuclear notch shaped like a club in *P. erythrinus* and *P. bogaraveo* and like a bell in *S. aurata*. Inside the nuclear notch occurs some electron-dense material in *S. aurata* is visible a cross-striated cylindrical body.

The two centrioles show a ”9+0” pattern and are oriented perpendicularly to each other in *S. aurata* and *P. bogaraveo* but not in *P. erythrinus*. The centrioles are linked to each other, as well as to the nuclear envelope, by electron-dense material. The distal centriole is linked to the nuclear envelope by means of a lateral plate and radial fibres in *S. aurata* and by means of basal feet, radial fibres and necklace in *P. bogaraveo*.

In all species the midpiece houses one mitochondrion. The flagellum contains the “9+2” axoneme, is perpendicularly to the nucleus and shows an unpaired fin in *P. erythrinus* but none in *S. aurata* and *P. bogaraveo*.

Knowledges obtained on sperm morphology may be an useful tool in aquaculture providing important references for evaluation of possible cell damages consequent to either cryopreservation procedures or exposure to contaminants.

1 Introduction

Sparids (porgies or sea breams) is one of the largest Percoidei families, predominantly marine and distributed in the Atlantic, Indian and Pacific oceans [1].

The family includes six subfamilies (Boopsinae, Denticinae, Diplodinae, Pagellinae, Pagrinae and Sparinae) [2] and 33 genera with about 115 species [1].

Most members of this family are economically valuable, attaining high market prices. For this reason, a number of them

are object of aquaculture; the most popular in the Mediterranean region is *Sparus aurata*, while other species, such as *Pagellus bogaraveo* and *Pagellus erythrinus*, are currently farmed at a pilot scale [3, 4] because seed production is still a problem. Therefore controlled reproduction, implying the availability of either fresh or thawed semen all around the year, is mandatory for the development of Sparid aquaculture.
In this context, the knowledge of sperm ultrastructure may provide important references for the assessment of the possible cell damage consequent to procedures of semen storage (i.e., cryopreservation). Moreover, ultrastructural features of fish sperm may be phylogenetically analyzed, thus providing important data for the elucidation of relationship patterns among fish groups [5, 6, 7]. Fish sperm shows a great variety in both morphology and ultrastructure, including the number and location of different organelles [8, 9, 10]. Within the family Sparidae, the sperm ultrastructure has been investigated in thirteen species [6] which share some ultrastructural features, but differ in a number of characteristics, such as the number of mitochondria, presence of sidefins and organization of cell organelles.
The present paper provides a short review of the sperm ultrastructural characters of Sparids Common pandora *Pagellus erythrinus* [11], gilthead sea bream *Sparus aurata* [12] and blackspot seabream *Pagellus bogaraveo* with the purpose of increasing data on Sparid sperm morphology, as well as to provide a basic knowledge for future cryopreservation studies.

2 Research methods

Sperm ultrastructure of Sparid fish has been investigated by means of scanning (SEM) and transmission (TEM) electron microscopy at the Department of Morphology, Biochemistry, Physiology and Animal Productions of Faculty of Veterinary Medicine (University of Messina).
Samples for research has been collected from fish held in captivity at the aquaculture experimental plant of the Institute for the Coastal Marine Environment (Territorial Unit of Messina, Sicily) of the National Research Council (IAMC-CNR).
Semen samples from adult male, *P. erythrinus* (total body length = $33.6 \pm 1.9 cm$, body weight = $768 \pm 29 g, n = 10$) *S. aurata* (total body length = $30.5 \pm 1.2 cm$, body weight = $620 \pm 15 g, n = 10$) and *P. bogaraveo* (total body length = 30.2 $\pm$ 1.6 cm, body weight = $650 \pm 15 g, n = 10$) were collected at the peak of spawning season, two weeks after the beginning of spermiation. Fish were anesthetized with MS-222 (0.1g/l), urine was extruded by gently squeezing the fish near the genital pore, faeces were carefully discarded, and the genital area dried. Milt was stripped from running males by gentle abdominal massage and collected in glass tubes.
Samples were fixed in 0.1M cacodylate buffer (pH 7.5) containing 4.5% paraformaldehyde, 2.2% glutaraldehyde and 5% sucrose for 2 h in ice bath, postfixed in 1% osmium tetroxide in 0.1M cacodylate buffer with 5% sucrose for 1 h in ice bath and centrifugated at 900 g for 10 min. The sperm samples were then processed for TEM or SEM. For TEM, sperm pellets were encapsulated in agar ([13]), dehydrated in an ethanol series and embedded in Araldite. Ultrathin sections were cut using an ultramicrotome (Ultracut-E,

Reichert-Jung), stained with uranyl acetate and lead citrate, and were examined under a Jeol Jem 100SX transmission electron microscope. For SEM, sperm pellets were glued on poly-L-lysine-coated coverslips [14]. After dehydration through an ascending ethanol series, samples were critical point dried using liquid argon (Balzers CPD 030), coated with 20 nm gold-palladium in an SCD050 sputter coater (BAL-TEC) and examined under a Cambridge Stereoscan 240 SEM operating at 20 kV.

3 Principal results

3.1 The *Pagellus erythrinus* sperm cell

The spermatozoon of *P. erythrinus* is a uniflagellated cell differentiated into a head and tail with a short midpiece and a flagellum. The head is spherical and no acrosome is observed (Figure 1a and b). The head is occupied almost totally by the nucleus. The nuclear chromatin appears finely granular and homogeneously dispersed. At the base of the nucleus, the nuclear envelope invaginates, forming a depression called nuclear fossa (Figure 1c and d). The two centrioles, arranged at right angle to each other, show a conventional “9+0” pattern. The distal centriole becomes the basal body from which the flagellum axoneme emerges (Figure 1c and d).

The midpiece is cone-shaped and contains a large, round mitochondrion with tubular cristae (Figure 1a and d). At the midpiece level, the axoneme is separated from the plasmatic membrane by a narrow cytoplasmatic canal which is formed by an invagination of the membrane itself (Figure 1d). The flagellum has a typical eukaryotic organization (”9+2” pattern, Figure 1c). The flagellum is inserted medio-laterally into the nuclear fossa and perpendicularly to the base of the nucleus (Figure 1d). It has a cylindrical shape throughout its length and shows one unpaired sidefin, which represents an evagination of the plasmalemma (Figure 1a).

3.2 The *Sparus aurata* sperm cell

The spermatozoon of *S. aurata* is a uniflagellated cell, differentiated into an acrosome-less head, a short midpiece and a long cylindrical tail (Figure 2a and b). The head is rounded and contains a nucleus with condensed granular chromatin. At the base of the nucleus, the nuclear envelope invaginates, forming a depression called the nuclear fossa. The centriolar complex is formed by the proximal and distal centriole that are located inside the nuclear fossa (Figure 2c). The two centrioles are approximately perpendicular to each other, although not in the same axis, and showed a conventional “9+0” microtubular triplet pattern. The proximal centriole is associated with a cross-striated cylindrical body, surrounded by an electron-dense material, lying inside a peculiar satellite nuclear notch, which appears as a narrow invagination of the nuclear fossa (Figure 2c and d). The two centrioles are linked to each other, as well as to the nuclear envelope, by an electron-dense material organized into diverse structures. A disk of electron-dense material is located between the two centrioles (Figure 2c). Moreover, the lateral surface of the distal centriole is attached to the nuclear envelope by means of a lateral plate and radial fibers made of an electron-dense material (Figure 2d).

The midpiece is short and irregularly-shaped (Figure 2b). One mitochondrion could be seen in the midpiece region, containing an electron-dense matrix and irregularly arranged tubular cristae. At the midpiece level, the axoneme is separated from the plasma membrane by a narrow cytoplasmatic canal which is formed by an invagination of the membrane itself. Vesicles and inclusions are irregularly distributed inside the cytoplasm (Figure 2e).

The axoneme has a typical eukaryotic organization, consisting of nine outer doublet microtubules and a central pair of singlet microtubules ("9+2"pattern) (Figure 2f). The doublets are connected to each other by microfilaments and radial spokes. The flagellum has a cylindrical shape throughout its length and is inserted perpendicularly to the base of the nucleus.

3.3 The *Pagellus bogaraveo* sperm cell

The spermatozoon of *P. bogaraveo* is a uniflagellate cell, differentiated into an acrosome-less head, a short midpiece, and a long cylindrical tail (Figure 3a and b).

The head is ovoid with the lateral axis greater than longitudinal axis. The nucleus shows a homogeneous, electron-dense chromatin compact in texture (Figure 3b).

At the base of the nucleus, the nuclear envelope invaginates, forming a deep depression, called the nuclear fossa, containing the centriolar complex. The nuclear fossa appears deep and bell-shaped in a sagittal longitudinal section. It appears smaller at the apical end and enlarging posteriorly (Figure 3c). The anterior region of the nuclear fossa is occupied by the proximal centriole, whereas the distal centriole (which serves as the basal body of the flagellum) is located in the posterior region.

A peculiar satellite nuclear notch shaped like a golf club is visible in cross-section, originating from the nuclear fossa, at the level of the distal centriole; some electron-dense material occurs inside the notch (Figure 3d and f).

The two centrioles are hollow, cylindrical structures with the conventional "9 + 0" microtubular pattern, lie in the same axis and are oriented perpendicularly to each other (Figures 3c and 4a).

The proximal centriole is connected by osmiophilic filaments (Figure 3a and b) to an osmiophilic ring surrounding the anterior end of the distal centriole. Two conical shaped basal feet are visible at the apical level of the distal centriole connecting it to the nuclear envelope; at the posterior end, the distal centriole is traversed by a basal plate (Figure 3b).

Radial alar sheets project peripherally from the distal basal plate (Figures 3e and 4b), connecting the caudal part of the centriole to the plasma membrane. Alar sheets are clearly visible, in a cross section, with the distal centriole resembling a cartwheel (Figure 3e). Below the basal plate electron-dense particles, termed the necklace, are leaned against the membrane (Figure 3b). The neck of flagellum cross sectioned, through the necklace region, shows Y-shaped bridges, a champagne glass-like structure, consisting in a short stem and a cup linking the doublets to plasma membrane (Figure 3e).

The midpiece is short and cylindrically-shaped (Figure 3a) and contains numerous electron-clear vesicles in the cytoplasm. One spherical mitochondrion can be seen in the midpiece region (Figure 3a and b). The mitochondrion contains an electron-

dense matrix and has irregularly arranged cristae; it is separated from the axoneme by a narrow cytoplasmic canal, which is formed by an invagination of the plasma membrane. A second membrane is located underneath the invaginated portion of the plasmalemma extending along the cytoplasmic canal (Figure 4b).
The flagellum has a cylindrical shape throughout its length and is inserted perpendicularly and eccentrically with respect to lateral and longitudinal axis of ovoid nucleus (Figure 4b).
The axoneme shows a typical eukaryotic organization, consisting of nine outer doublet microtubules and a central pair of singlet microtubules (”9+2” pattern). Transverse sections at different levels of the flagellum show radial spokes, tubule A and B of outer doublets of microtubules and dynein arms (Figure 4d). Neither intratubular differentiations (ITD, microtubules A e B of axonemal doublets are both hollow) nor lateral extension of the membrane (sidefins) are present in the flagellum.

4 Conclusions and perspective future

This study reports a description of ultrastructural features of spermatozoa of three species of Sparids considered important in the world of aquaculture products.
Within the family Sparidae, sperm ultrastructure has been investigates in all the 6 subfamilies (Boopsinae, Diplodinae, Pagellinae, Pagrinae and Sparinae) except Denticinae [15]. All species examined have a uniflagellate anacrosomal aquasperm, as is typically found in external fertilizing fishes [5] and share some ultrastructural features, such as the location of the centriolar complex inside the nuclear fossa and the perpendicular insertion of the flagellum with respect to the nucleus. However, they differ in some characteristics, such as the number of mitochondria, presence of sidefins and organization of cell organelles, especially as far as the “centriolar stabilization structures” is concerned. Morphological features of centriolar stabilization structures (alar sheet, basal feet, basal plates, etc.) vary considerably in different species and thus may have functional and evolutionary significance. Therefore, ultrastructural spermatozoon characters described, add new informations which could be useful to a better understanding of phylogenetic relationships between Sparidae.
Moreover, the data reported may be an useful tool in technologies applied to aquaculture because provide important references for evaluation of possible cell damages consequent to cryopreservation procedures. Some morfological features, indeed, as well as having an evolutionary significance seem to have a direct relationship with sperm ability to withstand cryopreservation by means of a better control of cryodamage [16].
Finally, ultrastructural studies on sperm morphology can help to understand the spermatic anomalies induced by the presence of environmental pollutants and may represent a valid predictive tool of environmental hazards. Indeed, sperms of fish caught from polluted sites show variables deformations including: changes in head morphology, incomplete chromatin condensation, malformed midpiece, altered axoneme structure [17]. It is believed that the observed alterations may result from metal accumulation and may lead to reproductive impairment with related consequences on the population as a whole [18, 19].

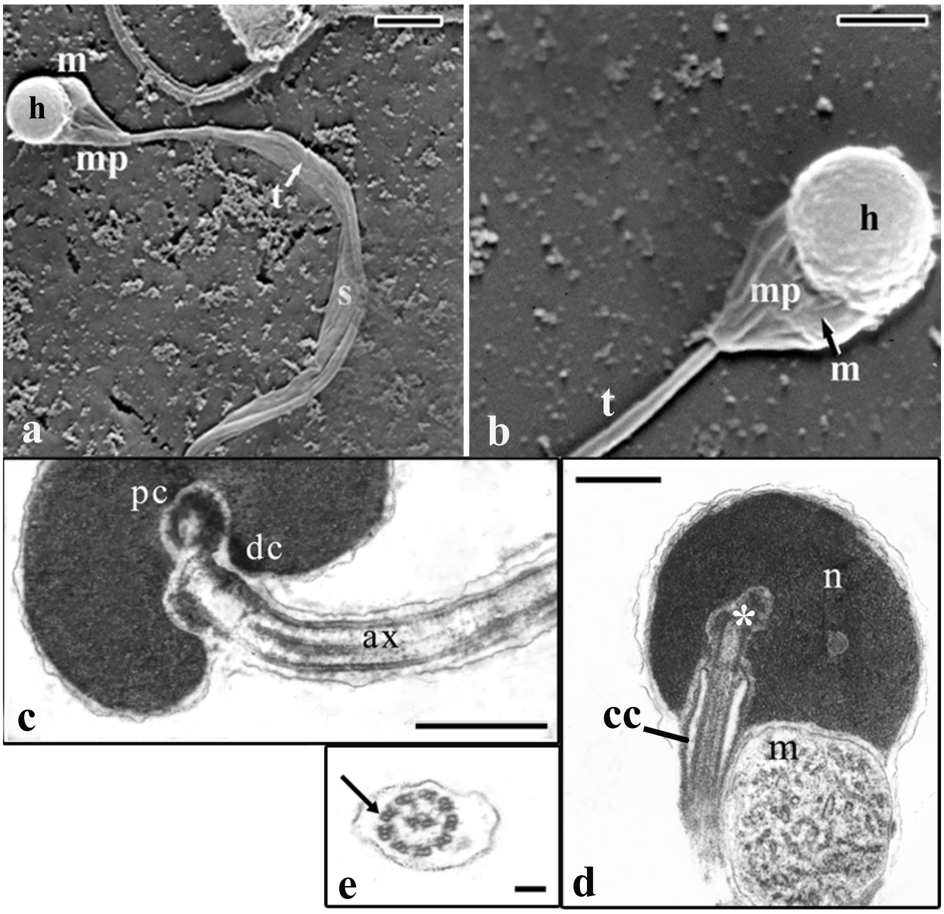

Figure 1: Scanning electron microscopy (SEM) micrographs of the spermatozoon of *Pagellus erythrinus*. a) A general wiew showing the distinction between the head, midpiece and tail. Scale bar: 1 μm. b) High-power view of the spherical head and cone-shaped midpiece. Scale bar: 0.5 μm. (c-e) Transmission electron microscopy (TEM) micrographs. c) Longitudinal section through the centriolar complex. Scale bar: 0.5 μm. d) Longitudinal section through the sperm showing the nuclear fossa (asterisk) and the medio-lateral inserting flagellum. Scale bar: 0.5 μm. e) Cross-section of the flagellum showing the 9+2 axonemal pattern (arrow). Scale bar: 0.1 μm. ax: axoneme; cc: cytoplasmic canal; dc: distal centriole; h: head; m: mitochondrion; mp: midpiece; n: nucleus, pc: proximal centriole; s: sidefin; t tail. Modified from Maricchiolo et al. [11].

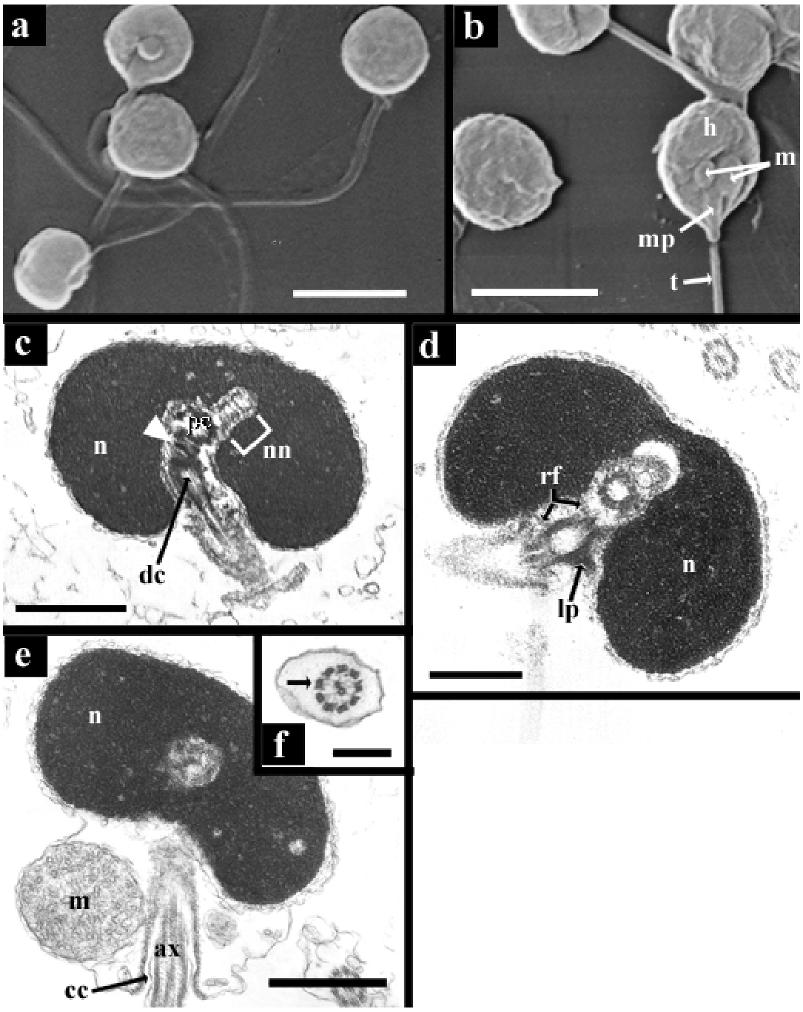

Figure 2: Spermatozoon of *Sparus aurata*. a-b. Scanning electron micrographs (SEM). Scale bars: 2 μm. a. General view of the spermatozoon. b. View of head and midpiece regions showing one mitochondrion. h: head; m: mitochondrion; mp: midpiece; t: tail. c-f. Transmission electron micrographs (TEM). c. Sagittal section through the centriolar complex showing the nuclear fossa containing the proximal and distal centrioles linked to each other by a disk of electron-dense material (arrowhead). The proximal centriole is associated with a cross-striated cylindrical body lying inside a satellite nuclear notch. Scale bar: 0.5 μm. d. Oblique section through the centriolar complex showing the distal centriole linked to the nuclear envelope by electron-dense structures. Scale bar: 0.5 μm. e. Sagittal section at head and midpiece level showing the mitochondrion. Scale bar: 0.5 μm. f. Cross section through the flagellum showing the 9+2 axonemal pattern (arrow). Scale bar: 0.25 μm. ax: axoneme; cc: cytoplasmic canal; dc: distal centriole; m: mitochondrion; n: nucleus; nn: satellite nuclear notch; pc: proximal centriole; rf: radial fibres, lp: lateral plate. Modified from Maricchiolo et al. [12].

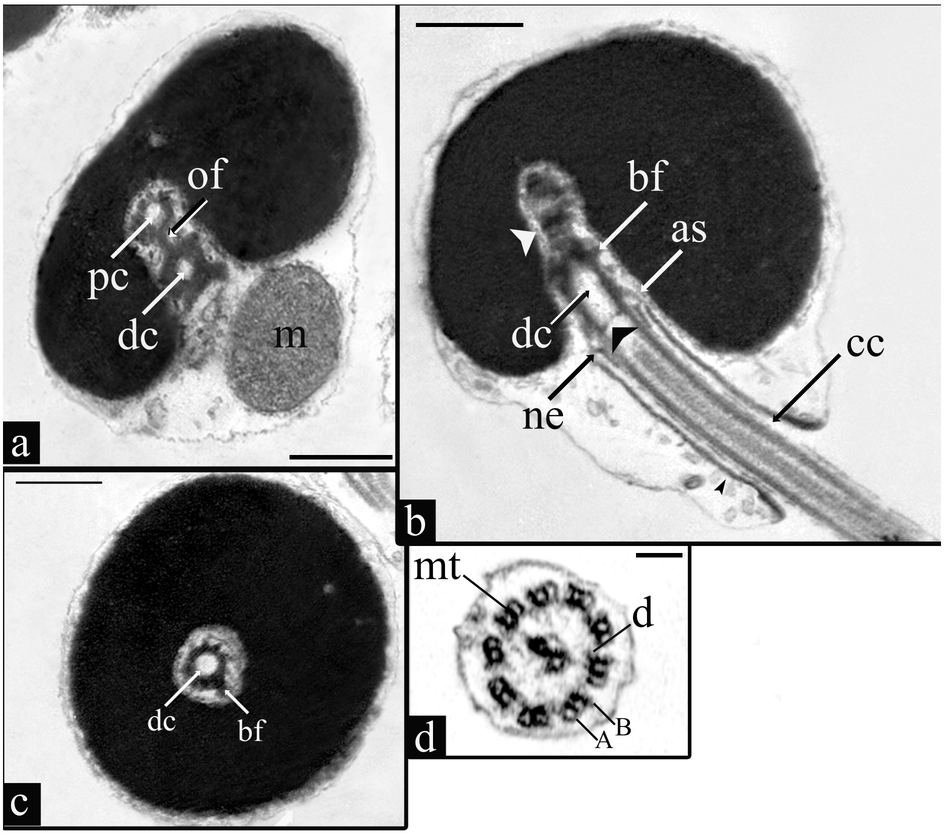

Figure 3: Spermatozoon of *Pagellus bogaraveo*. a. The proximal centriole (pc) is connected by osmiophilic filaments (of) to the distal centriole (dc). m: mitochondrion. Scale bar: 0.5 μm. b. Sagittal longitudinal section through the centriolar complex showing the osmiophilic ring (white arrow) connecting the centrioles to each other and the distal centriole (dc) linked to the nuclear envelope by diverse electron-dense structures. as: alar sheets; bf: basal foot; ne: necklace (black arrow: basal plate; cc: cytoplasmic canal). Scale bar: 0.5 μm. c. Cross section through the distal centriole (dc) showing a basal foot (bf) extending laterally. Scale bar: 0.5 μm. d: Cross section through the flagellum showing the 9+2 axonemal doublet configuration. Microtubules (mt) A and B, radial spokes (s) and dynein arms (d) are visible. Scale bar: 0.1 μm. Modified from Maricchiolo et al. [15].

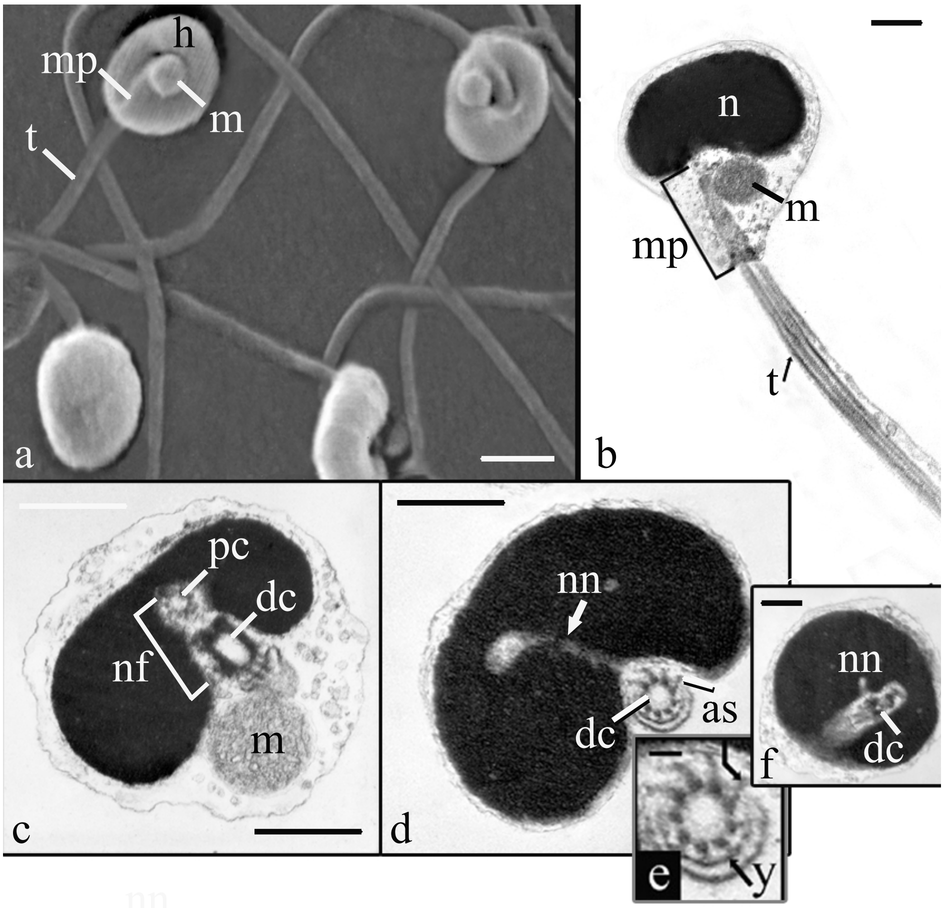

Figure 4: Spermatozoon of *Pagellus bogaraveo*. a. Scanning electron micrograph (SEM) of spermatozoa showing the head (h), midpiece (mp) and tail (t). m: mitochondrion. Scale bar: 1 μm. b–f. Transmission electron micrographs (TEM). b. Sagittal longitudinal section of a spermatozoon showing the ovoid nucleus (n), midpiece (mp) containing one mitochondrion (m), and tail (t). Scale bar: 0.5 μm. c. Sagittal longitudinal section through the centriolar complex showing the bell-shaped nuclear fossa (nf) containing the proximal (pc) and distal (dc) centrioles. m: mitochondrion. Scale bar: 0.5 μm. d. A peculiar satellite nuclear notch (nn) shaped like a golf club is located at the level of the distal centriole (dc). Scale bar: 0.5 μm. e. Cross section through the distal centriole of the flagellum, showing alar sheets (as) and Y-shaped bridges (y) connecting the doublets to the plasma membrane. Scale bar: 0.1 μm. f. The nuclear notch (nn) is located at the level of the distal centriole (dc). Scale bar: 0.5 μm. Modified from Maricchiolo et al. [15].

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Assessment of Small Pelagic Fish Biomass in the Western Adriatic Sea by Means of Acoustic Methodology

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Abstract

Since 1976, ISMAR CNR of Ancona has been conducting acoustic surveys in the north-western Adriatic Sea, with the aim to assess biomass of small pelagic fish, in particular anchovy (*Engraulis encrasicolus*), sardine (*Sardina pilchardus*) and sprat (*Sprattus sprattus*). Since 1987, acoustic surveys were also conducted in the central and southern Adriatic Sea. The sampling limit eastward is constituted by the Mid-Line or the 200 m bathymetry in the southern part. These surveys are carried out annually, in the warm season (summer-early autumn). The main results are the large temporal changes of the pelagic biomass as a whole and per species and the spatial distribution pattern of the pelagic biomass over the years. In recent years specific acoustic surveys have been conducted in this area in order to study the geographic distribution and the abundance of juveniles of small pelagic fish (sardine and anchovy) and improve the knowledge of the acoustic properties of late-larvae stages.

1 Introduction

The Adriatic Sea is one of the most productive systems of the Mediterranean. This high productivity sustains the biggest catches of small pelagic fish (anchovies and sardines) in Italy and one of the most important fisheries in the Mediterranean. However fishery in the Adriatic Sea, as in other very productive areas, have to deal with the problem of the enormous variability both in abundance level and in composition of these resources.

Small pelagic fish biomass varies greatly in space and time. This phenomenon is very important in the ecological and economic contexts and reflects the effect of climatic changes both on macro and micro scale [1, 2, 3, 4]. The most spectacular example of this phenomenon was the collapse of Peruvian "anchoveta" in 1984. Anyway similar situations were observed in the seas north-west and south-west of Africa [5], in California [6], in the Japan Sea [7] and in western Mediterranean Sea (Spain, Algeria, Morocco [8, 9, 10]. It is interesting to notice that these changes in the pelagic biomass happened both in presence (Chile, Peru, Japan, Spain) and in absence (Algeria, Morocco) of a big harvesting of these resources.

Adriatic Sea pelagic populations (mainly anchovies, sardines and sprats) present the classic characteristics of this kind of resources. These populations have been studied for decades by means of acoustic methodology (historical surveys). In this period they showed a series of dramatic

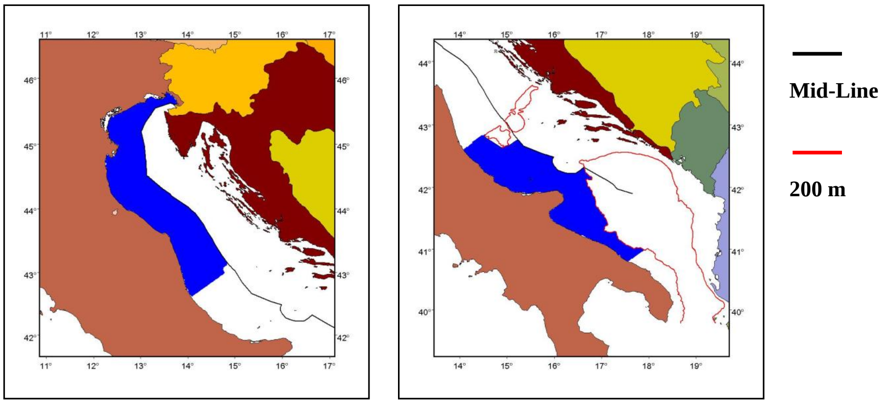

Figure 1: Study area of the acoustic survey performed by CNR ISMAR of Ancona in the Adriatic Sea. On the left north western Adriatic Sea, on the right south western Adriatic Sea.

changes in the abundance, composition and distribution [11, 1, 12]. The historical surveys series in the Northern Adriatic Sea that is the most important area in terms of pelagic fish biomass levels, started in 1976, while in the southern Adriatic Sea it started in 1987.

In this paper temporal fluctuations of anchovy, sardine and sprat biomass in the north-western Adriatic Sea derived from acoustic surveys conducted in 1976-2009 were reported. The main results are the large temporal changes of the pelagic biomass as a whole and per species and the spatial distribution pattern of the pelagic biomass over the years. The sampling limit eastward is constituted by the Mid-Line. This didn't allow to assess all the stock unit of the small pelagic species that is shared in the Adriatic Sea, however this is the main part exploited by the Italian fishery.

Moreover the preliminary results are reported of recent specific acoustic surveys conducted in this area in order to study the geographic distribution and the abundance of juveniles of small pelagic fish (sardine and anchovy) and improve the knowledge of the acoustic properties of late-larvae stages.

2 Materials and Methods

In 1976, the Marine Acoustics Unit of Ancona's CNR ISMAR started to carry out oceanographic surveys in the western side of the Adriatic Sea for the evaluation of small pelagic fish biomass by means of acoustic methodology (Figure 1). The areas covered by the surveys were on average respectively 7705 nm^2 in the north Adriatic Sea and 6493 nm^2 in the south Adriatic Sea, from Italian coast to Mid-line/200 m bathymetry. These surveys were carried out annually, in the warm season.

The biomass assessment and its spatial distribution for all the pelagic species to-

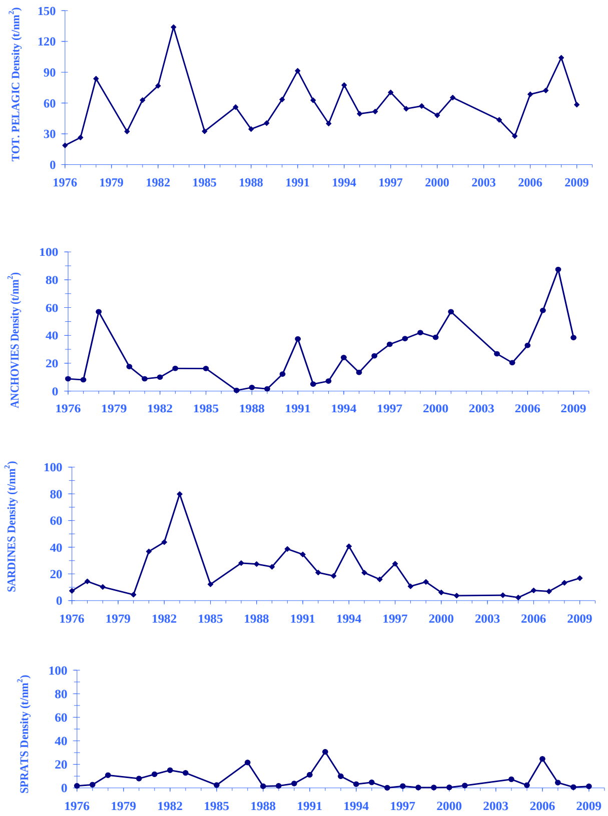

Figure 2: Trends of anchovy, sardine, sprat biomass and total pelagic biomass in north western Adriatic Sea from 1976 to 2009.

gether and per species separately (anchovies, sardines, sprats) was performed using the standard echointegration method [13], implemented by the multifrequency split-beam acoustic technology. Acoustic data were logged along systematic acoustic transects aboard the R/V G. Dallaporta using a SIMRAD EK 500 scientific echosounder working at 38, 120 and 200 kHz. Each year, before the cruise, the hydroacoustic system was calibrated following the standard sphere method [14].

During acoustic surveys net samplings on small pelagic fish have been undertaken extensively by a mid-water trawl (codend nominal mesh size: 18 mm) to determine size and species composition of the acoustically monitored pelagic biomass.

Since 2004 an environmental monitoring was performed during acoustic surveys. Oceanographic parameters were measured throughout the water column at discrete stations regularly distributed along the acoustic transects by means of a SEABIRD

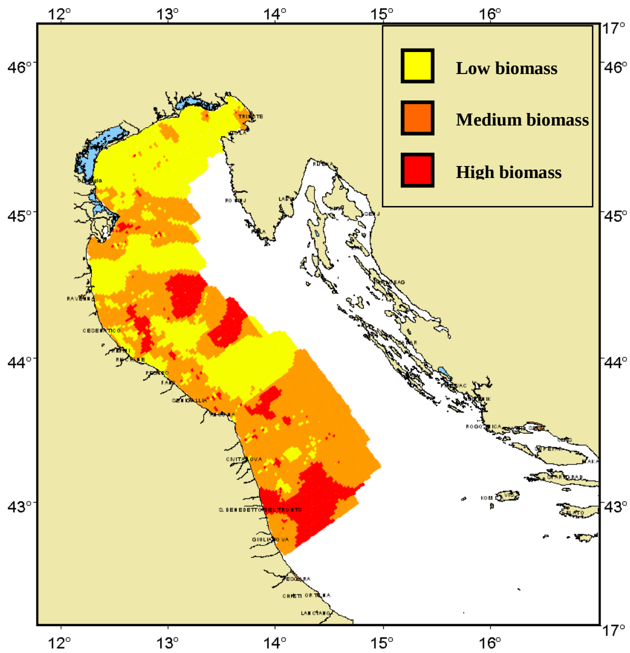

Figure 3: Map of the spatial distribution of total pelagic biomass in north western Adriatic Sea (September 2006).

911 plus CTD probe (temperature, salinity) equipped with a fluorescence sensor. In the framework of the EU project SARDONE, a re-analysis of the acoustic data on small pelagic species, from 2004 to 2008, was carried out in order to identify areas with anchovy and sardine juvenile presence and then to try to identify the most suitable habitats for these animals all over the Mediterranean joining these data with environmental data from satellite and CTD sampling. GAM analysis on the whole dataset is now in progress to find the potential habitats. The presence of juveniles was assigned mile by mile according to the nearest catch outcome and on the base of evidence of aggregations on the echogram that could be attributed to anchovy and sardine juveniles.

Anchovy juveniles are considered the individuals < 11 cm; size decided on the base of specific age-length keys. Sardine juveniles are considered the individuals < 12.5 cm.

During SARDONE acoustic surveys, net samplings were carried out with a midwater trawl capable to catch fish larvae (codend nominal mesh size: 5 mm); in this way it was possible to have synoptical data, acoustic and biologic, concerning anchovy and sardine post-larvae (individuals < 4 cm). The analysis focused on the comparison of data logged at 38, 120 and 200 kHz [15] and also on the comparison at each working frequency of biologic data (measured sizes) converted into Target Strength

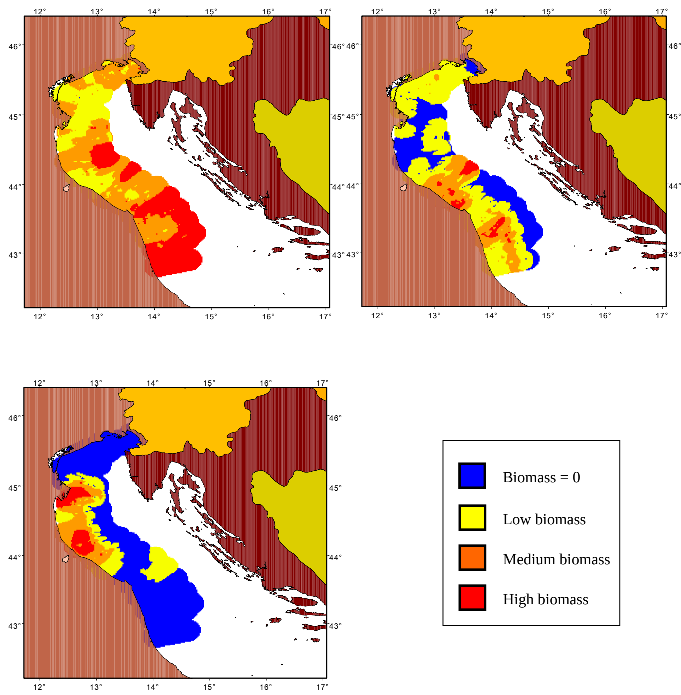

Figure 4: Maps of the spatial distribution of anchovy, sardine and sprat in north western Adriatic Sea (September 2006).

values through the Fish Bladder Resonance Model [16] and acoustic data relative to the water column stratum of the catch.

3 Results

3.1 Trends of anchovy, sardine and sprat biomass in the western side of the Adriatic Sea

The main outcome of the present research is the evident variability in time of pelagic resources both considering the species as a whole and considering species separately. Pelagic community of the Adriatic Sea is dominated by anchovies and sardines that constitute almost 80% of total pelagic biomass.

The trend of total pelagic biomass, derived from echosurveys in the time interval 1976-2009, is shown in Figure 2. The graph indicates that the total pelagic biomass fluctuates in a way which is almost periodical. The highest peaks around 134 $t{\cdot}\mathrm{nm}^{-2}$ and 104 $t{\cdot}\mathrm{nm}^{-2}$, were observed in 1983 and 2008, respectively. In recent years the biomass after decreasing below the mean increased again.

The trend of anchovy, sardine and sprat biomass in the Northern Adriatic Sea from 1976 to 2009 is shown in Figure 2. Anchovy stock has reached a peak in 1978, then, after a strong decrease it showed a slight recovery in the period 1983-85 just before the collapse of 1986-90. The recovery started in 1994 and after a new fall (2004-2005) the stock reached a high peak in 2008 and in 2009 it maintains a good level well above the mean of all the studied period. Anchovy is the species with the highest biomass level in the western side of the Adriatic Sea in these last years.

Sardine stock starting from a very low level of biomass (1976-80) reached the maximum value in 1983; then after a period of normality (1986-95), sardine population diminished and remained stable at very low levels with minor fluctuations. In these last years sardine population shows evidence of a slight recovery.

Sprat stock shows more drastic fluctuations than the other species even if the reference scale is much smaller. These last years are characterized by low levels of abundance apart for the peak of 2006 that was similar to the previous peaks of 1987 and 1992.

The distribution pattern of the total pelagic biomass in 2006 is shown in Figure 3. The high density areas represent 14.6% of the covered area and contain 36.5% of the total biomass. The medium density areas represent 47.7% of the investigated area and contain 50.9% of the total pelagic biomass. Finally the low density areas represent 37.7% of the investigated area and contain only 12.5% of the total biomass. The low density region is of no interest to fishermen. The maps of spatial distribution of the target species were represented in Figure 4. The intervals used for these maps were chosen with the same principle of the ones of total pelagic biomass.

3.2 Preliminary results on the spatial distribution of anchovy and sardine juveniles derived from past surveys (2004-2008)

Figure 5 and 6 show the distribution of anchovies and sardines juveniles in western Adriatic Sea during the historical surveys from 2004 to 2008. In red is marked the presence of anchovy juveniles along the acoustic cruise route and in blue the sardine juveniles presence.

Anchovy juveniles presence, in the study area, resulted mainly concentrated from Po delta southwards until Brindisi even if there's no continuity of distribution over the years. Anchovy juveniles were almost always concentrated near the coast (depth < 50 m).

Sardine juveniles are more sporadic and distributed mainly near Gargano Promontory and Manfredonia Gulf.

3.3 Preliminary results of the study on the acoustic properties of anchovy post-larvae

From a preliminary analysis based on some monospecific hauls for anchovy post-larvae (individuals < 4 cm), the impression is that the acoustic behaviour of these post-larvae is similar to that of adults of the same species, with a significant role of the swim bladder in sound reflection. The results are reported in Figure 7. The 38 kHz echogram shows the aggregations of anchovy post-larvae in correspondence of a monospecific haul.

The histograms show the comparison at each working frequency of biologic data (measured sizes) converted into Target

Strength values through the Fish Bladder Resonance Model [16] and acoustic data relative to the water column stratum of the catch.
Small pelagic fish late-larvae seem to form smaller aggregations than bigger sized individuals.

4 Conclusions

The main results of the acoustic surveys in the western Adriatic Sea show a difference between fluctuations of total pelagic biomass, presenting cycles of about five years, and the irregular variations of single species biomasses (anchovy, sardine and sprat), characterized by peaks and collapses that seem related with climatic events on a micro scale.
Anchovy is the most abundant species in the western side of the Adriatic since the late '90.
The analysis of the historical series of more then three decades puts in evidence that small pelagic populations in the western Adriatic Sea are characterized by remarkable fluctuations in time. Total pelagic biomass fluctuates in an almost regular and periodic way around the mean value due to the alternation in dominance between the species while single species seem to be affected by big and unpredictable variations. This research is limited to the Italian side due to a political agreement among the Adriatic Sea countries, thus it is not possible to obtain a complete picture of the stocks in the entire Adriatic Sea; anyway the eastern side is recently monitored by Croatian scientists and there is now a common effort, inside FAO AdriaMed project, to produce in the future a unique evaluation for all the GSA 17 (Northern and Central Adriatic Sea).
A first attempt was made with the acoustic estimates of both sides since 2004, using relative abundance indices in tuning catch at age analysis (VPA) for anchovy and sardine stock assessment in GSA 17 [17].
For what concerns south Adriatic Sea (GSA 18) since 2002 through AdriaMed project we began to study the eastern side (Montenegro coastal area) with acoustic methodology; in 2008 the study area was extended to Albania making the monitoring activity much more complete according to small pelagic fish distribution. Another basic question concerning small pelagic fish is their structure and spatial distribution that could be even more important for fishery respect to the simple total biomass estimation. Pelagic population biomass tends to concentrate in patchiness, that is to say high density strata containing the main part of the schools present at sea. In these areas fishery could get big catches. Around these areas there are wider zones with mean/low density.
The investigation on anchovy and sardine juveniles showed that in the period of the survey (July-September), at least in the last years, anchovy juveniles are well represented and distributed near the coast (depth < 50 m) mainly from the Po river mouth southwards until Brindisi, while sardine juveniles are quite rare and concentrated in southern Adriatic Sea near Gargano Promontory and Manfredonia Gulf.
The preliminary observations relative to the acoustic properties of anchovy late-larvae have put in evidence that the role of the swim bladder seems not negligible as in the adult individuals; these small organisms tend to form smaller aggregations respect to the adults of the same species.

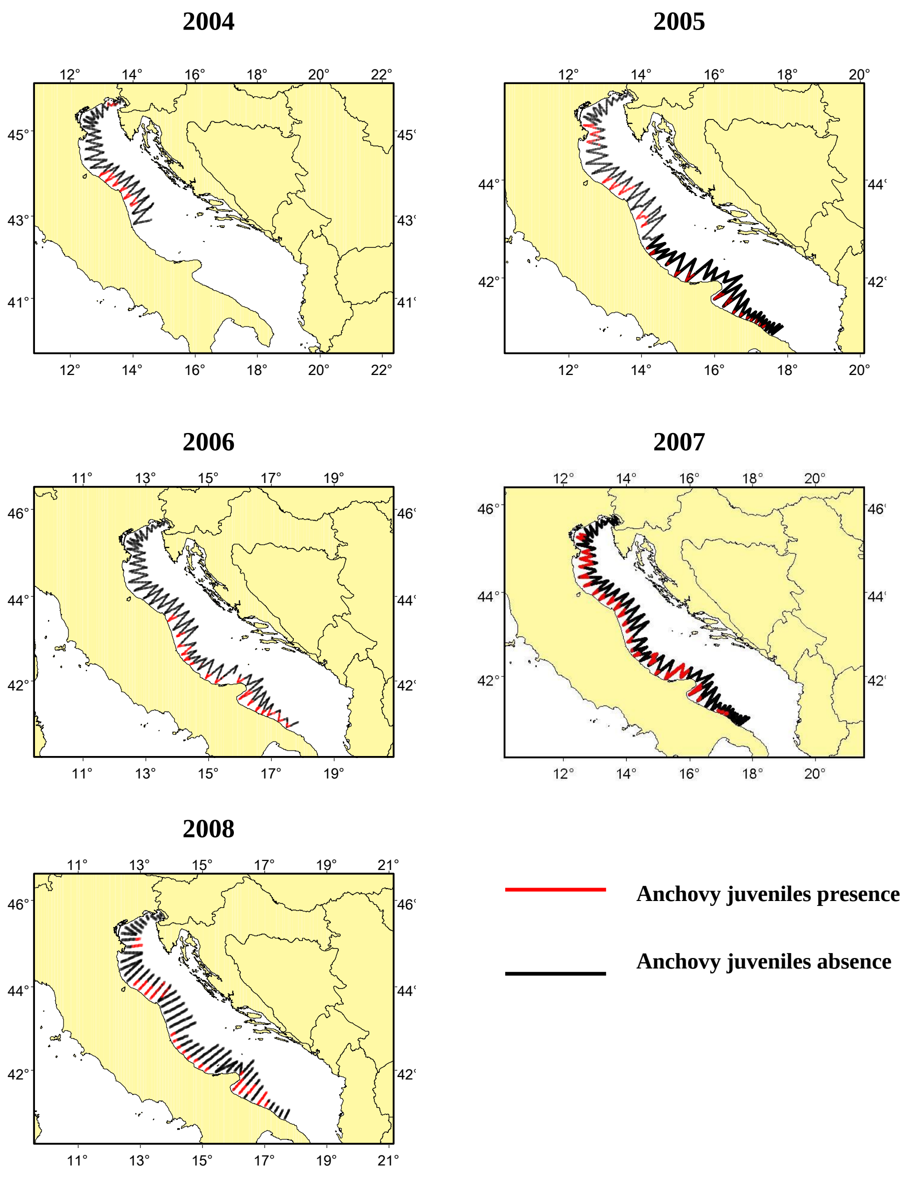

Figure 5: Anchovy juvenile presence along the acoustic transects derived from the historical surveys from 2004 to 2008.

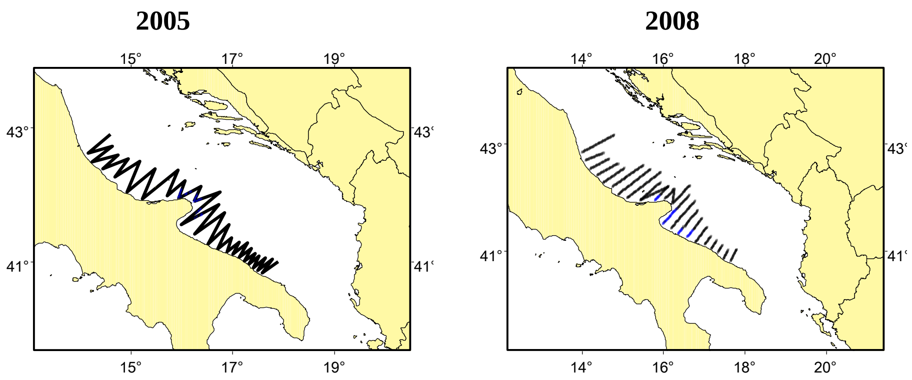

Figure 6: Sardine juvenile presence along the acoustic transects derived from the historical surveys from 2004 to 2008.

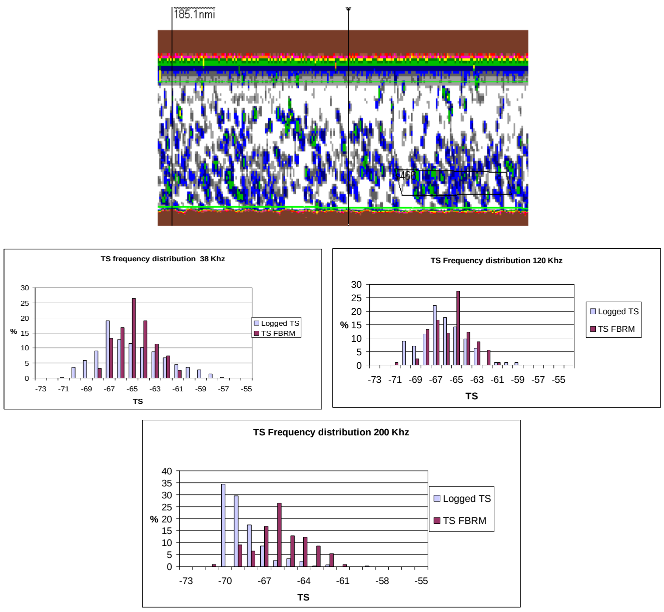

Figure 7: Echogram showing aggregations of anchovy post-larvae in correspondence of a mono specific haul and comparison of logged echoes and measured sizes converted into echoes on caught specimens.

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Growth and Biochemical Composition of Common Octopus (*Octopus vulgaris*) Fed Prey of Low Market Value

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Abstract

Octopus vulgaris is considered a possible candidate for industrial culture, in terms of its biological and market potential: easy adaptation to captivity conditions, high growth rate and high market price.
The aim of this study was to investigate the influence of diet on growth and biochemical composition of cultured *O. vulgaris*. Wild animals used as control were sacrificed immediately after capture, to provide the biochemical composition. The feeding trial lasted 30 days. Three fresh diets were evaluated: Group I (a mixture of crab *Carcinus mediterraneus*, bogue fish *Boops boops* and mussel *Mytilus galloprovincialis*), Group II (monodiet of *B. boops*) group III: (monodiet of *M. galloprovincialis*). Ingestion, growth and feed efficiency indexes of *O. vulgaris*, at the end of the feeding trial were determined. The best results were obtained with the monodiet based on bogue, that gave a weight gain (Wg) of 390.65±37.54 g, an absolute growth rate (AGR) of 13.02 $g \cdot day^{-1}$ and feed efficiency (FE) of 44.79 ± 0.96.
In respect to biochemical composition, the mussel-fed and bogue-fed groups showed the highest protein content. Animals fed on bogue showed the highest carbohydrates content, while the lowest value was observed in the mussels-fed group. The lipid content was found to be very low in all dietary groups. The highest value was obtained from the wild octopuses and the bogue-fed group. All the octopuses were rich in phospholipids.

1 Introduction

Cephalopods represent an important seafood, according to FAO [1, 2], they contribute to approximately 14% of the world fisheries. The market demand throughout different regions of the world and the price for *O. vulgaris* is increasing, due to the excellent palatability, quality attributes of this seafood. Due to some interesting biological characteristics and high market price, *O. vulgaris* represents an ideal candidate for aquaculture diversification [3, 4, 5, 6].

The main characteristics of *O. vulgaris*, which favour its potential for culture, are a short life cycle (12-18 months), high fecundity, rapid growth up to 13% body weight per day [7] and high food conversion [8, 9]. Furthermore, *O. vulgaris* may be easily kept in captivity, showing a rapid and easy adaptation, good acceptability of low-value natural or frozen foods and high survival rates (even 100%). Additional advantages are the social and economic relevance of this species, representing an important seafood for human consumption.

Nevertheless, the rearing techniques from hatchlings (0 day old paralarvae) to the benthic stage are not yet well established, limiting the development of its culture [3, 5, 10]. Therefore, in order to achieve a profitable long term commercial production of *O. vulgaris*, it is necessary to focus the efforts on growth and maintenance of sub-adult wild individuals, for which an adequate feeding development programme is necessary.
Several formulated artificial diets have been tested for feeding sub-adult individuals, but the cephalopod growth rates on those diets have been poor compared with natural diets [11, 12, 13, 14, 15, 16, 17], and this may be due to the lack of palatability of the diets or to their poor nutritional composition. However, some research groups have recently obtained formulas that octopus find acceptable and which produce significant growth [17, 18].
On the other hand the growth and feed efficiency of *O. vulgaris*, fed on natural diets, can vary widely depending on the exact species used as a diet [19, 20].
The aim of this study was to investigate the influence of diet on growth and biochemical profile of *O. vulgaris* fed with natural diets. In order to develop an optimal feeding programme, which could improve nutritional value of cephalopods, these results represent important basic knowledge for the future development of feeding techniques for *O. vulgaris*.

2 Materials and methods

2.1 Experimental rearing of *O. vulgaris*

The feeding trial was conducted at Institute for Coastal Marine Environment-CNR of Taranto, Italy. Octopus juveniles were caught in the coastal waters of Gulf of Taranto (Ionian Sea, Italy) by using trap nets. In the laboratory, each animal was weighed with a professional balance to the nearest g. Before the experiments started, the octopuses were kept together, for 24h, in a tank of 7 m^3 with an open seawater flow (37.5-39.0 $g \cdot L^{-1}$) system.
During the experimental period, the octopuses, weighing 500–650 g, were kept apart both to avoid cannibalistic behaviour and for an exact determination of food intake. For this reason, the octopuses were placed in cages of 1 cm mesh size and 2.5 m^3 capacity, having an open recirculating water system, so that dissolved oxygen was maintained at above 90% saturation.
In order to reproduce its natural habitat, in each tank was placed a PVC tube as refuge. The females were rejected, in order to avoid reproductive processes, such as gonad maturation, which influences the physiological state of the animals. The photoperiod, water temperatures (18±2°C) and salinity (38±2 $g \cdot L^{-1}$) with a diurnal variation were natural.

2.2 Experimental design of feeding treatments

The feeding treatments were conducted in the laboratory during the spring months (2008). Octopuses were divided in three experimental groups, each group consisted of six animals, each animal was kept individually in a cage. Each group represented a different feeding regime, and all diet has been composed by natural preys. The experimental diets were assayed in a feeding trial lasting 30 days.
Diet group I, fed on 80% crab *Carcinus mediterraneus*, 15% bogue fish *Boops*

boops and 5% mussels *Mytilus galloprovincialis*; Diet group II fed exclusively on fish *B. boops* over the whole feeding; Diet group III fed exclusively on mussels *M. galloprovincialis*. All the food furnished to each experimental group referred exclusively to the edible part. The "wild" group used as control (6 animals), was sacrificed immediately after capture, to provide the background of biochemical composition. Octopuses were fed once daily and each day the food was provided ad libitum between h12.00 and h14.00; fish was provided frozen, without tails or heads, and in the case of crabs were served alive and the edible fraction was estimated as 50% including legs. Mussels too were served alive. The feeding rate was 7% of the total weight of the animals in each cage and varied as a function of the animal's weight throughout the experimental period.

The uneaten food was removed from each cage the following day and dried, using adsorbent paper, before being weighed to calculate, by difference, the exact amount of food eaten. All samples of *O. vulgaris* were weighed once a week for adjusting the amount of food provided. Mortality and water quality parameters were recorded daily.

At the end of the experiment all samples were weighed (Wf=final weight in g). The following indexes were determined: Absolute growth rate AGR= (Wf-Wi)/t; Specific growth rate SGR = (LnWf-LnWi)x100/t; Feed efficency FE= (Wf-Wi)x100/IF; Feed conversion rate FCR=IF (Wf-Wi); Absolute feeding rate AFR= IF/t; where Wi= initial weight in g; Wf = final weight in g; t= time in days; IF=ingested food in grams.

2.3 Biochemical composition analysis

2.3.1 Sample preparation

At the end of the 30 days experiment, all octopuses of each feeding type were used for biochemical composition analyses. In particular the arm muscle of all animals were filleted, minced in small pieces and mixed until a homogeneous sample was obtained.

Protein content was measured, according to Bradford [21], in diluted homogenates, using the Bio-Rad protein determination kit (Biorad@-500-0006). Bovine serum albumin was used as a standard. Samples were read at 495 nm. Glycogen from the arm muscle was extracted in the presence of sulfuric acid and phenol [22]. Samples were read at 490 nm.

Total lipid content was determined gravimetrically after the extraction with a solvent mixture of methanol/chloroform/water 1:2:1 (v/v/v) following Folch method [23]. All analyses were made in triplicate for each sample.

Protein, carbohydrates and total lipids were expressed as mg/g of wet weight.

Triacylglycerols (TAG) and total cholesterol (CL) were measured by the colorimetric enzymatic Trinder method [24], using a commercial kit (SGM – Rome -Italy). Phospholipids (PL) were quantified by a colorimetric enzymatic method [25] with a commercial kit (SGM – Rome -Italy). Tryacylglicerols (TAG), phospholipids (PL) and cholesterol levels were expressed as a percentage of total lipids.

All analyses were repeated three times, and the results were expressed as mean values $\pm$ standard deviation (SD).

2.3.2 Data Analysis

Results are presented as mean values ± standard deviation (S.D.). Statistical analyses were performed using the SPSS 11.5 for Windows (SPSS Inc, Chicago, IL, USA). One-way ANOVA at the 5% confidence level, was conducted to test if there were significant differences among the three feeding trials. Normality and homogeneity were verified by Kolmogorov–Smirnov and Bartlett's tests, respectively. Having demonstrated a significant difference between the groups with the ANOVA, the Tukey's test for post-hoc multiple comparisons was applied.

3 Results

3.1 Growth performance

All dietary groups consumed the furnished food, and a survival of 100% was observed in all treatments. In Table 1 were reported mean values ±S.D. for every index of each dietary group.
The maximum weight increase was found in the bogue-based dietary group that gave an absolute growth rate (AGR) of 13.0 $g \cdot day^{-1}$. Octopuses fed the mixed and mussels diets had lower increases in weight. These equated to absolute growth rates of 8.9 and 7.2 $g \cdot day^{-1}$, respectively, although these differences were not significant ($p>0.05$).
The animals fed with the mixed diet showed a better ingestion rate (AFR) with respect to other groups, in a significant way ($p<0.05$).
Groups fed mussel and bogue showed the best feed efficiency. Considering FE, ANOVA showed significant differences amongst all three groups ($p<0.05$), with the best values obtained by octopuses fed mussels followed by those fed bogues.

3.2 Biochemical composition of *O. vulgaris*

Table 2 showed the biochemical composition of samples at the end of experiment. The protein concentration of animal fed the different diets varied from 101.8 ($g \cdot kg^{-1}$) in wild octopuses to 107.2 ($g \cdot kg^{-1}$) in those fed mussels. Statistical analysis showed that wild octopuses and those fed with mixed-diet had a significantly lower protein content than mussel-fed and bogue-fed groups ($p<0.05$). This last one showed the highest carbohydrate content, while the lowest value was observed in mussels-fed group.
The lipid content varied significantly among different feeding trials, ranging from 6.5 $g \cdot kg^{-1}$ in wild octopuses to 3.0 $g \cdot kg^{-1}$ in mussels-fed octopuses. The wild octopuses and the bogue-fed group had a lipid content significantly higher than the mixed and mussel dietary groups ($p<0.05$). The total lipid content in animals fed mussels was significantly lower than that observed in the other experimental diets.
All the octopuses were rich in phospholipids and cholesterol, the latter having a 34.5% of total lipids in octopus fed mussels. Octopuses fed bogue showed the highest cholesterol and lowest phospholipids contents with 52.0% and 26.9% of total lipids, respectively. Significant differences were also observed in phospholipids percentage between wild octopuses and those fed the mixed diet. The lipids of all *O. vulgaris* samples had poor TAG, accounting for 9.9-21.1% of total lipids. Compared with wild octopuses, all animals fed the experimental diets had a higher

	Dietary group I	Dietary group II	Dietary group III
WG (g)	267.83±29.3[a]	390.65±37.54[a]	215±145.67[a]
AGR	8.93±0.97[a]	13.02 ± 1.25[a]	7.17±4.85[a]
SGR	1.15±0.22[a]	0.95±0.16[a]	0.53±0.09[b]
AFR	51.79±16.16[a]	29.11±3.41[b]	13.6±10.1[b]
FE	17.92±3.4[a]	44.79 ± 0.96[b]	54.1±4.64[c]
FCR	5.73±1.21[a]	2.23±0.05[b]	1.85±0.16[b]

Values on the same line and different superscripts are significantly different ($p<0.05$)

Table 1: Mean values ±S.D. for every index of each dietary group.

	Wild	Dietary group I	Dietary group II	Dietary group III
Total Lipid (mg/g)	6.5 ± 0.18[a]	3.8 ± 0.02[b]	5.2±1.34[c]	3.05±0.53[b]
TAG (%)	9.89[a]	12.6[a,b]	21.15[c]	17.93[d]
PL (%)	51[a]	41.7[b]	26.92[c]	47.6[d]
CL (%)	39.1[a]	45.7[b]	51.92[c]	34.48[d]
Protein (mg/g)	101.81 ±1.93[a]	102.01±2.07[a]	105.62±1.69[b]	107.24±0.52[b]
Carbohydrates (mg/g)	12.5 ± 0.62[a]	12.2 ±0.11[a.b]	12.8 ± 0.57[a]	11.5 ±0.58[b]

Values for each sample with different superscript letters in the same row are significantly different ($p<0.05$).

Table 2: Biochemical composition of *Octopus vulgaris* under different feeding regimes.

TAG content (Table 2).

4 Discussion

O. vulgaris is a generalist predator and shows a wide feeding spectrum, eating on crustaceans, molluscs and fish [8], even if food availability determines the natural diet of common Octopus [26]. Literature reported that *O. vulgaris* under laboratory conditions, in presence of live prey, always preferred crabs, although in their absence it also fed fish and molluscs, but only after days and weeks spent in starvation conditions [8].

In this study octopus accepted all the furnished food very well and ingested it immediately after it was offered, confirming in this way that this species is a generalist predator. In particular in the presence of live crabs and mussels, and frozen fish, the octopuses immediately consumed fish with great voracity and in a short time. After they fed on all available fish, they also captured crabs and consumed them completely. Only as the last choice they fed on mussels as observed in previous studies [27, 28].

The results of this study show that the diet had a clear influence on growth, food intake and feeding efficiency, as also reported by Aguado Giménez & García García [29]. Better results were obtained with the monospecific diet, based on bogue. The growth and feeding efficiency indexes were similar to those observed by García García

& Aguado Giménez [20].

In the literature the better growth results and the higher food intake were obtained for crab-fed octopuses [19, 29]. García García & Cerezo Valverde [30] obtained a comparable growth with a monospecific diet based on crabs and with a mixed diet (crab and bogue), although this latter diet type showed the better feed efficiency. Smale & Buchan [31] and Cagnetta & Sublimi [19] stated that a mixed diet, based on crustaceans and fish, may better meet the nutritional requirements of octopuses for a high growth rate.

In this study, unexpectedly the octopuses fed on mixed diet showed a lower weight increase than those fed exclusively on bogues. It was observed that the maximum growth and the best FCR do not coincide with the highest AFR. The lowest FCR occurs at AFR below those at which maximum growth occurs such as what is reported in literature for fish [32, 33]. However, regarding growth, the statistical analysis did not show significant differences amongst all diet groups.

The low AFR value observed in *O.vulgaris* fed mussels may indicate that this food had a low palatability, associated with a possible lower nutritional value, even if the FE value was the highest.

Nutrition is one of the most important factors influencing the ability of cultured marine organisms to exhibit its potential for growth and reproduction. Food quantity and food quality are the critical factors affecting the juveniles growth under laboratory conditions [34, 35, 3, 36]. Despite the increase in the knowledge on nutritional requirements of the fast-growing young octopuses [4, 7] there is lack of information on these requirements for sub-adult growth [37]. García García & Aguado Giménez [20] in a study on the influence of diet on growth and nutrient utilization, showed a low growth in *O. vulgaris* fed diets with high lipids and low protein contents. This may be due to the fact that lipid digestibility is low in cephalopods [38, 37] for their limited capacity to metabolize lipid [39, 40].

In our case, all experimental dietary groups showed a low lipid content, with relatively large phospholipid and cholesterol fractions and triacylglycerols as minor components, similar to the observations reported in previous findings [41, 4, 27, 28]. This confirms that lipid digestion is low, probably due to the scarcity of an emulsifier in the digestive tract [20].

Diet had a clear influence on the octopuse's nutritional state. In a study on ongrowing of *O. vulgaris*, García García & Cerezo Valverde [30] analysed the furnished food composition, and they reported a lipid content of 0.79%, in the case of crabs (*Carcinus mediterraneus*), while for bogue a lipid content of 6.11%. At the end of the experiment, octopuses fed with bogues only, showed a lipid content of 0.34 ± 0.02%. This may be due to the fact that, even if *O. vulgaris* requires a low quantity of the lipids, this species stores significant quantities of lipids in order to guarantee high growth rates [30]. Probably their use is mainly involved in the cell membrane structure [37]. In cephalopods, lipids are stored in the digestive gland and then transported to the muscle to be used as an energy source [42]. The cephalopods are able to synthesize sterols, although Voogt [43] reported that mollusks, can carry out slowly this biosynthesis. For this reason, the main lipids source comes from food, even during reproduction [44].

The results indicate that *O. vulgaris* is a good protein source according to Ozogul et al. [45]. Although crabs offer a source of

protein similar to that of bogues [29, 20], the samples fed bogues showed a higher protein content than those fed mixed diet. The highest protein level was found in octopuses fed mussels followed by animals fed bogue. But, despite the good results obtained from this study with the monodiets, we think nevertheless, according to other authors [31, 19], that a single food should only be used exceptionally for a short period, and when there is not availability of other preys, since the mixed diets satisfies the nutritional requirement of octopuses better than a monodiet.
Regarding the lipid class composition (% of total lipid class) the results revealed that in all samples phospholipids were the dominant class, except for octopuses fed monodiet based on *B. boops* which showed a high cholesterol percentage. TAG was the least represented class in all samples.
Cholesterol level showed the highest concentration in octopuses fed *B. boops* and in those fed the mixed diet based. From a nutritional point of view, the low cholesterol content is a favourable attribute, considering the current dietary recommendation to reduce the daily intake of cholesterol in the human diet [46]. In this work we report the information about the influence of diet, based on preys of low market value or discards from commercial fishing which could improve the growth and nutritional value of cephalopods.
The biochemical composition of *O. vulgaris* confirms that this species has a great nutritional potential, also under culture conditions and on the other hand it does not show risks when it is kept in captivity (survival was 100%).
Nutritional problems are an important constraint to the development of many species commercial culture. It is known that the common octopuses like a varied diet, although the results obtained from this study allow us to state that the single food (bogue) are a real possibility, during a limited period of rearing or in particular situations (such as the availability of prey and their high costs in determined period).
Naturally, a diversified diet better covers the nutritional requirements than a monodiet. The most important step is certainly the possibility to set up preliminary basic aspects, referring to the productive responses of *O. vulgaris* fed in captivity, using natural single foods. A profitable long term industrial culture of common octopus depends on the development of a satisfactory diet for a high growth performance. In this study the better results obtained with *B. boops* diet, support the possibility that this species could be a valid component for other mixed diets to produce balanced and inexpensive formulations.
Further studies on this subject appear necessary also to promote an optimal choice of prey of high nutritional quality, improving the development of feeding techniques.

5 Acknowledgements

This work has been financed by Apulia Region (POR). We are also grateful for coordination and collaboration in the organization of the project to Federcoopesca and Cirspe.

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Bioactive Molecules from Marine Biomasses and their Potential Employment in Pharmaceutics and Dietetic: the Project ACTI.BIO.MAR.

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Abstract

The isolation of natural therapeutic substances is one of the new challenges for the biomedical research. Moreover, the raising of aquaculture made the use of biological fodder necessary, in order to fulfil the European normative law. In this respect, in the last years, many new bioactive compounds from marine organisms useful to both pharmaceutics and fodder industry have been identified. In this framework, the project "Bioactive molecules from marine biomasses and their potential employment in pharmaceutics and dietetic (ACTI.BIO.MAR.)", granted by the Apulian Region (Italy), is focused on the development of new technologies aimed toward the exploitation of marine biomasses. The selected marine organisms (polychaetes, cnidarians and macroalgae) have been investigated as sources of antibiotics as well as high nutritional value compounds useful to the fodder industry as an enrichment and/or an alternative innovating fodder for nourishment of fishes in intensive aquaculture. Since industry requires high quantity of biomass to extract active compounds, the selected species have been reared in a fish farm to ensure an uncontaminated biomass clear of pollutants. The involvement of private enterprises in the project is aimed toward the valorisation of the results through the technology transfer of the know-how to pharmaceutical and aquaculture firms and their transformation into an economic value by the production of both new antimicrobials and an innovative fodder.

1 Introduction

1.1 Antimicrobial compounds

It is well known that the diversity of the living organisms represents an immense source of natural compounds that might potentially be active against virus, bacteria and carcinogenic cells. At present, although several pharmaceutical compounds are chemically synthesized, about 45% of the therapeutic agents employed in

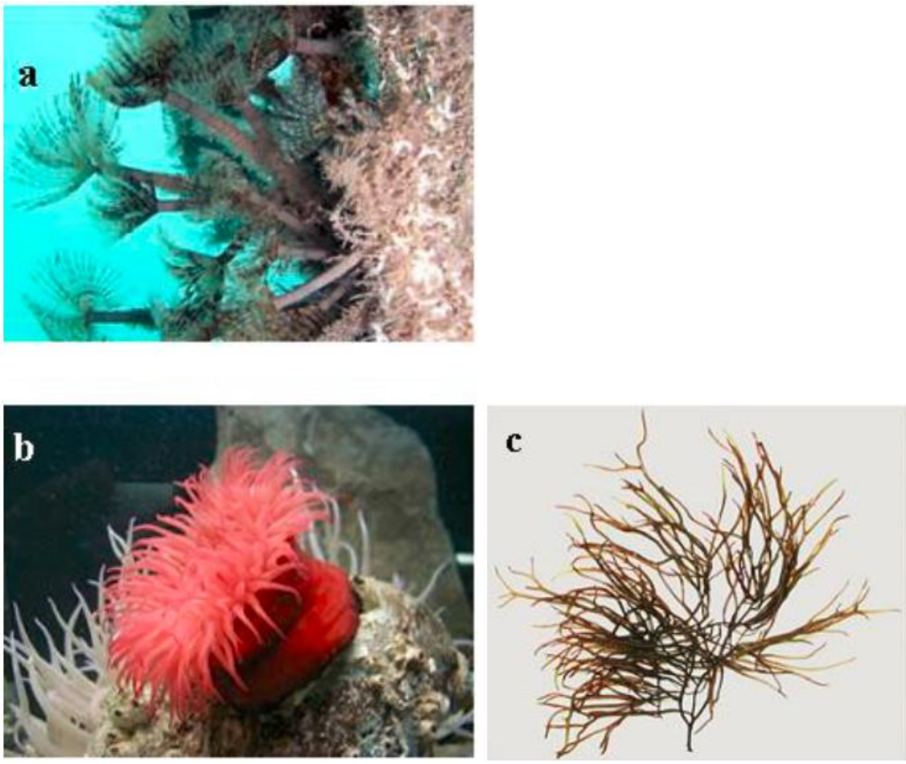

Figure 1: Target marine organisms: (a) Polychaetes; (B) Cnidarians, (C) Macroalgae.

the pharmacopoeia are of natural origin. During the last decades about 5000 novel metabolites with diverse pharmacological activities have been isolated from various marine sources. The antibacterial compounds present in the marine environment and derived from bacteria, algae and several species of invertebrates show a wide range of antimicrobial activity also toward human pathogenic microorganisms. The probability to discover new bioactive compounds in marine invertebrates is high. In fact, the production of bioactive molecules by these organisms is related to the defence towards predators and pathogens and ensures their survival in the marine environment. After screening 1020 marine invertebrate species, Shaw et al. [1] and Rinehart et al. [2] constantly found antibacterial and antifungal compounds particularly in echinoderms. Antibacterial activity was evidenced and characterized in *Marthasterias glacialis* eggs, in seminal plasma, in different planktonic larval stages, in coelomocytes lysate and in eggs of the sea urchin *Paracentrotus lividus* [3, 4, 5, 6, 7]. Cnidarian mucous contains several molecules of pharmacological interest and with antibiotic activity, but detailed studies are still scant. The mesoglea of the medusozoan jellyfish is pathogen free thus leading to sup-

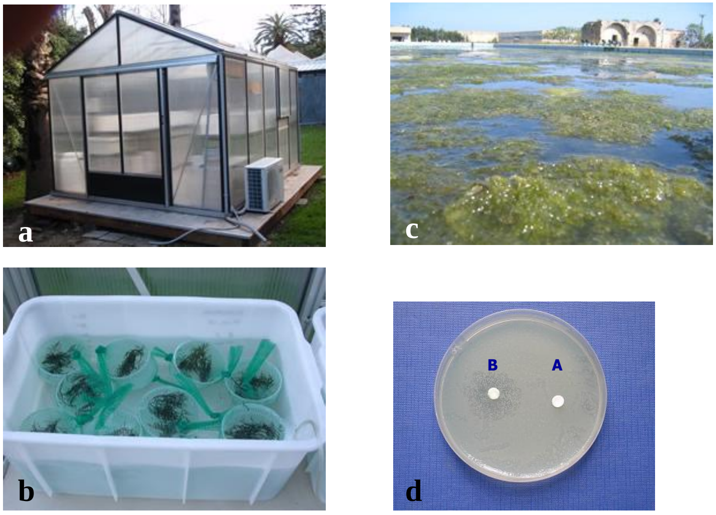

Figure 2: Research activity on macroalgae: (a, b) macroalgae rearing under laboratory conditions; (c) macrolagae rearing in aquaculture farm; (d) antibacterial activity of an algal lipidic extract (Kirby and Bauer method: A=Control, B= disc imbued with algal extract; note the inhibition of the bacterial growth around the disc.).

pose the presence of diffusible substances with antimicrobial activity. The extracts derived from many seaweeds show antimicrobial activity against several microorganisms, such as bacteria, fungi, viruses. In fact, the red seaweed *Sphaerococcus coronopifolius* [8] and the green seaweed *Codium iyengarii*, this last coming from the Arabic Sea, showed antibacterial activity; in particular, in *C. iyengarii* an action similar to the tetracyclines against both Gram-positive and Gram-negative bacteria was observed [9]. Also extracts from *Caulerpa racemosa, C. cupressoides, Ulva lactuca, Gracilaria foliifera, Hypnea musciformis, Sargassum myricocystum, S. tenneerium, Padina tetrastomatica* showed antibacterial activity against both Gram-positive and Gram-negative bacteria [10]. *Haligra* spp. showed high antimicrobial activity against *Staphylococcus aureus* [11], *Stocheospermum marginatum* against *Klebsiella pneumoniae* and *Gracilaria corticata* against *Proteus mirabilis* [12]. In particular, very good results came from lipid-soluble extracts of marine macroalgae against multi-antibiotic resistant bacteria [13, 14]. Moreover, recently, from the alginates, polysaccharides abundant in the brown seaweed cell wall, some fraction of both mannuronic and guluronic acid were extracted. These extracts showed a high antibacterial activity against *Escherichia coli, Salmonella paratyphi, S. aureus and Bacillus subtilis* [15].

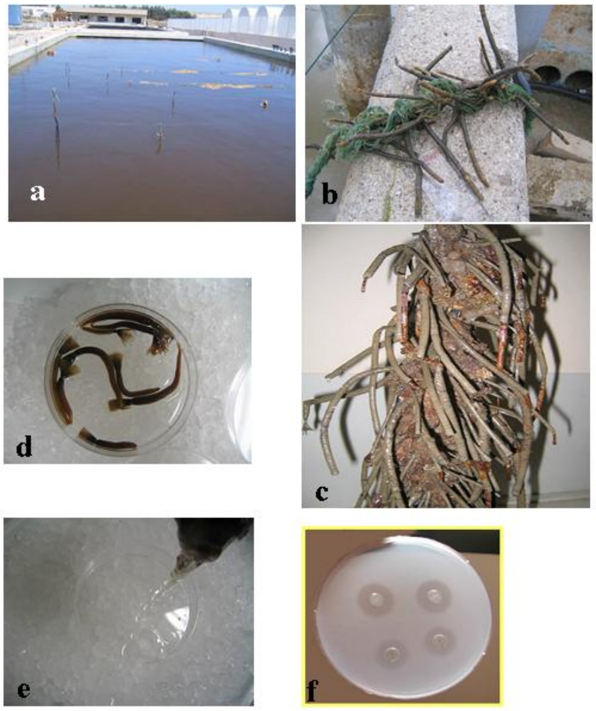

Figure 3: Research activity on polychaetes: (a, b, c) polychaetes rearing in aquaculture farm; (d, e) mucus collection; (f) antibacterial (lysozyme-like activity) of polychaete mucus (Standard assay on Petri dishes).

1.2 Fodder industry

The feeding of aquatic organisms is a crucial point in aquaculture. The fodder represents not only a source of important nutritional oligodynamic components [16] but also a modulator of immune defence. The use of animal proteins in fish feeding produces negative feedback (potential pathogenic transmission, increasing of the over fishing on the fish-stocks, BSE warning). In this context, the research has to be focused on the individuation of new protein sources as already proved by our previous projects on the biological treatment of wastes from aquaculture farms through bioremediators (e.g. VI Triennial Plane, Italian Fishery and Aquaculture Ministry; regional project Bioimpact "Pilot project for the diversification of the mariculture production and the reduction of the environmental impact"; regional project PO.IN.T. "Polyculture Activity Integrated to mussel-culture: pilot project in Taranto"). Data from these projects pointed out that the target organisms (i.e. *Sabella spallanzanii, Branchiomma luctuosum, Cladophora prolifera, Gracilaria dura*) represent bioremediators highly efficient in removing organic and inorganic

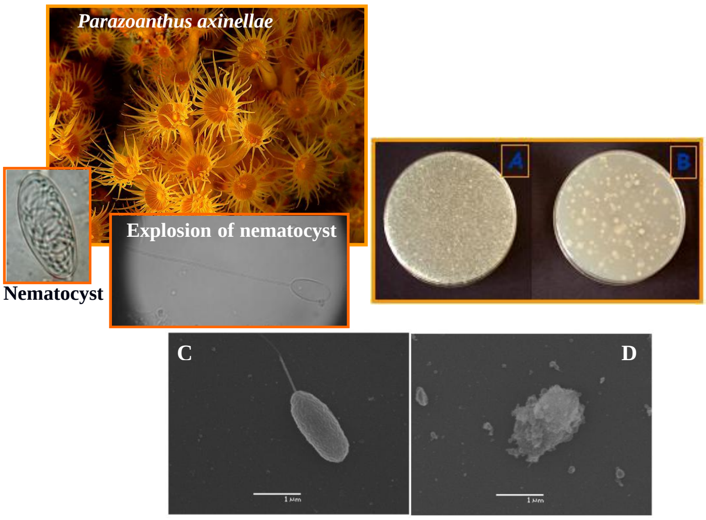

Figure 4: Research activity on cnidarians. Antibacterial assay on Petri dishes using *Paraxoanthus axinellae* nematocysts extract: (A) control, (B) bacteria treated with nematocyst extract. Note the inhibition of bacterial growth in the treatment in comparison with the control. Scanning electron micrographs of the bacterial strain employed in the antibacterial test: (C) control, (D) bacteria after the treatment with nematocyst extract. Note the bacteria lysis and the loss of the flagellum. Scale bar 0 1 μm.

matter from aquaculture wastes other than a resource of marketable value (e.g. as a source of agar exploitable in the food industry, as a fertilizer in agriculture, as a valuable substitute for soya, as a valuable component of omnivorous fish fodder, as a source of essential microelements and OMEGA-3). In the framework of applied research, studies on these species are highly recommended in order to assess the qualitative and organoleptic features and to evaluate the potential of low-cost farming for harvesting high-value products.
The ACTIBIOMAR project is focused on the development of new technologies aimed toward the exploitation of marine biomasses. The research purpose is to investigate the potential employment of some marine organisms [polychaetes, cnidarians and macroalgae (Figure 1)] as sources of:

- biologically active secondary metabolites, matter of interest to pharmaceutics;
- foodstuffs at high nutritional content useful to the fodder industry as enrichment and/or alternative innovating fodder for nourishment of marine fishes reared in intensive aquaculture farms.

2 Methods

The scheduled scientific activities of the project were:
- evaluation of the growth capability of

the polychaete *Sabella spallanzanii* under laboratory conditions as well as in an aquaculture farm in different feeding conditions, in order to produce biomass suitable for both the extraction of antimicrobial compounds and the fodder production;

- evaluation of the growth capability of macroalgae in laboratory, greenhouse (Figures 2a, 2b) as well as in an aquaculture farm (Figure 2c) with different concentrations of nitrogen salts, in order to produce biomass suitable for both the extraction of antimicrobial compounds and the fodder production. The following algal species were examined: *Cystoseira barbata, Chaetomorpha linum, Gracilaria bursa-pastoris, G. dura, G. gracilis, Hypnea cornuta, Gracilariopsis longissima* and *Grateloupia turuturu*;
- biochemical analyses of the selected marine invertebrates and macroalgae (i.e. proteo-lipid content, content in $\omega3$, comparison of monounsaturated and polyunsaturated fatty acids, determination of the $\omega3/\omega6$ fatty acid ratio) in order to evaluate their nutritional value by gas chromatography;
- research of natural compounds showing antimicrobial activity was performed on the mucus produced by the polychaetes *S. spallanzanii* and *Branchiomma luctuosum*, nematocyst extracts of the cnidarians *Parazoanthus axinellae, Condylactis auriantiaca* and *Actinia aequina* as well as on the lipidic extracts of macroalgae. Scanning electron microscopy (SEM) was also used to evaluate the damage on bacteria treated with nematocyst extracts. The effectors responsible for the antimicrobial activities were characterized by gas-chromatography, gel-chromatography and high pressure liquid chromatography.

3 Results

Concerning the growth capability of polychaetes reared both in laboratory and in aquaculture farm, we obtained a conspicuous biomass (Figure 3a, b, c). Moreover, the selected macroalgal species showed high growth rates both in laboratory and in greenhouse culture, displaying also a good capacity in dissolved nitrogen uptake especially in the ammoniacal form.

As regards the biochemical analysis (Table 1), polychaetes exerted an interesting proteo-lipidic profile. In particular, the polychaete *S. spallanzanii* is composed of proteins for 26% of the total dry weight, of lipids for 7% (0.32% cholesterol, 0.84% phospholipids, 0.40% triglycerides and 5.49% other) of water for about 79%. Of particular interest is also the presence of long-chain polyunsaturated fatty acids of the $\omega3$ series.

Saturated fatty acids (SAFAs) were the most abundant fatty acid class in all the examined macroalgae and the palmitic acid methyl ester (16:0) was the prevalent SAFA followed by myristic acid methyl ester. Monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) usually showed the same proportion. As regards MUFAs, oleic acid methyl ester (18:1 $\omega9$) prevailed. The $\omega3/\omega6$ fatty acids ratio, which represents an interesting nutritional indicator, was > 1 in several species. Concerning the antimicrobial activity, the mucus of *S. spallanzanii* (Figure 3d, e, f) showed a lysozyme-like activity. The maximum diameter of the lysis observed was 9.6±0.02 mm corresponding to 1.31 mg/ml of lysozyme of chicken egg white. A lysozyme-like activity was also evidenced in *B. luctuosum* with the maximum diameter of lysis of 5.5±0.50 mm corresponding to 0.75 mg/ml of lysozyme

Sabella spallanzanii	
Water (%)	79 ± 0.41
Total proteins (mg/ml)	26 ± 0.21
Total lipids (mg/ml)	7 ± 0.002
Triglycerides (mg/ml)	0.40 ± 0.0003
Cholesterol (mg/ml)	0.32 ± 0.0002
Phospholipids (mg/ml)	0.84 ± 0.0004
∑ Saturated fatty acids (SAFAs)	70.28± 0.42
∑ Monounsaturated fatty acids (MUFAs)	13.65± 0.22
∑ Polyunsaturated fatty acids (PUFAs)	16,07± 0.31
ω-3 fatty acids	4.5 ± 0.22
ω-3/ω-6 fatty acid ratio	0.5 ± 0.27
Macroalgae - Fatty acids	%
14:0	7.69 ± 2.44
16:0	41.27 ± 12.53
18:0	4.42 ± 2.87
18:1ω9	5.94 ± 3.35
18:2ω6	3.11 ± 4.16
18:3ω3	1.13 ± 0.64
18:3ω6	2.79 ± 2.65
20:4ω6	5.45 ± 3.54
20:5ω3	4.54 ± 5.0
22:6ω3	2.27 ± 0.91
∑ Saturated fatty acids (SAFAs)	58.61 ± 13.39
∑ Monounsaturated fatty acids (MUFAs)	18.52 ± 9.30
∑ Polyunsaturated fatty acids (PUFAs)	22.92 ± 10.35
ω-3/ω-6 fatty acid ratio	1.04 ± 0.47

Table 1: Main biochemical characteristics of the polychaete *Sabella spallanzanii* and macroalgae.

of chicken egg white.

In cnidarians an antibacterial activity was recorded in the nematocyst extracts of *P. axinellae, C. auriantiaca* and *A. aequina*. SEM observations in *P. axinellae* showed a lytic mode of action of nematocyst extracts on treated bacteria (Figure 4). The proteins involved in the antimicrobial activity exerted by the marine invertebrates were then chemically characterized and resulted influenced by the same physical-chemical parameters (ionic strength, pH, and temperature) as other lysozyme molecules.

By disc diffusion assay, used to assess the antimicrobial activity, we observed that among the examined macroalgae, the lipidic extracts of *G. longissima, C. barbata* and *C. rupestris*, containing mainly the palmitic acid (as revealed by the gas-chromatography), showed a bacteriostatic activity against several *Vibrio* species (Figure 2d).

4 Discussion and conclusions

The revaluation of natural products is not surprising since secondary metabolites are the adaptive product of the long-term evolution of competitive interactions between organisms. By contrast, in the combinatory chemical synthesis the possibility to synthesize a biologically active product is merely probabilistic. Our results indicate that the target marine organisms are suitable for potential exploitation in pharmaceutics and dietetics on account of their observed interesting antibacterial activities as well as their proteo-lipidic profiles. Indeed the finding of a lysozyme-like antibacterial activity in the mucus of *S. spallanzanii* as well as in the nematocysts of the studied cnidarians have implications for future investigations related to the employment of these matrices as a source of compounds of pharmaceutical interest. As regards pharmaceuticals, we have to consider that by now the use of antibiotics is a common routine in the cure of bacterial infections. However, the increasing development of bacterial resistance to traditional antibiotics, even to cocktails of different products, has reached alarming levels, thus necessitating the strong need to develop new antimicrobial agents. Furthermore, in these last years, the occurrence of fungal infections in the humans is greatly increased due to the rise of people affected by immunodeficiency. Lysozyme exhibits antimicrobial activity against different microorganisms, and it was chosen recently as a model protein to develop more potent bactericidal agents with broader antimicrobial specificity. Several strategies are attempting to convert lysozyme to be active in killing Gram-negative bacteria other than Gram- positive bacteria thus introducing, for the first time, a new conceptual utilization of lysozyme which would be an important contribution for medicine and modern biotechnology [17]. Thus, *S. spallanzanii* mucus and nematocyst extracts of the studied cnidarians provide an accessible, renewable resource that could reward wider exploration. Moreover, the isolated bioactive metabolites could constitute new leads on which the research of new pharmacologically active compounds through synthetic way should be based. The fatty acid profiles of the examined macroalgae showed that palmitic acid methyl ester (16:0) was the predominant saturated fatty acid. Since the palmitic acid represents the main component of fatty acids, it is presumably responsible for the antibacterial activity observed in the target algal species. In several studies, indeed, palmitic acid has been reported to be the major antibacterial compound in a mixture of fatty acids from other algal species. The ability of fatty acids to interfere with bacterial growth and survival has been known for several decades [18]. The antibacterial effects of fatty acids are frequently observed during bioassay-guided fractionation of extracts from a variety of organisms [19, 20, 18]. Their broad spectrum of activity, non-specific mode of action and safety makes them attractive as antibacterial agents for various applications in medicine, agriculture, aquaculture and food preservation, especially where the use of conventional antibiotics is undesirable or prohibited. In this framework, an interesting issue of the present project is that the lipidic extract of *C. barbata, G. longissima* and *C. rupestris* inhibited the growth of several *Vibrio* species, which include some aquaculture-relevant pathogens. These results are relevant considering both the resistance against antibiotics developed by bacteria [21] and the need to control fish and shellfish diseases due to bacterial infections, including vibriosis, common in aquaculture plants [22]. The aquaculture industry is growing worldwide with an estimated production, referred to 2004, of about 45.5 million tonnes [23] and the emergence of microbial diseases is of major concern, implying serious financial losses [24].

The fatty acid profile of several examined macroalgae revealed also an interesting composition in PUFAs, particularly regarding the $\omega 3$ and $\omega 6$ acids, which in humans play a role in the prevention of cardiovascular diseases as well as in the protection against chronic inflammatory diseases such as arthritis, psoriasis, and diabetes [25, 26, 27, 28, 29]. At present, traditional European human food products possess a $\omega 3/\omega 6$ ratio of approximately 1/15–17. This is mainly due to the low consumption of seafood set up against the increasing intake of $\omega 6$ fatty acids from vegetable oil. As a consequence, the Western diets are deficient in $\omega 3$ fatty acids and exceed in $\omega 6$ fatty acids [28]. In this project several algae showed a $\omega 3/\omega 6$ fatty acid ratio >1, thus suggesting that these macroalgae may be used as a natural source of $\omega 3$. In addition, also the occurrence of the essential C18 PUFAs in the algal extracts is important for both human and fish nutrition since these organisms are not able to synthesize them [30, 31, 32]. Also the $\omega 3/\omega 6$ fatty acid ratio of the polychaete *S. spallanzanii* is interesting and accounted to 1:2. Moreover, in this species the average protein content is 26g/100g in dry weight. This value is very attractive for animal feed considering that the concentration of protein in traditional fodder is 44% [33] and that in other exploited marine invertebrates is even lower [34]. In addition, the worm proteo-lipidic composition in comparison to the traditional fodders showed no significant differences for proteins, lipids, fibres and ashes. Therefore, we can suggest the potential employment of the studied marine organisms as a dietary supplement for fishes nourishment. Indeed, the rising cost of fish feed worldwide necessitates the consideration of every possible natural resource as a potential ingredient in their preparation. Finally, the results on the growth capability of the studied invertebrates and macroalgae under laboratory conditions as well as in an aquaculture farm revealed that the biomasses of the target marine organisms can be easily obtained from a polyculture farm system.Therefore, polyculture rearing may become a routine thus obtaining an increase of exploitable biomass, avoiding environmental injuries and even reducing environmental impacts (e.g. the reduction of the excess of nitrogen salts). The energetic surplus is thus converted into biomass useful in animal feeding and extraction of bioactive substances with positive effects from economical and ecological points of view.

As a consequence, the impact of the project ACTIBIOMAR, dealing with innovation within the biotechnological sector, is of major importance on industrial production, aquaculture and commercial trade concerning human and reared species health.

The involvement of private companies in the project is aimed at transforming the knowledge into an economic value by means of the technological transfer of the scientific results to the pharmaceutical and aquaculture firms.

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Juvenile Anchovy, *Engraulis Encrasicolus*, Habitat Conditions and Daily Growth in the Central Mediterranean Sea

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Abstract

This study investigates the juvenile anchovy life stage in their habitat during the recruitment period. Daily otolith age readings permitted to evaluate the birthdate distributions which, compared with the spawning effort obtained from monthly samples, highlighted a higher mortality of larvae and postlarvae during the first part of the spawning period. Differences in the growth rate were also recorded during the first half of the spawning season by measuring microincrements and backcalculating the length at age. The link between mortality and growth rates has been discussed along with temperature as the main habitat feature regulating growth in the postlarval and juvenile phases. Data were collected in the Straits of Sicily in three multidisciplinary surveys (2001, 2004 and 2005), while growth performances and the birthdate analyses were carried out on data from the 2005 survey. The oceanographic parameters and juvenile spatial distributions, showed a negative linear relationship between water temperature and juvenile anchovy average length for each trawl suggesting a preference for smaller organisms to inhabit warmer waters. The birthdate peak was observed between the end of July and early August with individual ages ranging from 46 to 186 days. The obtained mean growth was 0.4 mm$\cdot$d^{-1} whilst the daily backcalculated growth rates range between 0.4 and 1.06 mm$\cdot$d^{-1}.The estimated mortality rate for juvenile anchovy in the age range 60-100 days old, was 0.03 d^{-1}.

1 Introduction

Anchovy fishery represents the main world fishing resource and constitutes almost 30% of the total Mediterranean fish production [1, 2]. Clupeoid fish populations, especially anchovies and sardines, exhibit large fluctuations in abundance in several distributed areas in the world [3, 4, 5]. In the study area, acoustic and ichthyoplankton-based biomass evaluation methods showed how the high interannual variability in biomass levels were mainly associated to environmental variability [6, 7, 8].

Growth and mortality, the principal processes that determine population dynamics and the high variability in recruitment success, are generally linked to the mortality of early life stages from hatching to recruitment time. Estimation of these life history parameters at different life stages

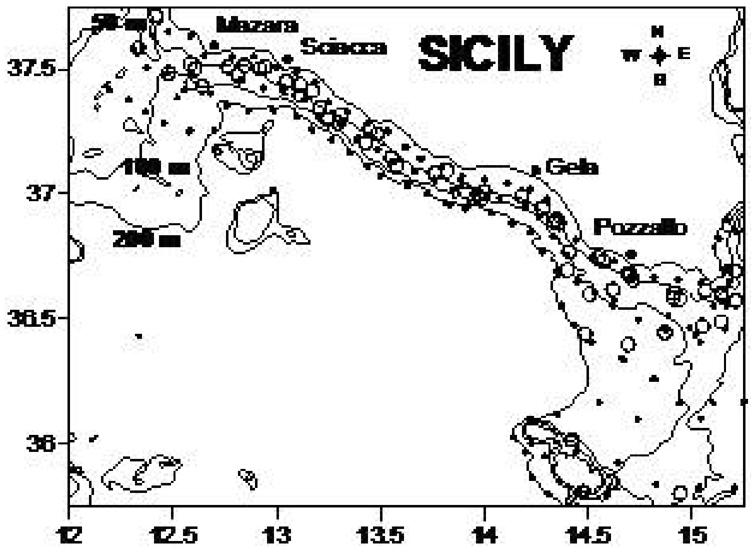

Figure 1: Map of the Strait of Sicily with geographical positions of performed trawls and CTD stations: (●) = CTD stations; (○) = trawls with anchovy.

and time intervals is fundamental for interpreting changes in population abundance and structure. The reproductive season for anchovies in the study area was observed to start in May and to finish in October [9]; during this six-month period the environmental conditions may vary drastically affecting the spawning fraction of mature females as well as the mortality of the spawning products. It was observed how the timing of the reproductive season onset may be linked to broader water temperature variations. Furthermore the peak of the spawning period was observed to take place when higher water temperatures are reached usually between the end of July and early August [9]. Growth studies have already been carried out on adult anchovy fishery linking temperature and primary production to growth parameters [10] but no data has ever been presented on juvenile growth and environmental features in the study area. Even in other Mediterranean areas, very few studies have dealt with clupeoid juvenile growth and mortality estimations [11, 12, 13, 14]. This is mainly because the direct approach (daily growth readings and increment measurements) is time consuming and also requires relevant sampling efforts although it provides highly valued information for the understanding of recruitment variations in fishes.

Birthdate analyses of juvenile fishes represent a useful tool to determine which time periods allow high survival for spawning products [15, 16, 17, 18, 11, 19, 20, 21, 22]. Birthdate analyses are based on daily otolith micro-increment counts under optical microscopy [23] validated for this species by previous studies [24, 25, 26, 27]. It is well known how daily growth micro-

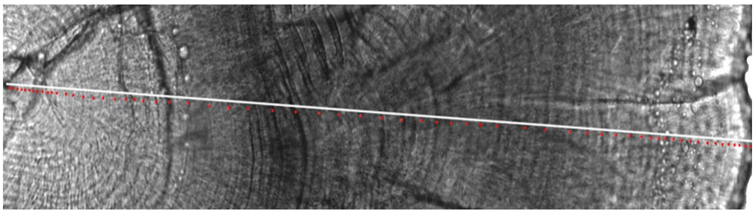

Figure 2: Otolith image with its nucleus and daily rings.

increments in otoliths are used to determine age for several anchovy species [28, 23, 29, 30, 31, 32, 24, 26] and allow, in juvenile fishes, to record growth patterns in the first year of life.
In the present paper, data from three oceanographic surveys together with growth data were analyzed to study the spatial distributions and processes regulating recruitment such as growth and mortality. Moreover, we posed the question if habitat features can be associated to favourite anchovy juvenile growth areas, and if growth differences between areas or time lags may affect recruitment strength.

2 Materials and methods

2.1 Juvenile data

Juvenile samples of European anchovy were collected during the recruitment surveys conducted on board of R/V "Dallaporta" during 2001 (4-17 October), 2004 (7-19 October) and 2005 (5-13 October). The catches were performed by means of a mid-water trawl-net with an opening of 14 x 7 m, and with the cod-end mesh-size of 19 mm (stretched) in the Straits of Sicily (Figure 1). The mesh size is within the range of cod-mesh size for these studies [33]. For each positive trawl an anchovy random sub-sample was measured on board in order to obtain total (TL) and standard length (SL) to the nearest 1 mm, and total body weight (TW) to the nearest 0.1 g by means of a scientific portable marine scale (Marel S182). Otoliths were extracted from specimens up to 130 mm TL, i.e. the mean measurement which represents the length when anchovy juveniles are expected to become adults (one year old juveniles; [10]). For each trawl 25-50 sagittal otolith pairs – five specimens for each 0.5 cm size class – were extracted, cleaned and dry-stored in black plastic moulds. The trawl cod-end mesh-size did not allow retaining specimens less than 45 mm SL.

2.2 Oceanographic data and juvenile habitat characterization

A CTD probe (Minisonde 4a, Hydrolab) was deployed up to a 100 m depth (Figure 1) to obtain temperature, salinity and oxygen profiles along the water column in each station. Data interpolation and mapping were achieved by means of Ocean Data View software [34]. Habitat measurements were obtained averaging the data of the two-three CTD stations nearest to each trawl.

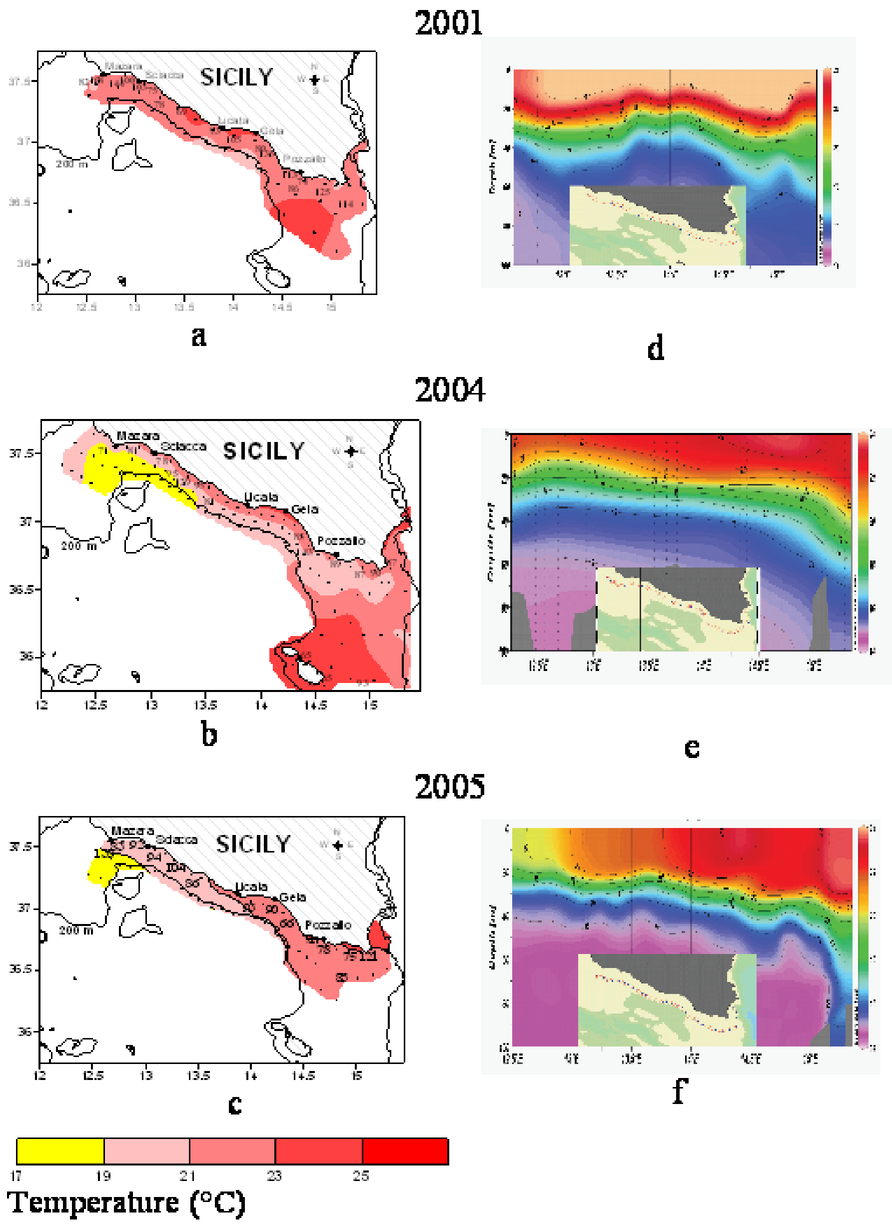

Figure 3: Depth averaged temperature surface contour plots (0-40 m) with the CTD stations (♦) and the anchovy juveniles total lengths average for each trawl and year (a, b and c); the depth section plots (d, e and f), are the respective NW-SE temperature contour sections.

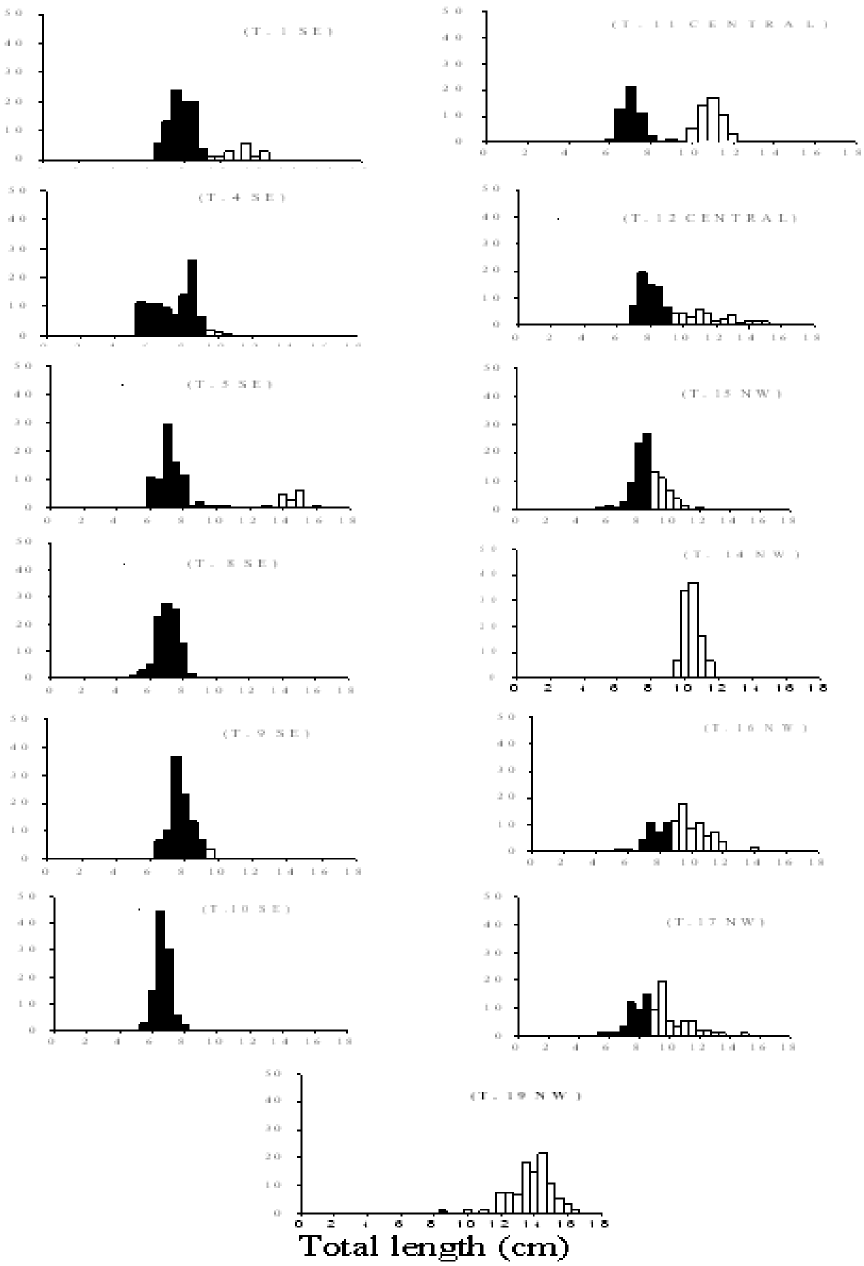

Figure 4: Length frequency distribution for each 2005 trawl and their respective geographic position (SE= South-east; NW= North-west); black bars = specimens < 95 mm TL (mean juvenile length). Trawls 14 and 19 were not represented here because specimen lengths were beyond the upper limit of the adopted graph scale.

2.3 Otolith preparation and readings (2005)

The otolith preparation for micro-increment and birthdate analyses were performed on data from the 2005 survey and among the available trawls only 6 were sampled because the collected specimens were enough to cover the complete size range of juvenile size distribution. A total amount of 310 otoliths, were collected for polishing; each otolith was fixed on a microscope glass slide by means of resin and thin sagittal sections were produced by grinding with decreasing grain paper. Readings and interpretation of daily micro-increment growth were carried out by transmitted visible light at 400x magnification.

2.4 Age, growth and mortality of juveniles during 2005

Thin sagittal sections were obtained as shown in Figure 2. Among the examined otoliths (n=310), about 21% (n=65) were rejected because they were not readable. Two independent observers accomplished daily increment readings, counting twice per otolith: first from the nucleus to the edge and then, from the edge to the nucleus; the average of the readings was adopted as the final result. Counts that differed more than 5% of the total amount of the increments were rejected [24]. In this study less than 3% of the otoliths were rejected and the remaining 238 were used for daily reading purposes. The ageing criteria followed Campana and Jones [35], while for the increment counting the individual mark reading (IMR) method was followed [36].

The mortality rate was estimated by means of the “Catch curve analysis”. A non-linear regression fitting was performed on data without considering the descending abundances. The fitted model was the negative exponential function:

$$N_t = N_0 e^{-Zt},$$

where Z is the instantaneous mortality rate, N_t and N_0 are respectively the number of fishes at age t and 0.To yield parameter estimates with the smallest variance, a weighing factor equal to the inverse of age was introduced in the least-squares analysis, as the variance in length was not homogeneous [37, 38]. A goodness of fit measure was provided by the examination of the residuals distribution.

Daily increment width evolution and growth patterns were obtained by measuring a subsample of 121 specimens – 15-25 for each trawl – selected among those which showed the clearest rings. The measurements were performed on live images by means of an image-processing system calibrated at 400x (Optimas 4.10) and following transects located within 5° of the longest radius from the core to the posterior margin of the otolith.

The length growth of each juvenile was backcalculated from micro-increment width data. The backcalculation method, revisited by Francis [39] was applied. The dimensions of one or more marks in hard body parts of the fish, together with its current body length, are used to estimate its length at the time of formation of each of the marks. This technique is based on the relationship between the fish body size (length or weight) and the size of the hard body part considered (radius of otoliths, scales, etc). The employed equation in backcalculation was the following:

$$L_i = \left(\frac{a + bR_i}{a + bR_c} \right) L_c,$$

where L and R represent fish length and otolith radius, respectively; c and i the time of capture and the time of ring i formation, respectively. In the equation, the estimates of a and b were obtained from the linear regression of the otolith radius versus the body length . This technique is generally applied on both annual [10] and daily growth increments [13]. The juvenile growth pattern was obtained as the average of daily increment width of each calendar day, while the average growth rate was obtained as the slope of length-at-age data linear regression.

3 Results

3.1 Juvenile habitat characterization

Thermal conditions during the recruitment period in 2001 indicate a quite uniform situation up to the shelf break warm and well stratified waters with temperature averaged in the upper layer (0-40 m) in the range 19.6-24.5°C as showed by the depth-averaged contour plots (Figure 3a). No upwelling phenomena are present and the thermocline is still well developed at a constant depth (15-20 m) as showed in Figure 3b. In this stable and homogeneous environment geographical distribution of juveniles is also uniform, as juvenile specimens inhabit all the coastal zones with no relevant differences among areas in terms of their mean size among trawls (Figure 3a). During 2004 in the study area were recorded lower temperature than during 2001; less intense stratification was observed with a shallower thermocline especially in the north-western part of the study area. Despite the lower temperature no upwelling or cooler jet injection were evidenced by the increasing temperature shoreward. During 2004 the juvenile distributions did not appear skewed towards NW or SE as they were caught on both sides of the study area. The physical characteristics of the study area during 2005 differed strongly from the previous survey, mainly due to an increasing temperature gradient, NW-SE (Figure 3c and 3f). The thermocline in 2005 was deeper than in the previous year and the mixed layer extended down to a depth of 20-30 m. During 2005 the juveniles mean length per trawl appears related to the temperature patterns as smaller sizes were recorded in the south-eastern warmer area (Figure 3c). Evidence of the length shift towards the southeast area is given in Figure 4, where the length frequency distributions for each trawl show an increasing fractions of juveniles (total length <9 cm) moving towards the south-eastern part of the Sicilian continental shelf. Taking in mind that the mean length at age 0 for anchovy in the study area is 90-95mm TL [10], the ratio between, respectively the n° of specimens with lower (juveniles) and higher 90 mm total lengths, were compared between the north-western and the south-eastern areas (north and south of Licata). The Chi square test shows a highly significant greater presence of juveniles ($<$ 90 mm) in the southern region (84.3% vs. 43.6%; $p < 0.001$.These findings may support the preference of smaller specimens for the warmer southernmost waters (Figure 3c and 3f).

In order to understand how the distributions of juveniles were related to temperature or to the other measured environmental factors, the mean temperature, salinity, oxygen, and bottom depth, were plotted against the mean juvenile size for each trawl along the study period. The regres-

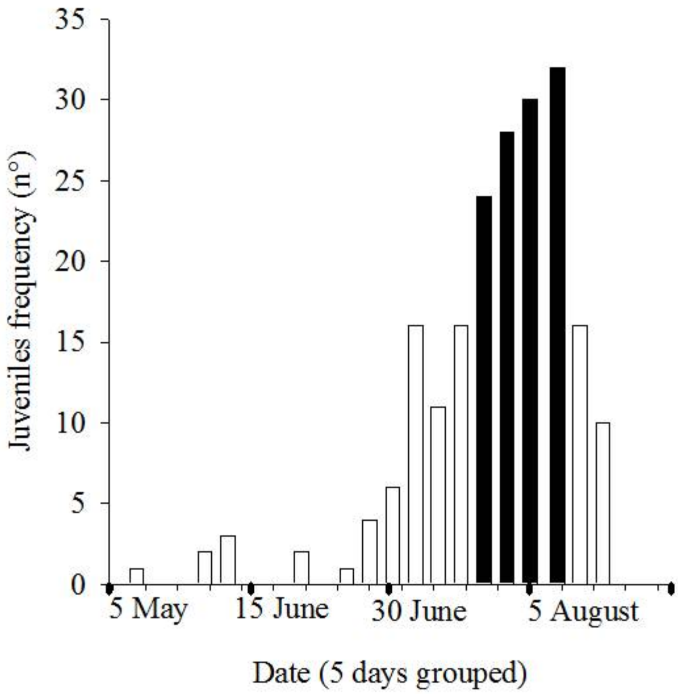

Figure 5: Birthdate frequency distribution obtained from the 2005 survey data; black bars = peak of survivorship.

sion analysis singled out a significant negative linear relationship ($F2,42 = 13.08$; $p = 0.001$; $r^2 = 0.24$) only between temperature and the juveniles anchovy mean size for each trawl.

3.2 Birthdate, growth and mortality of juveniles 2005

The frequency distribution of all anchovy specimens collected in 2005 showed a clear peak between 65 and 85 mm (TL), also observed in the sub-sample selected up to the size of 130 mm TL which was used for the birthdate analyse. A two sample Kolmogorov-Smirnoff test showed that the sub-sample distribution was representative of the whole population ($p>0.3$). The age of examined fishes ranges between 50 and 152 days with respectively 55 and 100 mm observed TL. In the stability test for birthdate distribution, the "abundance ratio" was high (0.67) indicating a relatively stable distribution [40]. The examination of mortality model residuals showed that they were not randomly distributed around the regression, particularly at older ages. Then, to avoid heteroscedasticity, it was decided to fit only data between 60 and 100 days. Within this age range, the estimated mortality rate was 0.0346 ± 0.01 d^{-1}. The overall birthdate distribution presents its modal value in early August while the bulk of anchovies hatched mainly between end-

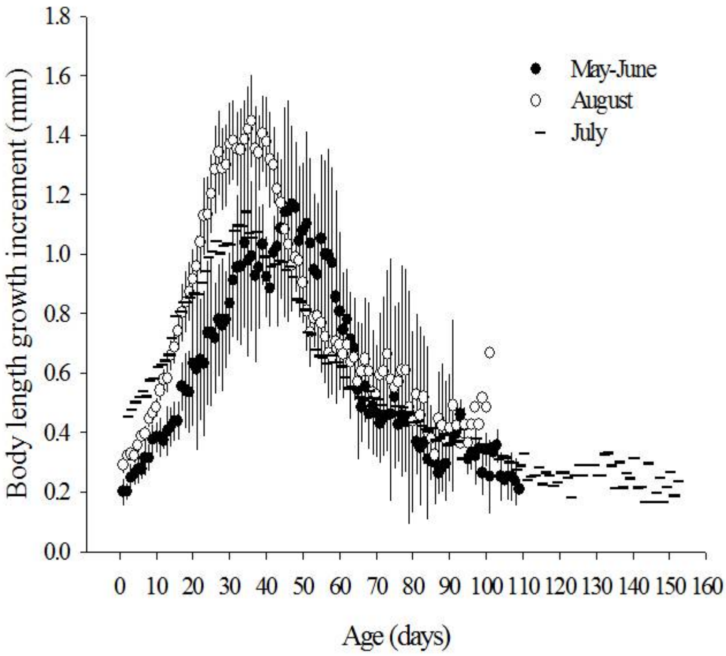

Figure 6: Backcalculated body length averages, obtained by grouping measurements from specimens belonging to the start of the spawning season (May-June), the middle (July) and the peak (August). The coefficient of variation (CV) were hidden for the July series to show better the differences between the other two series.

July and mid-August (Figure 5). The reproductive season, as reported in literature [9], extended from April to October with a clear peak in July-August. Nevertheless birthdate results showed that very few specimens survive from those born during the first half of the spawning season (90-150 days old) and that the recruitment of the 2005 year class derived mainly from the July-August spawning period (Figure 5). The backcalculated lengths, from otolith micro-increments, permitted to reconstruct the overall daily growth pattern, expressed as total body lengths, with their confidence limits till the age of 152. The daily body growth rate starts around 0.4 mm$\cdot$d^{-1}, rising to 1.4 mm$\cdot$d^{-1} between 30 and 40 days old, while at 80 days old, after this high growth period the values decreased again to 0.4 mm$\cdot$d^{-1}. The values after 110 days, which fall under 0.3 mm$\cdot$d^{-1}, have to be considered only as partly indicative of the subsequent pattern evolution because they are based on few specimens. The plots of monthly average series, with their confidence limits, showed how the growth rate in length, changes during the spawning period. Namely, the May-June growth se-

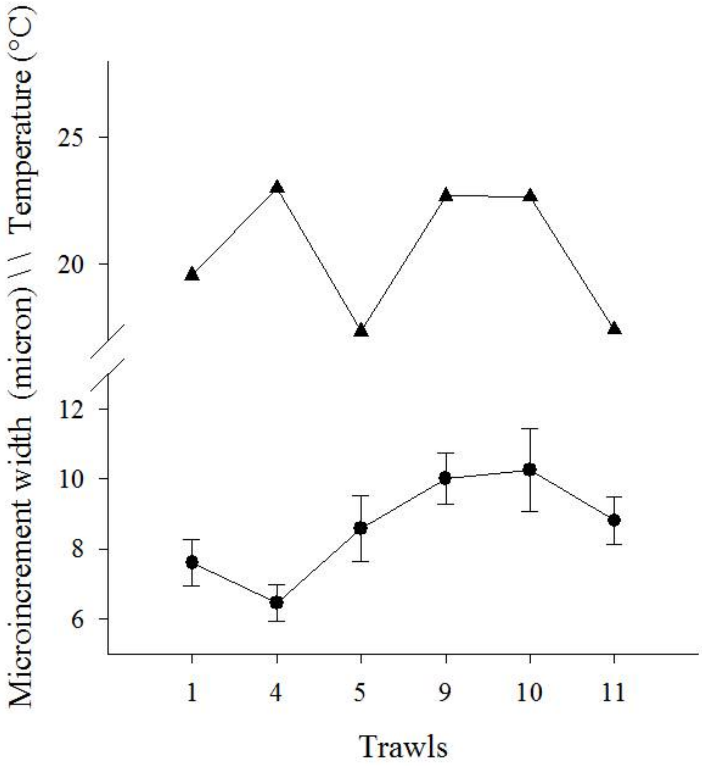

Figure 7: Average growth rate for 2005 trawls (●) (increment width data) and their confidence limits; average column temperature in the trawls (▲).

ries, during the first 50 days, showed significant lower levels compared to August (Figure 6). The overall average otolith micro-increments width pattern showed a first zone with an increasing width starting from 4 and reaching maximum of 14 microns at 35 days, while after this age the width decreases slowly to reach 3 micron at 115 days. The average growth rate obtained by the linear regression of length at age data (observed) was 0.35 mm·d^{-1} (F 1,116 = 56.02; $p < 0.001$) (Figure 8), which compared with the higher growth rate (backcalculated) over the whole age range, suggests that the estimated slope reflects more the first and last growth phases between 30 and 40 days and above the 80th day onwards.

4 Discussion and conclusions

Scanning electron and optical microscopes based studies highlighted how important the sectioning process is for age readings and interpretation purposes [24, 26].The differences in growth rates ob-

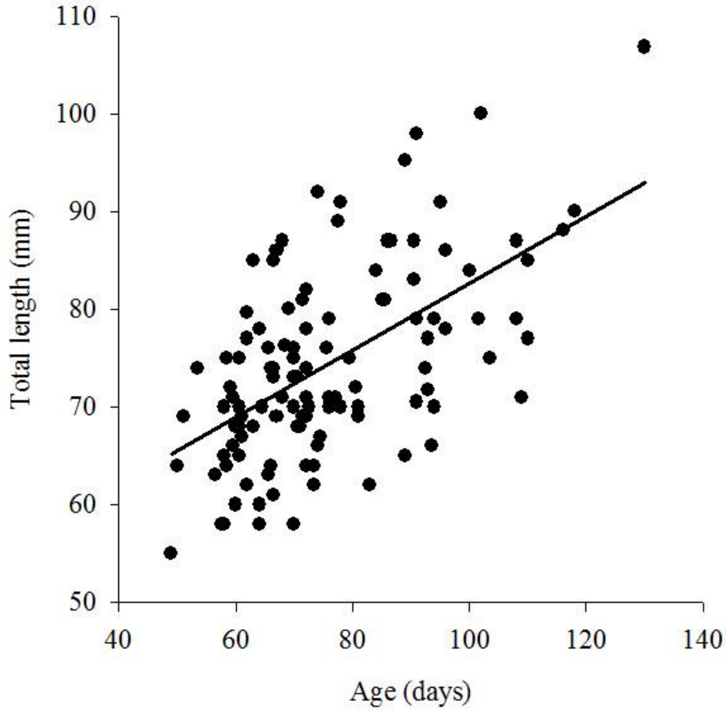

Figure 8: Total fish length at age linear regression in juveniles sampled during 2005; regression coefficients: Intercept=48.41; slope 0.34; Pearson coefficient (r^2) 0.33. ($F=56.02$; $p < 0.001$).

served among the May-June and August born specimens may suggest that they go through a faster growth period reducing their mortality in the very vulnerable larval and post-larval phases. Small pelagic population dynamics, driven by the survival of their offspring, appear to be heavily influenced by environmental variability affecting the different life stages from eggs to juveniles. The incidence of mortality during the pre-recruitment period is not yet clear. The estimated low natural mortality rate in the analysed age range, suggests how growth trajectories in that life phase don't heavily affect the recruitment strength while other factors such as the fishing mortality could play a more important role. On the other hand, it's well known how the post-larval and juvenile mortality sometimes may affects recruitment. In the present paper, the analyses of juvenile spatial distributions and oceanographic data further support the role of temperature in selection of a favourable area. The underlying mechanism is probably the increment of metabolic rates due to higher temperatures which in turn increase growth rates and thus reduce mortality due to higher predation rate at smaller lengths. In our study the estimated average growth rate for each trawl appears related to the temperature recorded in the corresponding area.

The growth–mortality hypothesis ‘bigger is better’ [41] suggests that the bigger larvae have a faster growth rate reaching the juvenile stage at a younger age and have a higher probability of surviving to recruitment. In previous studies the role of temperature in the anchovy population, inhabiting the study area, was linked to adult reproductive biology as an index of the goodness of the spawning site [42] or as a cue for the onset of the breeding season [9]; this paper showed how temperature appears to play an important role also in the selection process of the juvenile habitat.

The water column structure during the study periods appears mainly related to temperature summer season regime, which determines a strong thermocline. A stable well developed thermocline, would have important effects on plankton dynamics. Gray and Kingsford [43] suggested that the thermocline needs to be stable and persistent during a prolonged period of time for consistent plankton relationships to be developed because fish larvae and zooplankton may display a time lag in their response to changes in thermocline depth and strength. The juvenile habitat appears to be a very stable environment with high temperature regimes in which no upwelling events have been recorded. Finally it could be resumed that the presence of juveniles are not related to a well defined geographical nursery area but juveniles were caught along the entire south Sicilian coast, which appears to be a suitable area for them. Otherwise during 2005 the length frequency distribution changes according to the NW-SE- direction, suggesting the CE-SE area as most favourable to juveniles.

The Hatchdate analysis too, was confirmed as an important tool to evaluate the environmental processes which may affect recruitment; the data discussed in this paper has shown how a lower growth rate affected the spawned products which belong to the early part of the spawning season, and, moreover, how their small recruited number suggests that mortality for those cohorts was also high. The multiple spawning fish has a temporal dimension which is sometimes overlooked. Further investigations dealing with larvae and post-larvae together with synoptic environmental data, such as water temperature during the spawning period may better elucidate the mechanisms which affect the larvae survival rates.

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Evaluation of Demersal Resources as Essential Tool for a Long Term Common Fisheries Policy (CFP): the Role of the Scientific Research

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Abstract

The Common Fisheries Policy, the European Union's instrument for the management of fisheries, was reformed in 2002 to ensure sustainable exploitation of fisheries stocks. The reform introduced a precautionary long-term approach to protect and conserve living aquatic resources and to minimise the impact of fishing activities on marine ecosystems. The most part of fish stocks are overexploited; these populations could increase if fishing pressure decreased for only few years. Fisheries stocks today are constituted of young and small fish that are mostly caught before they can reproduce. In 2009 the EC published a Green Paper aimed to stimulate a debate on the reform. On this topic, the researchers of IAMC of Messina are involved in several national and European projects aimed to the evaluation of the demersal resources. The focal points undertaken are: stocks monitoring by trawl surveys and commercial landings: two case-studies are reported, the evaluation of *Helicolenus dactylopterus dactylopterus* and of *Aristaeomorpha foliacea* and *Aristeus antennatus*; gear selectivity: the effect of mesh configuration and mesh size on the codend selectivity of the species *A. foliacea* and *Mullus barbatus* have been investigated; nursery areas distribution have been identified using GIS approach; no-taken zones monitoring; analysis of demersal assemblages; evaluation of parasitosis effects on ichthyic populations and genetic identifications of demersal stocks. A synthesis of the main themes treated during last years is reported together with the more interesting results obtained.

1 Introduction

The Common Fisheries Policy, the European Union's instrument for the management of fisheries, was reformed in 2002 to ensure sustainable exploitation of fisheries stocks. The reform introduced a precautionary long-term approach to protect and conserve living aquatic resources and to minimise the impact of fishing activities on marine ecosystems. The most part of fish stocks are overexploited; these populations could increase if fishing pressure decreased for only few years. Fisheries stocks today are constituted of young and small fish that are mostly caught before they can reproduce. In 2009 the European Commission published a Green Paper aimed to stimulate a debate on the reform. The Green Paper reports: "Scientific knowledge and data are of vital importance to the CFP, because policy decisions must be based on robust and sound knowledge on the level of exploitation that stocks can sustain, of the effects of fishing on marine ecosystems and on the impacts of changes such as climate change". On this topic, the researchers of IAMC of Messina are involved in several national and European projects aimed to the evaluation of the demersal resources. The focal points undertaken are: stocks monitoring by trawl surveys and commercial landings, gear selectivity, nursery areas distribution, no-taken zones monitoring analysis of demersal assemblages, evaluation of parasitosis effects on ichthyic populations, genetic identifications of demersal stocks. On this paper a synthesis of the main themes treated during last years is reported together with the more interesting results obtained.

2 Stocks monitoring by trawl surveys and landings

Data coming from international (MEDITST project, [1]) and national trawl surveys (GRUND project, [2]) and from landings data collection (CAMPBIOL project funded by EU/MIPAAF) carried out in the Southern Tyrrhenian Sea (FAO Geographical Sub Area 10b) were elaborated to produce indicators (biomass index temporal trend, length frequency distributions, sex ratio, mature proportion, maturity length, recruits indices, adults-recruits relationship) and stock assessment models of the resources status. The status of the fished stock could be assessed by comparing an estimate of the current fishing mortality with an estimate of the fishing mortality rate that produces the maximum yield, Fmax.

Two case-studies are here reported: the evaluation of the scorpionfish *Helicolenus dactylopterus dactylopterus* and of the two red shrimps *Aristaeomorpha foliacea* and *Aristeus antennatus*.

2.1 *Helicolenus dactylopterus dactylopterus*

The blue-mouth is a scorpionfish widespread in the whole Mediterranean basin, where it plays an important ecological role in deep-sea fish communities. The rarefaction of this large-size sedentary and slow-growing fish can be an index of overexploitation. A total of 1412 specimens of *H. dactylopterus* weighting 20.051 kg were caught during 11 bottom trawl surveys carried out in the Southern Tyrrhenian Sea from 1995 to 2005 (MEDITS Project) [3]. The species appeared in 40% of the 296 hauls analysed, throughout the whole depth range surveyed. The highest values of frequency of occurrence ($> 67\%$) were obtained in the slope, whereas in the shelf they fell to around 16%. From the Von Bertalanffy growth model the following parameters for the whole population were obtained: Linf.: 30 cm; K: 0.16 year^{-1}; t_0: -0.02. The sampled population is composed mainly of the young-of-the-year fraction, which can reach up to 98% of the whole catch. Recruits (age 0+) and juveniles (age up 4 years) are limited to waters shallower than 500 m, whereas adults are present below this depth. The length structure of the whole population as well as the distribution pattern of recruits were quite homogeneous in the studied area. The recruits seem to be concentrated in the proximity of promontories and in particular in their eastern part. This phenomenon could be correlated to the western–eastern water flows in the Southern Tyrrhenian Sea and to the steep slope of the sea bottom [4]. In particular, the northern coasts of Sicily are influenced over the continental shelf by Modified Atlantic Water (MAW), characterized by temperatures of 14–15 °C, and in the slope by the Levantine Intermediate Water (LIW), marked by lower temperatures and higher salinity [5]. The Calabrian coasts, where the recruits have a wider bathymetrical distribution, are probably influenced by local down-welling events, caused by wind forcing [6], which causes a major availability of trophic resources in deeper strata.

2.2 The giant- red shrimp *Aristaeomorpha foliacea* and the blue-red shrimp *Aristeus antennatus*

The application of statistical models on the data time series arising from MEDITS and GRUND surveys allowed us to compare some of the biological and ecological aspects of the two shrimps, *A. foliacea* and *A. antennatus*. Their spatial and temporal distribution, abundance, size structure, maturity, growth and state of exploitation have been studied in the Southern Tyrrhenian Sea.

The abundance of both species, during the last 14 years, showed a significant decrease. Although fluctuations of aristaeidae are known in literature, as periodic events not easily explainable [7, 8, 9, 10], the reason for this suffering could be related to trophic and hydrological factors and to fishing effort increasing in the Southern Tyrrhenian Sea [11].

The estimated exploitation rate (E) for *A. foliacea* and *A. antennatus* showed high values, hence both species seems to be under an overexploitation status. The situation of overexploitation is confirmed by the Virtual Population Analysis (VPA) conducted on commercial catches sampled from 2006 to 2009 inside the fisheries Data Collection Regulation (EU-DCR) Program "CAMPBIOL". The estimate of the current values of the mortality rate for *A. foliacea* varies between 0.43 and 0.60 and for *A. antennatus* varies between 0.88 e 1.30. To assess the effects of fishing on stocks of red shrimps, fishing mortality rates found were compared with the values given in the case of a sustainable exploitation (Biological Reference Points) [12]. Based on the results obtained, to bring the fishing of *A. foliacea* and *A. antennatus* from the current situation of overfishing (Fc) to a situation of bio-economy sustainability (F0.1), it is suggested a reduction of fishing mortality by about 35%. This reduction could be achieved either by reducing the fleet operating in the area and with the reduction in fishing days.

3 Technical measures: gear selectivity

The basic aim of technical measures (such as selectivity of trawl fishing gear) is to avoid or limit the capture of immature fish to allow them to contribute to stock renewal as adults, of unwanted fish because of their lack of commercial value or fish for which fishermen have no more quotas, of marine mammals, birds and other species such as turtles. The EU legislation for the Mediterranean Sea recently introduced a 40 mm square-mesh codends for trawl nets.

A study to analyse the effect of mesh configuration and mesh size on the codend se-

Figure 1: The cover used during the selectivity experiment.

lectivity was recently carried out in Sicilian waters [13]. We investigated the effect of mesh configuration and mesh size on the codend selectivity of the species *Aristaeomorpha foliacea* and *Mullus barbatus*, commonly captured in the Sicilian trawl fisheries. These fisheries are noteworthy for the large number and variety of commercially important species caught. They traditionally operate using small diamond-shape meshes in the codend, which tend to retain almost all animals. At the moment, in Italy the MMS (Minimum Mesh Size) of trawl codends is 40 mm (EC Reg. 1967, 2006), resulting in the capture of many fish below the MLS (Minimum Landing Size). Improving trawl net selectivity is therefore of prime importance. Relatively little scientific work has been done to assess the selectivity of square-mesh codends in the highly variable multi-species conditions prevailing in the Sicilian trawl fisheries. In this experiment four codends with two different mesh sizes across two mesh configurations have been tested: two diamond- (DM44mm and DM54mm) and two square-mesh (SM44mm and SM54mm). Therefore mesh size and mesh configuration were included as explanatory variables in the statistical analysis. Consequently we can discuss the advantage/disadvantage of using e.g. a 40-mm square-mesh codend instead of 50-mm diamond-mesh codends as requested in the new Council Regulation. An ad hoc "cover" was made and used to quantify the fraction escaped from the cod-end (Figures 1 and 2). From the literature we know that the increase in mesh size produces both an increment in L50 (the length at which the specimens have 50% of escape probability) and an unwanted increment in SR (selection range: L75 – L25 where L75 and L25 represent the length at which the specimens have 75% and 25% of escape probability). The results attained in the present study allow to conclude that the use of 44 mm square-mesh codend results in

Figure 2: Cod-end and cover on board.

L50 similar to that of the 54 mm diamond-mesh codend and better SR. The species analysed make the best use of the square mesh opening either because of their body shape or because of forcing the mesh to penetrate their body through. In conclusion, enforcement of installation of square-mesh cod-ends in Mediterranean demersali trawl fisheries can be suitable technical solution to decrease the capture of immature individuals.

During GRUND project the selectivity for the deep water rose shrimp, *Parapenaeus longirostris* (Lucas, 1846) was analysed [14]. A selectivity experiment, using the "covered cod-end technique", was carried out in the Southern Tyrrhenian Sea. Fifty-four hauls were carried out between 10 and 800 m of depth from a commercial vessel equipped with the "Italian Tartana" commonly used for both commercial fishery and experimental survey in this Mediterranean area. 36 and 20 mm diamond (stretched) mesh size were mounted in the cod-end and a cover respectively. On the basis of shrimp carapace length structure, observed both in cod-end and cover, a logistic model was used in order to estimate the retention length at 50%, the selection factor and the selection range. The selection process showed to be highly affected by the bottom location, in the shelf and in the slope. In any case, present selection performance seems to be inadequate for a sustainable and efficient exploitation of the resources. However, the selection parameters obtained were comparable to those found in other Mediterranean and Atlantic areas.

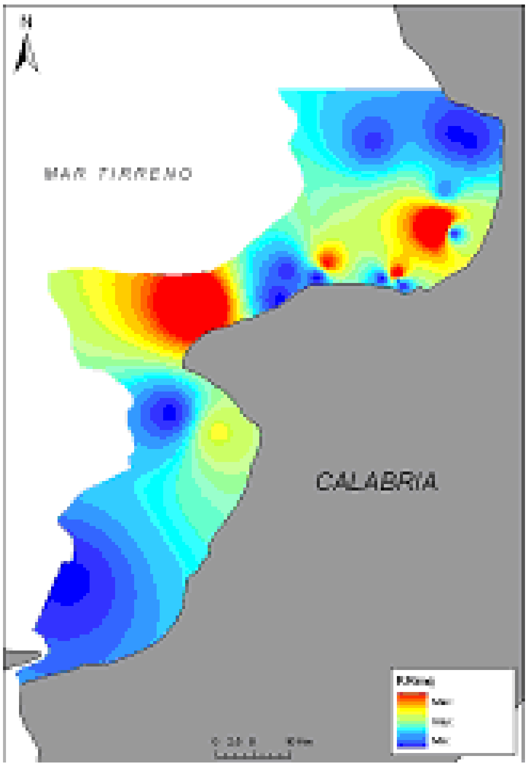

Figure 3: Nursery areas identified along Calabrian coasts.

4 Nursery area and No-Taken Zones (NTZ)

4.1 Distribution of hake (*Merluccius merluccius*) nursery area along Tyrrhenian Calabrian coasts

Data come from eleven seasonal trawl survey carried out from C.po Suvero to P.ta Pezzo. In particular eight GRUND survey (spring-summer 1994-2002), eight MEDITS surveys (autumn1994-2002) and three POR surveys (autumn and summer 2004-2005) were analysed [15]. On the whole 140 hauls were carried out, between 10 m and 800 m of depth, during daylight from the dawn to the sunset. All hake caught were measured to the nearest 0.5 cm (TL) and counted. To separate the recruits from the older specimen a cut-off, elaborated for each survey, is used. On the basis of swept area, the number of recruits "R" of each haul were transformed into R per km^2. The nursery area were identified using a GIS approach. Despite the variability recorded among the different season and year, the density of recruits showed stable areas characterized by an high concentrations of recruits. Two well defined area, over the time of recruits concentration, although characterized by spatio-temporal variability, were identified between 100 and 300 m. The first one is localized in the correspondence of C.po Vaticano and the second one inside the S. Eufemia Gulf (Figure 3).

The surface occupied by nursery area was larger in the southern part of the zone (C.po Vaticano) than in the northern one (S. Eufemia Gulf). The average density of the recruits recorded in each survey was statistically different (ANOVA $p < 0.005$). During the autumn (GRUND data) two main patches were identified. A large nursery area between 100 and 300 m has been localized in the correspondence of C.po Vaticano and it is quite stable during the year; infact only during 1997 and 2000 survey it was not found. The highest density of recruits was observed in this nursery area during 2001 (2662 $\mathrm{R{\cdot}km^{-2}}$). The second area, localized inside the S. Eufemia Gulf always at depth comprised between 100 and 300 m, was observed only in three years (1997, 2001 and 2002). The highest density of recruits was recorded during 2001 (6877 $\mathrm{R{\cdot}km^{-2}}$).

4.2 Monitoring No-Taken Zones: Gulfs of Patti and Castellammare

The implementation of no-taken zones (NTZs, i.e., areas in which general or specific fishing is temporarily or permanently restricted) might represent a proper management tools especially in situation of severe stocks depletion and conflicts between users. These two basic aims induced the Sicilian regional administration in creating two NTZs, in terms of bottom trawling banning, in 1990, in the Gulf of Patti and the Gulf of Castellammare, respectively. The two areas are located along the northern Sicilian coasts, and interested all the shelf bottoms ($<$ 200m) and part of the slope (200-500m). The two areas have been routinely explored by experimental bottom trawl surveys by the Operative Unit of CNR of Messina within both national (GRUND) and international (MEDITS) programs. The possibility to use bottom trawl surveys routinely carried out in Italian Seas as a possible tool to monitor the evolution of protected areas has been evaluated [16]. The shelf hauls performed in two gulfs, Castellammare and Patti (Northern coast of the Sicily), where bottom trawling has been banned since 1990, were used. The standing stock for the pooled species and for *Pagellus erythrinus* showed high variability but not significant temporal trend. *Mullus barbatus* decreased in both gulfs, although only in Castellammare the trend is significative. The mean size in the catch vs the mean size at maturity indicates a better situation for *Pagellus erythrinus* than *Mullus barbatus*. The impressive stock recovery, observed in the Gulf of Castellammare in the first four years after the introduction of the fishing ban ([17]), have been likely attenuated (*Pagellus*) and slightly decreased (*Mullus*) respectively. These results are anyway in accordance to what observed [18] after eight year of the ban. The strong year by year variability, likely reflecting also the interaction between the life cycle and the survey periodicities [19], can be smoothed out by the analysis of GRUND data.

5 Analysis of demersal assemblages

In the Mediterranean Sea, trawl catches are composed of a highly diversified mix of fish, cephalopods, crustaceans and several epifaunal macrobenthic species [20]. Monitoring and management of Mediterranean trawling requires a multispecies and ecosystem-based approach. In this context the study of demersal assemblages is a fundamental and priority aspect. This kind of analysis permits to collect some important information which can be correlated with the "status" of the studied biotopes and that can be useful for the management of the alieutic resources. The demersal assemblages off the Southern Tyrrhenian Sea have been analysed [21, 22]. Specifically, the structure of fish demersal assemblages has been analysed on a temporal (10 years) and spatial scale using a multivariate approach; ecological indices (Shannon-Wiener and Pielou) for each group resulting from Multidimensional Scaling Ordination were calculated; areas supporting high values of diversity and evenness has been identified by a Geographical Information System (GIS) method.

Multidimensional scaling ordination showed four groups distributed according to the depth gradient: a coastal group (< 100 m depth), a group in the lower part of the continental shelf (101 – 200 m), an epibathyal group (201 - 500 m) and the last one derived from hauls made in the middleslope. All the study area was characterised by diversity values quite heterogeneous. The persistence index was generally low. Results could be useful from an ecological point of view and for the management of fisheries activities [22].

6 Parasitosis effects on ichthyc populations

Parasites are the main cause of pathologies in fishery products. Parasites may be cause of zoonosis, may contain toxic substances in their body fluids, can cause breathing difficulties, severe anemia and emaciation in fish infested with consequent economic damage, may reduce the reproductive performance, can cause changes in chemical and nutritional profile of meat.

Studies on evaluation of parasitosis effects on ichthyc populations have been carried out in cooperation with the Department of Veterinary Public Health - University of Messina. Specifically, several species of pandalid shrimps were examined for occurrence of isopods. *Pseudione affinis* (Epicaridea: Bopyridae) parasitized *Plesionika martia*, *P. antigay* and *P. edwardii*. Parasitic effects on the hosts included carapace dilation and gill atrophy [23].

The presence of Unitubulotestis sardae (Trematoda: Didymozoida) in the gills of Atlantic bonito (*Sarda sarda*) populations caught in the strait of Messina has been reported. The histopathological change were neovascularisation and hyperplasia of the gill epithelium, leading to the formation of a bistratified cystic wall; SEM investigations and tissue damage due to Gnathia vorax (Isopoda Gnathiidae) larvae have been reported [24]. Severe infestations affected different teleosts species. The amount of blood sucked up by single parasite was evaluate. Haematophagia may play an important role in the pathogenesis of such infestation [25].

A research on Aggregata sp. infection in Sepia officinalis and the influence of parasitosis on biometrics was carried out. Parasites were found in the 100% of samples (gills 96.7%, caeca 90%, intestina 43.3%). The frequency and gravity of infection were not related to mantel length, weight and thickness. Potential implications of infection on cephalopods quality were discussed [26].

7 Genetic identifications of demersal stocks

In fishery management, a unit of stock is normally regarded as a group of fish exploited in a specific area or by a specific method [27]. The stock concept has two central argument: that fish species are subdivided into local populations and that there may be genetic differences between local populations which are adaptive. Genetics and fishery management can interact in several ways [28]. When the genetic population structure of a species is known, the distribution of subpopulations in mixed fisheries can be estimated [29]. The general goals of population genetics studies are to characterize the extent of genetic variation within species and account for this variation [30]. The amount of genetic variation within and between populations can be determined by the frequency of genes and the forces that affect their frequencies, such as migration, mutation, selection and genetic drift [31]. Fluctuations in environmental conditions and population density may cause considerable variability. Important factors in genetic variability, like selection, migration and genetic drift are affected by human activities. For example, selection, in general, is a process by which the future contribution of some genotypes to the next generation is limited and it is the dominant mode of human interaction with fish population [32]. Size selective fishing gear, destruction of habitat, alteration of prey availability, pollution stress and other such activities can impose new selection pressure on a stock or may alter the existing selection forces. The dangers inherent in subdivision of fish populations are that imbreeding and genetic drift will lead to fixation of genes, loss of fitness (vigour, fecundity, resistance to disease) and ultimately extinction of local populations [33]. Stress such as exploitation may augment these dangers through effects on effective population size [32]. A low level of gene flow can serve to prevent genetic differentiation. Marine fish stocks exchange between one or two orders of magnitude more migrants per generation than fresh species [34]. Gene flow rates of a few individuals per generation would mean that populations cannot be distinguished genetically and would appear to be panmictic, yet for fisheries management an exchange of up to 10% between populations may justify their treatment as separate stocks [35].
The IAMC Institute of Messina is now involved in determining the genetic differences on *Mullus barbatus* populations coming from different Mediterranean sites.

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A Multimarker Approach to Assess the Environmental Pollution on Biological Resources Subject of Commercial Fishing: the Case of *Mullus barbatus* (Linnaeus, 1758) in the Southern Tyrrhenian Sea

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Abstract

A set of biomarkers were used to evaluate the environmental quality of a marine area along the Sicilian Tyrrhenian coast, subjected to a high anthropic impact. Biological responses to environmental stress were investigated in a commercially valuable fish, the red mullet *Mullus barbatus* (L. 1758), which has also been recommended as one of the most important sentinel organisms for the Mediterranean sea (UNEP/RAMOGE, 1999). Liver cytochrome P4501A levels, measured as ethoxyresorufin-O-deethylase activity, glutathione S-transferase and UDP-glucuronosy transferase activities were detected as specific biomarkers of exposure to PCBs and PAHs. In order to investigate the impact of other anthropogenic chemicals widely present in marine coastal areas due to agricultural run-off, acetylcholinesterase activity was also measured. Vitellogenin gene induction was assayed in adult male specimens as a biomarker of exposure to estrogenic compounds. Gonad developmental stage and gonad alterations, such as intersexuality and/or fibrosis, were also investigated histologically to assess potential disturbances in reproduction.

1 Introduction

The NW Mediterranean Sea is the recipient of extensive urban and industrial wastewater discharges from bordering countries, through continental runoff, sewage sludge disposal, and atmospheric deposition. It is estimated that up to now more than 100.000 chemical compounds and xenobiotics have been produced by chemical industry, many of which are persistent: because of their ability to bind to organic matter, they may accumulate up the food chain, especially in species at the apex of the chain itself [1, 2], constituting a potential risk factor for the entire marine biotic component, but also for humans, as final consumer. The primary toxicity of a contaminant is exercised primarily at biochemical and molecular levels and only later the effects may be observed, by a cascade mechanism, through the hyerarchical ladder of

the organization (organelle, cell, tissue, individual) until reaching the population [3].
Regarding biochemical responses of fish to aquatic pollutants, the mixed function oxidase system P450 is known to play a major role in the oxidative metabolism/biotransformation of toxic compounds such as chlorinated and aromatic hydrocarbons [4, 5, 6]. The induction of EROD activity has been successfully used in assessing environmental pollution as specific markers of exposure to PCBs and PAHs in a variety of fish species [5, 7].
The inhibition of AChE activity is a specific biological effect of exposure to agricultural pesticides including organophosphates (OPs) and carbamates (CBs) [8] but more recently, its field application in biomonitoring programmes on fish species has been reported to be less specific, thus it is now emerges as a more general marker of exposure to neurotoxic contaminants including heavy metals and organochlorines [9, 10].
Some xenobiotics compounds, called Endocrine Disruptors Compounds (EDCs), can interfere at various levels and through different mechanisms, with the normal hormonal regulation of organisms. Numerous studies on EDCs highlight the reproductive disorders induced by them, such as decrease in the number and quality of eggs [11, 12, 11], changes in the number and viability of sperms [13, 14], alterations in sexual differentiation and development [15], intersexuality [16, 17, 14], stimulation of estrogen receptors in immature males, stimulation of hepatic vitellogenesis in males and juveniles [18, 19, 17, 20].
In ecotoxicological studies the use of a wide battery of biological responses is recommended since single biomarkers cannot reflect impairment of an organism's health and/or adaption to impaired environmental conditions [21, 22].
The selected bioindicator fish species, *Mullus barbatus*, is widely used in environmental monitoring programs in the Mediterranean Sea [23, 24, 25, 26, 14, 27, 28, 29]. The red mullet is a benthic species, wide distributed and easy to find, considered of high commercial value and with well-known biological features [30, 31], which because of its territorial characteristics (habitat, feeding mode and degree of mobility),is suitable for biomonitoring investigations [32, 33]. Due to their close association with sediments these fish tend to concentrate contaminants to a higher degree than other species.
In this paper, a set of biomarkers were used to evaluate the environmental quality of a coastal marine area with high anthropic impact. The effects of exposure to chemical contaminants were investigated in a sentinel species, the red mullet *M. barbatus* (L., 1758) and responses to environmental stress were detected.

2 Materials and methods

2.1 Study area

The area selected to carry out this study lies in the Tyrrhenian sea, along the northern coast of Sicily (Figure 1). The selection of the sampling sites was as follows: site 1, a low impacted area, close to cape Tindari, characterized by being a not taken zone; site 2 (7 Km far from site 1), a moderately impacted area within the gulf of Patti, recipient of both urban and agricultural drains; and site 3 (30.65 Km far from site 1), just in front of Milazzo, affected by the presence of an oil refinery, as well as by a most important commercial harbour.

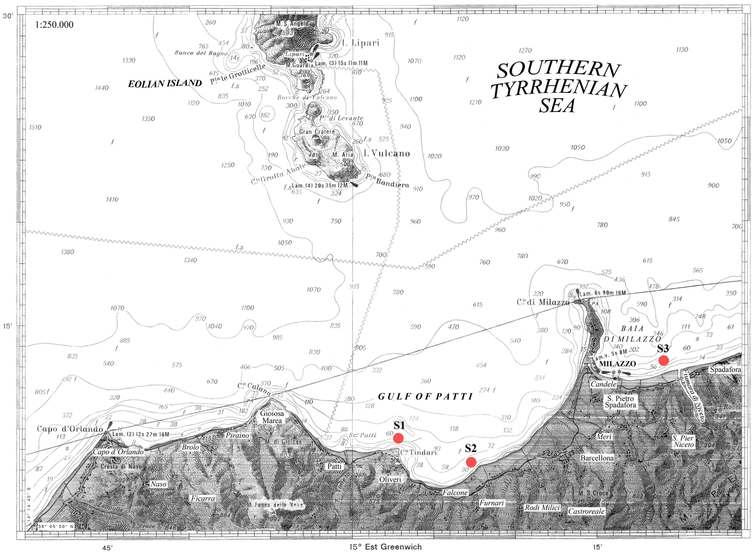

Figure 1: Study area with sampling sites.

Target gene	Primer sequence		Amplicon size	Annealing
	Foward	Reverse	(nucleotides)	temperature (°C)
Vtg	CAGGGAGAAGATGACCCAGAT	AGAGATGCCCTCACCCTTTG	126	59
β-actin	CAGGGAGAAGATGACCCAGAT	GATACCGCAGGACTCCATACC	~430	57

Table 1: Primer pair sequences, amplicon size and annealing temperatures used for vitellogenin expression analysis.

2.2 Sampling

Red mullets, *Mullus barbatus* (mean LT $\pm$ SD) were collected by trawl surveys in June 2007 and January 2008 at 50 m depth. After sexing macroscopically, total length (TL, cm) and body weight (BW, g) of males were recorded. Liver and brain were frozen in liquid nitrogen and stored at -80 °C, until laboratory analysis. Gonads were fixed for 24 h in Bouin's solution and stored in 70° ethanol for subsequent histological analysis.

2.3 Biochemical and catalytic assays

Cytosolic and microsomial fractions were prepared according to Corsi et al. [34]. Liver microsomal 7-ethoxyresorufin-O- deethylase (EROD) activities were measured in duplicate according to the spectofluorimetric assays of Burke and Mayer [35] using a Perkin-Elmer LS50B luminescence spectrofluorimeter. UDP-glucuronosyltransferase activity was performed according to Aitio[36] modified by Collier et al. [37]. The glutathione S-transferase activity was measured by the

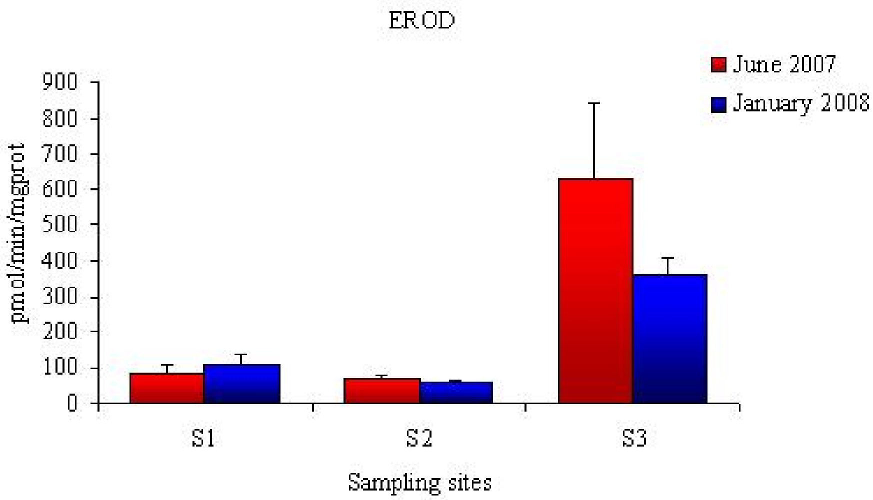

Figure 2: EROD activity levels (pmol min^{-1} mg prot^{-1} ± s.d.) in *M. barbatus* in three different sites and two sampling periods.

spectrophotometric method described by Habig et al. [38] modified for microplate readers. AChE activity was measured on microplate by the method of Ellman et al. [39]. Total proteins were measured according to Bradford [40] and total proteins concentration was expressed in mg·ml^{-1}.

2.4 Total RNA isolation and reverse transcription polymerase chain reaction (RT-PCR)

The expression of the vitellogenin gene was qualitatively investigated on 45 and 14 male specimens from the summer and winter surveys, respectively. In particular, in June 16 from site S1 (size range 13.5-22 cm), 21 from site S2 (size range 15-22), and 8 from the site S3 (size range 15.5-20 cm), and in January 8 from the site S1 (size range 12-19.5 cm), 3 from site S2 (size range 16-19 cm) and 3 from site 3 (size range 12.5-16.5 cm) were collected. The small number of individuals analyzed for the winter survey was caused by the loss of some samples. Six females, one per survey and site, were analyzed as positive control. Total RNA from liver tissue was extracted in Trizol reagent, following the procedure recommended by the manufacturer (Invitrogen). The total RNA was digested with RNase free DNases to remove endogenous DNA contamination. Primer pair sequences, their amplicon size and annealing temperatures are shown in Table 1. All primer pairs gave a single band pattern for the expected amplicon size in all reactions, and no amplification occurred in RT reactions without enzymes.

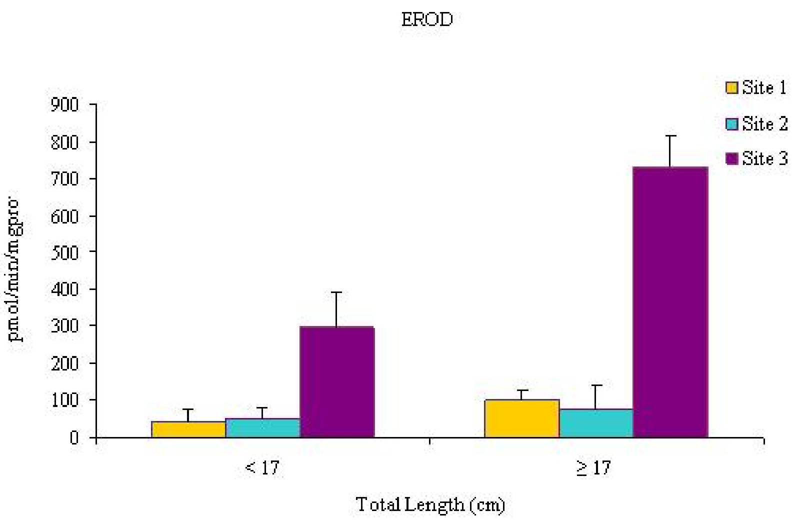

Figure 3: EROD activity levels (pmol min^{-1} mg prot^{-1} $\pm$ s.d.) in different size classes of *M. barbatus*.

1 µg of total RNA from each liver sample was used to synthesize cDNA. The fragments of genes of interest, vitellogenin and β-actin (used as housekeeping gene), were subsequently amplified in Thermal cycler MX3000 (Statagene). PCR was carried out in a 20 µl reaction mixture and conducted at 95°C for 15 min, 32 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, extension at 72 °C for 1 min, and terminated for 10 min at 72 °C. The PCR products were visualized by electrophoresis in agarose with ethidium bromide staining.

2.5 Histological analysis

Gonadal samples were dehydrated in a graded series of ethanol, cleared in xylene, embedded in resin, cut in 5 µm-thick sections and stained with haematoxylin-eosin. Testes were staged for maturity as follows: immature/resting (only spermatogonia) (stage I); developing (spermatocytes and spermatids, no spermatozoa) (stage II); maturing (all spermatogenic stages, including spermatozoa, in the seminiferous lobules (stage III); running (spermatozoa in the main sperm duct) (stage IV); spent-recovering (residual spermatozoa and spermatogonia) (stage V). The gonads were also examined for histopathological alterations, such as necrosis, degeneration, feminization, and/or delayed or arrested gamete development.

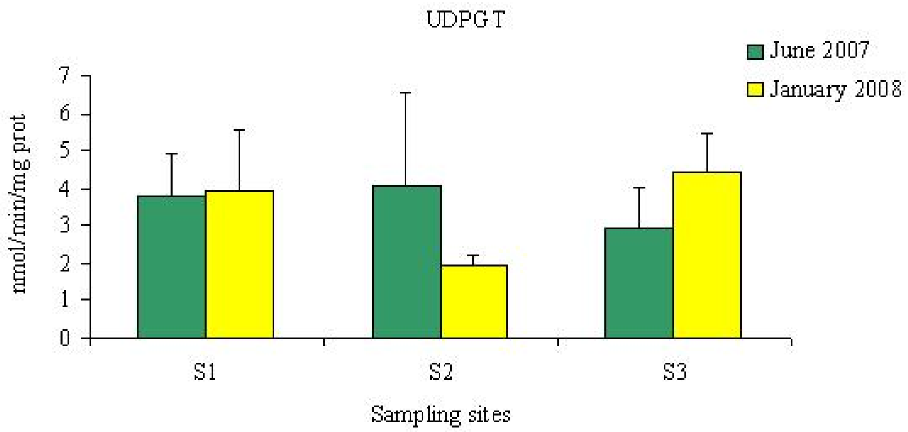

Figure 4: UDPGT activity levels (nmol min^{-1} mg prot^{-1} $\pm$ s.d.) in *M. barbatus* in three different sites and two sampling periods.

2.6 Statistical analysis

Biochemical activities were determined individually in 3-8 specimens per station, and the data are reported as mean $\pm$ S.D. The statistical analysis of data was done using one-way analysis of variance (ANOVA). The homogeneity of variances and the normality of data were checked using respectively Levene's test and Kolmogorov-Smirnov test, respectively. A p value $\leq$ 0.05 was considered as statistically significant. Data on EROD, GST, UDPGT and AChE were compared between survey, sites and size classes.

3 Results

7-ethoxyresorufin-O-deethylase activities for the three sampling sites in both survey (June 2007 and January 2008), are shown in Figure 2. These were significantly higher ($p<0.001$) in the samples from site 3 than in the other sample sites, both in summer and winter survey. There were no significant differences between sites 1 and 2 (p=0.97). No significant differences were noted in EROD activity between the summer and winter sampling, in either site 1 (p=0.34), 2 (p=0.42), or 3 (p=0.50). Comparing enzyme activities between two size classes ($\leq$ 17 cm and $\geq$ 17 cm), corresponding to younger and older than 1 year, respectively (Project CAMP-BIOL, 2006), it was evidenced that larger individuals showed a greater induction of EROD activity (Figure 3).

Particularly, there was a significant difference between size ($p \leq 0.001$) in sites 1 and 3, but not in site 2 (p=0.50). Comparing the two size classes in the two sampling periods and different sites investigated, there was no significant difference in summer for site 1 (p=0.22) and site 2 (p=0.74), while in site 3, the two size classes were significantly different ($p \leq 0.05$). In twinter sur-

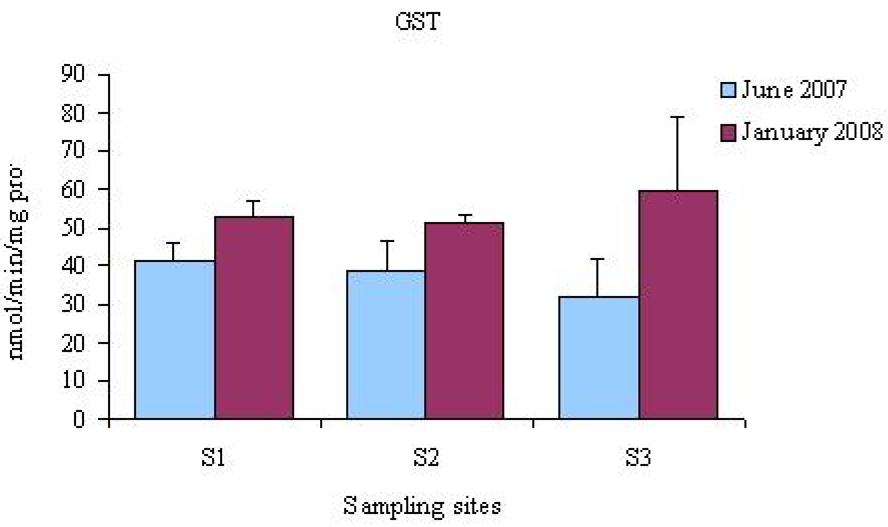

Figure 5: GST activity levels (nmol min^{-1} mg $prot^{-1}$ $\pm$ s.d.) in *M. barbatus* in three different sites and two sampling periods.

vey EROD activities were significantly different between sizes in site 1 ($p \leq 0.01$) but not in site 2 (p=0.06). The number of samples in site 3 was insufficient for comparison.

The results of UDPGT and GST activities are presented in Figures 4 and 5, respectively. The UDPGT activities ranged between 2 and 4.4 nmol min^{-1} mg $prot^{-1}$, and they did not show significant differeces between sampling sites (S1: p=0.84, S2: p=0.55, S3: p=0.72). There were no significant differences between the levels of UDPGT activity in the summer and winter sample period, either in site 1 (p=0.85), 2 (p=0.19) or 3 (p=0.10). Comparing the different size classes, no significant difference was found in any of the three sites (S1: p=0.84, S2: p=0.55; S3: p=0.72). Comparing the UDPGT activity for the two size classes in the two sampling periods and in different sites, there was no significant difference either in the first year (S1: p=0.66, S2: p=0.52, S3: p=0.62), nor in the second (S1: p=0.90, S2: p=0.22, S3: insufficient number of samples for comparison).

The GST activities ranged between 32.2 to 59.4 nmol min^{-1} mg $prot^{-1}$ and revealed significant seasonal differences ($p<0.001$), but no differences between sites (p=0.64). No significant difference between size classes was found in any of the three sites (S1: p=0,31; S2: p=0,31; S3: p=0,62). Comparing the GST activity for size classes in the two sampling periods and in different sites, there was no significant difference either in the first year (S1: p=0.23, S2: p=0.10, S3: p=0.30), nor in the second (S1: p=0.87, S2: p=0.26, S3: insufficient number of samples for comparison).

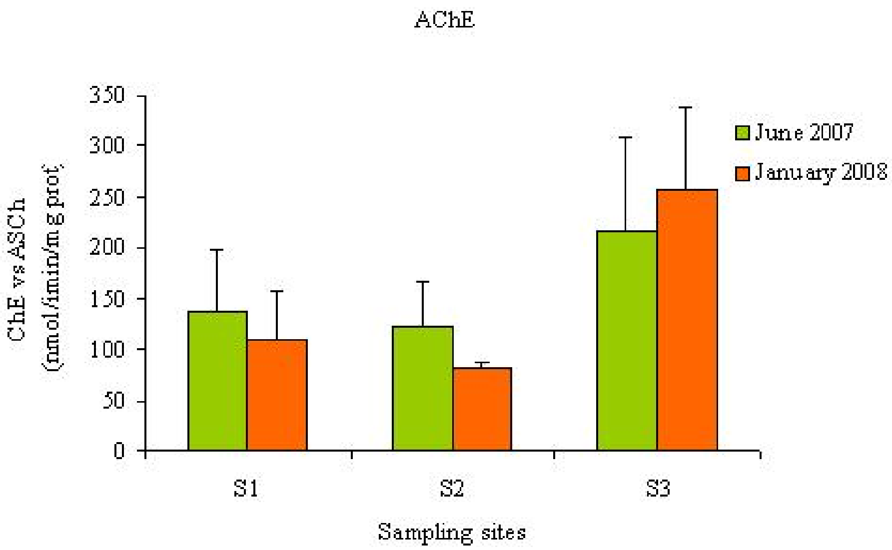

Figure 6: ChE vs ASCh activity levels (nmol min^{-1} mg prot^{-1} $\pm$ s.d.) in *M. barbatus* expressed in three different sites and two sampling periods.

The results of the analysis of acetylcholinesterase activity of *M. barbatus* are shown in Figure 6. Inhibition of AChE was significantly greater in site 1 and 2 (Gulf of Patti) both in first (p¡0.05) and in the second survey (p¡0.01) than in the site 3 (Milazzo refinery). No significant difference was observed between the AChE activities of the specimens collected from these two sites, either in the June (p=0.92) nor in January (p=0.70).
Regarding the comparison of data for the two surveys, there were no significant differences in this enzyme activity between summer and winter survey, either in site 1 (p=0.42) or site 2 (p=0.13) or site 3 (p=0.53).
Regarding the comparison between size classes, it wasn't significant difference in any of the three sites (S1: p=0.30; S2: p=0.76; S3: p=0.46). Comparing the AChE activities for the two classes of size in the two sampling periods and in different sites, there was no significant difference either in the first (S1: p=0.64, S2: p=0.85, S3: p=0.68), nor in the second survey (S1: p=0.35, S2: p=0.14, S3: insufficient number of samples for comparison).
Vitellogenin gene was found to be expressed only in a specimen of 16 cm taken from site 3 in summer survey (Figure 7).
All the examined individuals from the three sites had testes at maturity stages III and IV, in both summer and winter samplings (Figures 8 and 9). None of the examined specimens showed any signs of histopathological alterations, such as necrosis, degeneration, feminization, and/or delayed or arrested gamete development.

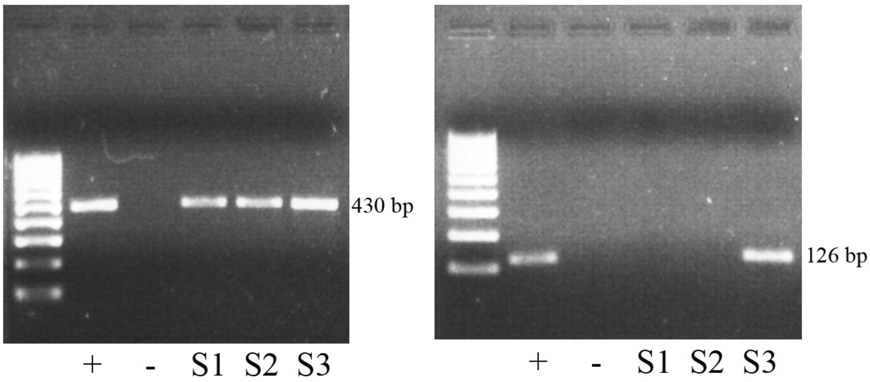

Figure 7: Vitellogenin gene expression in a male from S3 site (right). On the left, expression of the housekeeping gene (β-actin). + and - indicate respectively the positive and negative control. A marker of 100 bp was used.

4 Discussion and conclusions

The use of biological markers, measured at a molecular or cellular level, has been proposed in the last ten years as a sensitive early warning tool for the measurement of biological effects in environmental quality assessment [41]. The analysis of induction of cytochrome P450 enzyme activities in fish is one of the most effective biomarkers in the measurement of environmental quality, and several studies of environmental monitoring have found an induction of the activities of this multi-enzyme complex in relation to presence of anthropogenic sources of pollution, such as industries, urban sewage and harbours.

It is known that the mixed-function monooxygenase is induced by PAHs, but also from other compounds, such as polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and PCB congeners in many species of fish [42, 43, 44, 45, 4, 46, 47].

In the present study, the highest EROD activity was measured in both summer and winter surveys in samples from site 3 (Milazzo), with values comparable to those reported by Porte et al. [28] in an area influenced by both urban and industrial wastes from Marseilles (about 650 pmol/min/mgprot). In fact, the Milazzo area is known as one of the most contaminated in the Mediterranean sea [24], being characterized by continuous discharges of pollutants deriving from oil processing., as well as from ship traffic, manoeuvring for loading and unloading of oil tankers and discharging of ballast waters. Moreover, a little further east of the oil refinery, the river Niceto flows, another possible source of contaminants inducing CYP450. Contamination by PAHs and heavy metals has subsequently been reported in sediments from the same area [48].

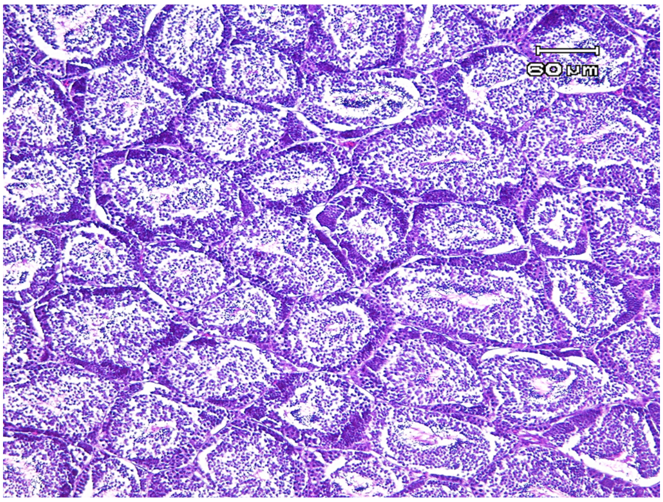

Figure 8: Histological section of a *M. barbatus* testis at stage III (maturing).

Sites 1 and 2 showed EROD activities significantly lower than site 3, but they were however moderately impacted, because the values in these sites are still high, compared to those reported in the literature from Burgeot, [24] (4.9-19.4 pmol/min/mg prot) and Corsi [25] (2.5-17.5 pmol/min/mg prot) for areas not industrialized. There were no significant differences between these two sites, probably because, although distant from each other (7 Km), both fall within the Gulf of Patti. This closed area, as reflected in the data provided by stations of the Air Weather Service of Messina, is characterized by winds coming mainly from the northwestern quadrant, which at low depth (up to 100 meters), are responsible of the South-East currents insisting on the coast. This causes a movement of water within the Gulf of Patti that goes from site 1 to site 2.

It is known that gonad activity may influence P450 cytochrome activity in females, as demonstrated by EROD activity decreasing [49], or being totally inhibited [50] during the spawning season. For this reason, enzyme activities were assayed in males, and, as expected, they did not show any significant seasonal differences. On the other hand, the size of the fish seems to have an influence on EROD activity, with significantly higher values in larger specimens (¿17 cm). These are older individuals (¿ 1 year), which may have been exposed to contaminants for a longer time, with consequent increase of detoxificating activity.

GST and UDPGT enzymes, involved in phase II detoxification process, are sensitive to exposure to PAHs [51, 52]. Although GST and UDPGT activities are used as biomarkers in environmental monitoring programs, these indicators are less sensitive than the enzyme activity of phase I. From the results obtained in this study the GST and UDPGT activity appeared lower than those reported in the literature by Regoli et al. [21] and Burgeot et al. [24]. In particular, UDPGT did not change

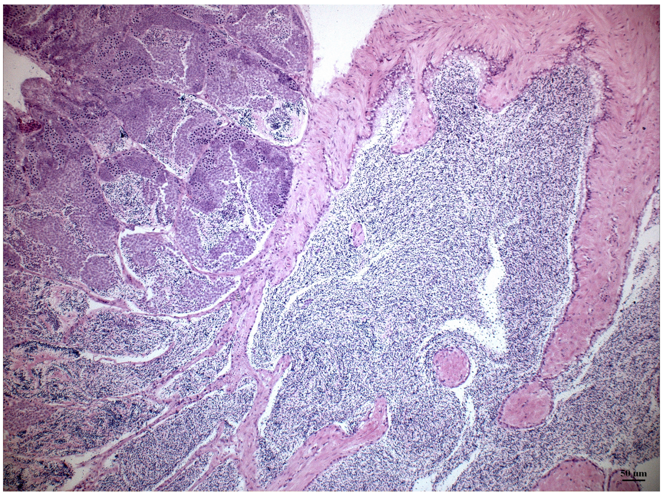

Figure 9: Histological section of a *M. barbatus* testis at stage IV (running).

significantly either between sites or seasons, unlike GST, which appeared to be affected by seasonality.

A significant inhibition of cholinergic enzyme AChE was recorded in brain tissue of red mullets in site 3, with values around 250 nmol/min/mg prot. AChE activity showed no significant differences between sites 1 and 2, where there have been similar values to those reported by Lionetto et al. [26] (70-130 nmol/min/mg prot) in harbour areas. More than organophosphate and carbamate insecticides, the AChE can also be inhibited by heavy metals, which could explain the values so high (almost twice) of this enzyme activity in site 3 highly industrialized and adjacent to the harbour of Milazzo. The results may suggest the exposure of red mullets to the AChE inhibitor compounds in sediments in front of the refinery and the harbour area of Milazzo.

Most studies on the aquatic environment have evaluated the content of specific endocrine disruptors in surface waters [53, 54], in effluents from sewage treatment of civilian discharges [55, 56] and industrial discharges. Martin-Skilton et al. [14] investigated gonadal changes caused by endocrine disruption in *M. barbatus* in the North-West Mediterranean. These Authors reported delayed gamete maturation, intersexuality and fibrosis in fish from a highly impacted area, near the city of Marseille. These phenomena were more evident during the reproductive period (spring). In our study, however, although biomolecular analysis revealed the induction of vitellogenin gene in a single specimen from the Milazzo site, suggesting exposure to xenoestrogens, histology showed no pathological changes of the gonads, such as fibrosis or feminization, or inhibition of gamete maturation. Therefore, the reproductive potential of these specimens was not affected by exposure to contaminants. Histological analysis of female gonads is currently under way in order to validate further the results obtained so far.

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Recruitment Dynamics of Young-of-the-Year Marine Fish in the Coastal Lagoon of Lesina

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Abstract

Coastal lagoons are aquatic ecosystems characterised by a high productivity. The artisanal fishing has always been practiced in these areas due to the high yield. This kind of fishery exploits the migratory movements of euryhaline marine fish species between the open sea and the lagoon. The recruitment of these economically valuable marine species to the tidal channels of Lesina Lagoon, Italy, was monitored during their maximum recruitment period and the distribution of the juveniles in the lagoon itself was studied for a year. Sampling was conducted using winged 2-mm mesh fyke-nets and a manual beach seine with a 2-mm mesh central bag. The majority of the marine species juveniles entered in lagoon during the late autumn and winter. The Mugilidae family was found to be the most abundant. *Liza ramada* recruited in the largest numbers, followed by *Liza aurata* and *Mugil cephalus*. The Sparidae family was represented by only two species, *Sparus aurata* and *Diplodus vulgaris* with few individuals. The highest abundances of Mugilidae juveniles were found at stations situated near the mouths of freshwater channels. These findings may be useful tools for the conservation and management of lagoon fish stocks in terms of essential fish habitat protection and suitable fishery management.

1 Introduction

Euryhaline marine fish species use coastal lagoons and estuaries as nursery grounds [1, 2, 3, 4, 5, 6]. These ecosystems provide the juveniles of euryhaline marine species with abundant food and optimal environmental conditions for rapid growth, thus helping them to quickly get through the critical phase of their life history [7, 8, 9, 10]. In addition, the shallow depth of these basins and the presence of extensive seagrass beds mean that the pressure on these organisms from predators is lower than in the nearby marine environment [11].
Many of these species (including *Chelon labrosus, Liza aurata, Liza ramada, Liza saliens, Mugil cephalus, Dicentrarchus labrax, Sparus aurata*, some *Diplodus* species and the diadromous species *Anguilla anguilla*), which enter Mediterranean coastal lagoons during their early life stage, have significant economic value. Juvenile fish start to enter lagoon basins at 10-30 mm standard length (SL) [12]. When they have reached sexual maturity, they return to the sea to reproduce [13]. Artisanal fishing in Mediterranean lagoons exploits the migratory movements of euryhaline marine species between marine and lagoon environments.
Once inside these complex ecosystems, juvenile fish colonise the areas which are most favourable to their survival and

growth [14, 15]. Many studies have been conducted on the functional role of shallow water environments, and correlations have been found between abundance of juveniles and environmental factors such as temperature, salinity, turbidity, availability of food, presence of predators and competitors and the structural characteristics of the habitat, especially plant coverage and substrate, i.e., type of sediment [11, 16, 17, 18, 19]. The effects of these factors seem to differ from species to species depending on the case study. Species distribution is often the result of a combination of several factors [20].

Coastal lagoons and transitional water systems in general are often strongly affected by human activities (agriculture, urbanisation, industrialisation, fishing and aquaculture) and by their position between sea and continent. The artisanal and non-selective fishing traditionally practised in lagoon systems affects the recruitment of migratory fish assemblages, as does pollution, to which lagoon ecosystems are particularly vulnerable. The high residence times of both waters and sediments mean that they tend to accumulate pollutants, often causing reduction (or degradation) of essential habitats for fish species, such as nursery grounds. As a consequence, the conservation and management of lagoon fish populations now require continuous monitoring of fishing and the designation and conservation of nursery grounds for commercially important species [4]. However, proper management is hindered by the lack of important information on the ecology of each species (including the volume of recruitment and the criteria adopted by fish juveniles for habitat selection in the lagoon basin), information which needs to be combined with data on the estimated catch [4].

In this context, the objectives of this study were 1) to verify the migration calendar of economically important marine species entering Lesina Lagoon, 2) to assess the volume of recruitment and 3) to describe the spatial distribution patterns of these species in the lagoon.

2 Materials and Methods

2.1 Study area

Lesina Lagoon is situated on the Adriatic coast of Southern Italy (15°45′ E, 41°88′ N) (Figure 1). It has been declared a Site of Community Importance and is included in the Gargano National Park. The lagoon is oblong in shape, 22.4 km long and 2.4 km wide on average. It has an area of 5136 ha and a catchment basin of about 460 km^2 [21]. The average depth is about 0.7 m and the maximum depth is about 1.5 m. It is separated from the sea by a sandbar about 16 km long and 1-2 km wide. At a point approximately 7 km from the western end, the lagoon narrows, effectively creating two sub-basins.

The lagoon is connected to the sea by two tidal channels, Acquarotta and Schiapparo. The Acquarotta Channel, which links the western sub-basin of the lagoon with the sea, is about 3 km long, 6 to 10 m wide and from 0.8 to 2 m deep. At its seaward end there is an unauthorised landing stage for fishing boats. The Schiapparo Channel, which joins the eastern part of the lagoon to the sea, is about 1 km long, about 25 m wide and between 2 and 4 m deep. The two channels have sluices which serve to regulate exchanges between the lagoon and the sea and complex fishing systems called *lavorieri*, which trap adult fish moving to the sea but allow juveniles and small-sized

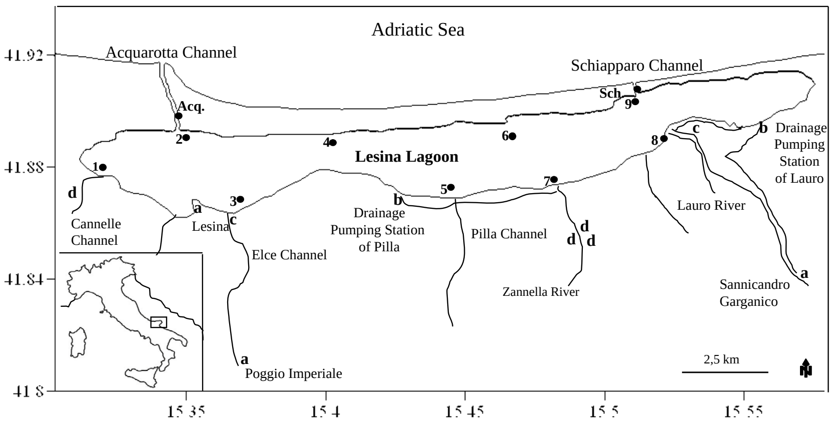

Figure 1: Location of sampling stations in Lesina Lagoon. Population centres, a; drainage pumping stations, b; urban wastewater treatment plants, c; fish farms, d.

adult fish to enter.

Freshwater inputs are supplied mainly by two year-round tributaries, Lauro and Zannella, whose waters enter the lagoon at the eastern end. There are also numerous other intermittent channels and two drainage pumping stations, Lauro and Pilla, also located in the eastern part of the lagoon, which pump waters drained from the surrounding countryside. Lesina Lagoon has a characteristic E-W salinity gradient due to the concentration of freshwater inputs in the eastern area of the lagoon, which becomes more pronounced during summer. Salinity also varies from one year to another depending on the volume of water exchange with the sea, the amount of precipitation and the pumping of surface waters for the irrigation of nearby agricultural land. Since the construction of the *lavorieri* in 1999, salinity has decreased continuously and currently varies from 6 to 38 [22, 23]. The gradient has also become less intense.

Fishers' cooperatives run the fishing areas under concessions which are granted at the beginning of the season. The artisanal fishing they practice in the lagoon is based mainly on the use of fixed nets, known locally as *paranze*. These are nylon seines with a 6-mm mesh stretched across the lagoon from one side to the other, which channel the fish into fyke-nets (also with a 6-mm mesh) positioned at regular intervals along them. These are installed in September and kept in use until February of the following year.

2.2 Sampling and data analysis

Between September 2006 and May 2007, every fifteen days, a fyke-net with a 2-mm mesh and wings about 130 cm long was placed near the lagoon end of each channel for 24 hours with the mouth facing towards the sea (Figure 1). The catch was removed about every six hours to prevent the animals from dying. Young-of-the-year

(YOY) fish of euryhaline marine species were separated from the rest of the sample and fixed in 4% buffered formaldehyde. In the laboratory, the specimens were identified at species level and counted. Sub-samples were taken at each sampling and the SL of each specimen in the sub-sample was then measured. Between January and March 2007, the recruitment period for *A. anguilla* in Lesina Lagoon [24], the migration of glass eels through the Acquarotta Channel was measured daily: two fyke-nets, modified for the capture of the glass eels (1.6 m long, 0.43 m wide, with a 2 mm mesh and wings of different lengths - 1.3 m and 1.65 m) were placed on the two sides of the channel. The glass eels were counted in situ and a sub-sample of these was taken to the laboratory. Here the specimens were anaesthetised in Eugenol diluted in water, and their total length (TL) was measured. On waking the glass eels were returned to the lagoon.

Sampling in the lagoon basin was carried out monthly from October 2006 to August 2007 in nine sampling stations (Figure 1). Stations 1, 3, 5, 7 and 8 were located near the mouths of freshwater channels. Specifically, stations 3 and 8 were situated in areas that received the partially treated waste waters of three municipalities (with a total of about 30,000 inhabitants). Station 5 was located near the mouth of the Pilla Channel which discharges waters drained from nearby arable land. Stations 1 and 7 were positioned in areas affected by the discharge of effluent from buffalo and fish farms, by small channels on the western side and by the Zannella River on the eastern side. Stations 4 and 6 were located on the seaward side of the lagoon. Finally, stations 2 and 9 were located near the two tidal channels.

The fish were captured using a manual beach seine (11 m long and 1.30 m high, the central section having a 2-mm mesh and the two lateral sections a 4-mm mesh) with a central bag (about 3 m long and 0.8 m in diameter with a 2-mm mesh). The area sampled in each haul was about 150 m^2. The fish were fixed in 4% buffered formaldehyde. In the laboratory, specimens were identified at species level and counted. Only the YOY individuals of euryhaline marine species were measured for SL.

At the same time as the fish sampling, water temperature, salinity, pH and dissolved oxygen in both tidal channels and the lagoon basin were recorded using a YSI 6920 multi-parametric probe equipped with a 650 MDS data logger in order to establish the sampling stations' hydrological features.

For each species, fish collected in all samplings of the same month were pooled to study temporal variations in juvenile abundance in both tidal channels. The recruitment patterns of marine species juveniles were investigated by the construction and subsequent visual inspection of length-frequency distributions. For each species, fish from the fyke-net samplings were pooled and length-monthly frequency distributions were constructed from the observed size distributions in the samples and from the total number of individuals. At each station and for each species, the abundances from replicate seine hauls were pooled in order to study the spatial variation in juvenile abundance in the lagoon basin.

Species	September		October		November		December		January		February	
	Acq	Sch	Acq	Sch	Acq	Sch	Acq	Sch	Acq	Sch	Acq	Sch
Anguilla anguilla	0	0	0	0	0	0	0	0	0	1	0	0
Chelon labrosus	0	0	0	0	0	0	0	0	0	0	0	0
Dicentrarchus labrax	0	0	0	0	0	0	0	0	0	0	0	0
Diplodus vulgaris	0	0	0	0	0	0	0	0	0	0	0	0
Liza aurata	0	0	9	58	4	231	654	78	192	103	2099	297
Liza ramada	0	0	0	0	0	8	2253	48	5240	6155	1332	463
Liza saliens	1	0	46	0	0	0	0	1	1	0	0	0
Mugil cephalus	0	4	1107	716	0	1639	52	15	6	0	0	0
Sparus aurata	0	0	0	0	0	0	1	0	0	4	9	1
Total	1	4	1162	774	4	1878	2960	142	5439	6263	3440	761

Figure 2: Total number of individuals for each species caught at each tidal channel of Lesina Lagoon. Acq, Acquarotta Channel; Sch, Schiapparo Channel.

3 Results

3.1 Environmental characteristics

Temperature ranged from 5.8 °C in January to 22.9 °C in September in Acquarotta Channel and from 6 °C to 22.9 °C in Schiapparo Channel. Salinity ranged from 13.8 in January to 38 in March in Acquarotta and from 7.8 in January to 38.6 in September in Schiapparo. pH ranged from 8.6 in May to 9.1 in April in Acquarotta and from 8.5 in May to 8.9 in October in Schiapparo. Dissolved oxygen ranged from 60.2% saturation in May to 125% saturation in December in Acquarotta and from 60.6% saturation in May to 119.7% saturation in April in Schiapparo.

In the lagoon, temperature values ranged from 9.4 °C in December (station 9) to 29.3 °C in July (station 6), salinity ranged from 8.0 in March (station 8) to 37.6 in August (station 2), pH ranged from 7.63 in October (station 8) to 9.32 in March (station 1) and dissolved oxygen ranged from 49.8% saturation in May (station 9) to 200.9% saturation in December (station 3).

3.2 Catch composition

In the tidal channels, a total of 24,047 YOY fish of euryhaline marine species were captured entering the lagoon: 14,218 specimens were captured in the Acquarotta Channel and 9829 specimens in the Schiapparo Channel. The Mugilidae family had the highest number of species (i.e. five: *C. labrosus*, *L. aurata*, *L. ramada*, *L. saliens* and *M. cephalus*) and was the most abundant (Figure 2). The species that recruited to the lagoon in the largest numbers was *L. ramada*, which accounted for 66.7% of the total catch, followed by *L. aurata* (16.9%) and *M. cephalus* (15.2%). *L. saliens* and *Sparus aurata* had a lower number of recruits (0.5% of the total catch) and *C. labrosus*, *Diplodus vulgaris* and *D. labrax* accounted for very few specimens. Finally, a total of 306 glass eels were captured between January and February 2007.

In the lagoon basin, a total of 2,277 YOY fish of euryhaline marine species were captured during the year of study. *L. saliens* was the most abundant species, accounting for 37.4% of the total catch, followed by *L. ramada* (29.8%), *M. cephalus* (27.3%), *L. aurata* (4.1%), *C. labrosus* (1.1%) and *S. aurata* (0.3%).

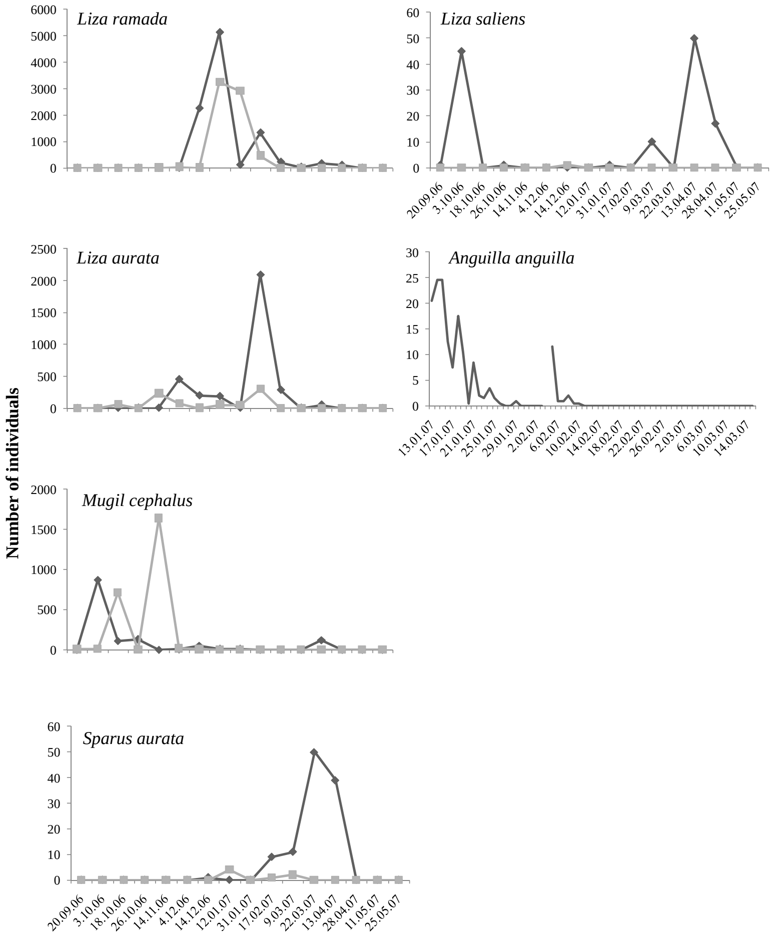

Figure 3: Abundances of young-of-the-year marine fish captured in each sampling in each tidal channel. Acquarotta Channel, black line; Schiapparo Channel, grey line.

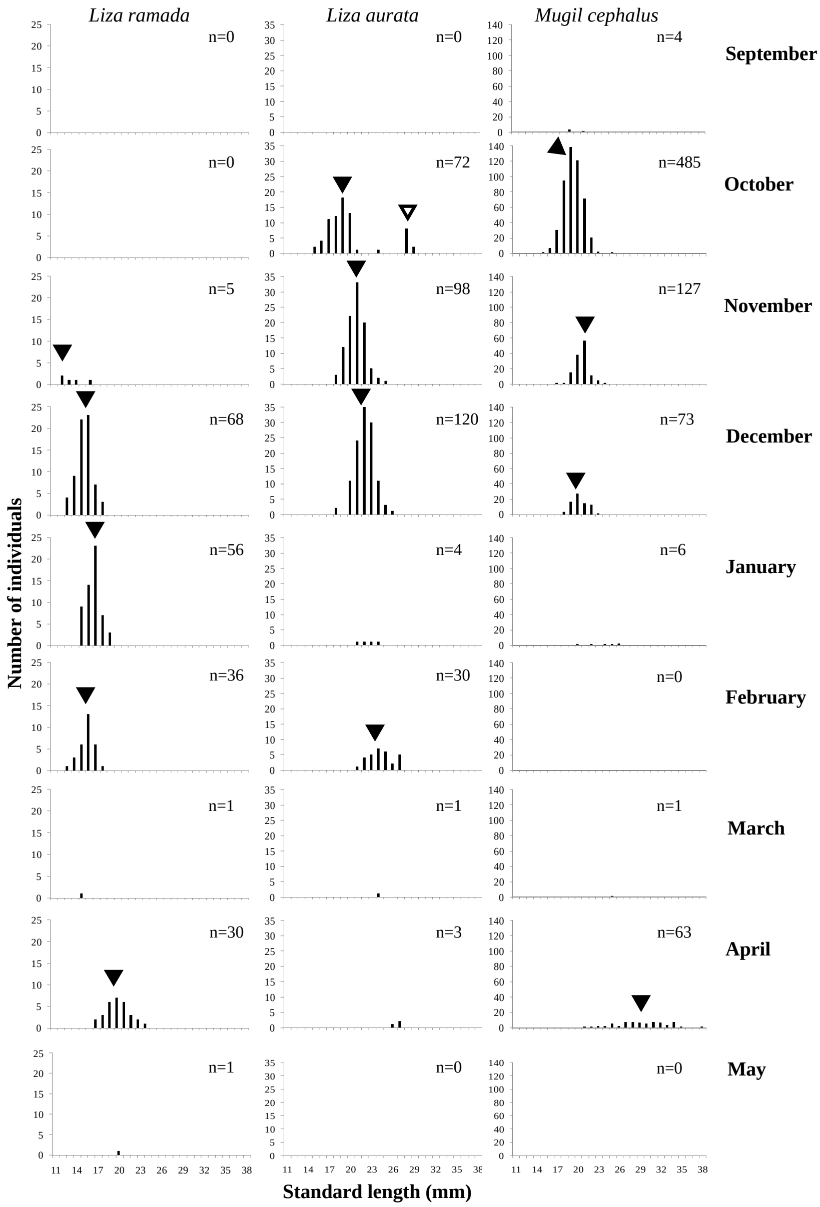

Figure 4: Monthly length-frequency distributions of *Liza ramada*, *Liza aurata* and *Mugil cephalus* juveniles collected in tidal channels of Lesina Lagoon. Arrowheads identify modes. Y-axes are scaled differently across species.

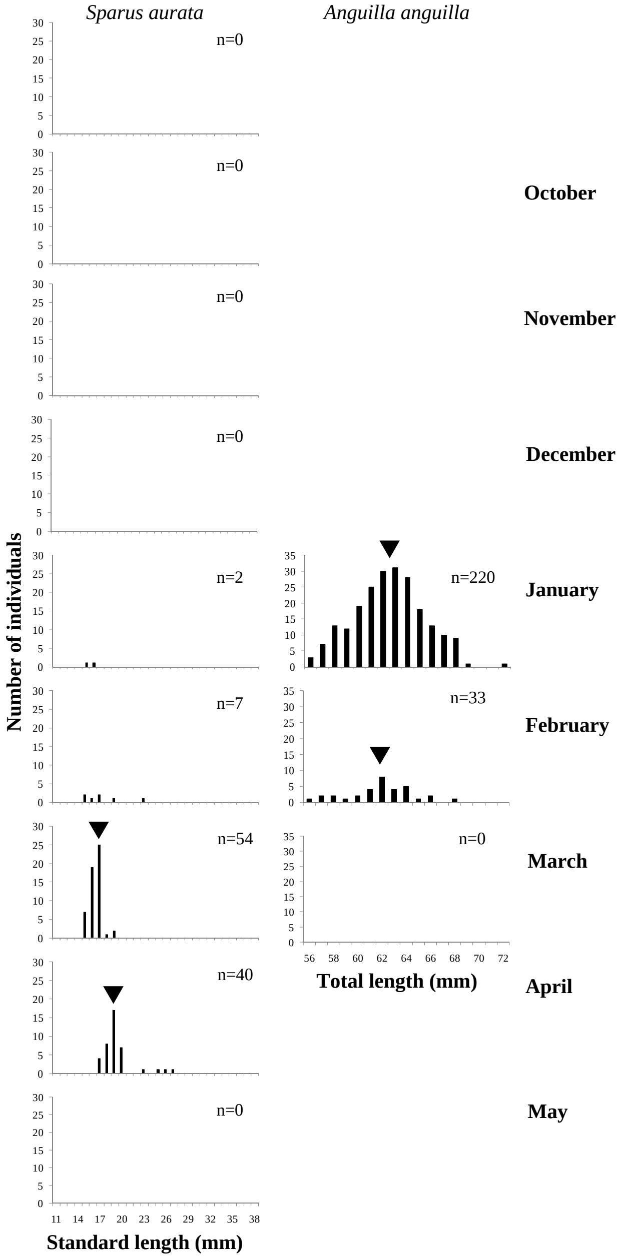

Figure 5: Monthly length-frequency distributions of *Sparus aurata* and *Anguilla anguilla* juveniles collected in tidal channels of Lesina Lagoon. Arrowheads identify modes. Y-axes are scaled differently across species.

3.3 Recruitment calendar, size distributions and spatial abundance patterns

Liza ramada
L. ramada juveniles were collected from November 2006 to May 2007 with SL ranging from 12.5 to 24 mm. In both tidal channels, abundance peaked in January (Figure 3). Small individuals (<15 mm) were found in the catch from November to December and in February. The length-frequency distributions showed the presence of two modes: one from December to January and one in February. Despite the presence of two modes, the length-frequency distributions showed the progression of a single abundant cohort (Figure 4).
Juveniles were caught at all stations sampled in the lagoon basin (Figure 6). The highest juvenile catches occurred at station 5 (52.9%), situated near the mouth of the Pilla Channel, and in much lower numbers at stations 2 (16.7%) and 9 (11.2%), located near the tidal channels, and at stations 1 (8.1%) and 7 (6.3%), located near the mouths of the Cannelle Channel and the Zannella River respectively.

Liza aurata
L. aurata juveniles were collected from October 2006 to April 2007 with SL ranging from 15 to 27.9 mm. Recruitment of small individuals (<20 mm) occurred from October to December. In each of the two tidal channels, two abundance peaks were recorded (Figure 3). In the Acquarotta Channel, the first peak was observed in December, and a second, higher peak was observed in February. In the Schiapparo Channel, the two peaks, of similar magnitude, were recorded in November and February. The length-frequency distributions showed the presence of two modes in October: one which progressed until February, and a second which disappeared in the following distributions, indicating the presence of two distinct cohorts (Figure 4). The collection of a few larger individuals in October suggests that recruitment of a second cohort might have occurred, but of very low abundance.
In the lagoon, YOY fish were caught at all stations investigated except station 3 (Figure 6). The highest juvenile catch occurred in station 5 (41.9%).

Mugil cephalus
M. cephalus juveniles were collected from September 2006 to January 2007 and from March to April 2007 with SL ranging from 15.6 to 38.1 mm. Recruitment of small individuals (<20 mm) occurred from October to December. In each of the two tidal channels, the highest abundances were recorded in autumn: in October and December in the Acquarotta Channel, and from October to November in the Schiapparo Channel (Figure 3). Additionally, in the Acquarotta Channel, a smaller peak was observed in April. The length-frequency distributions showed the progression of a single abundant cohort (Figure 4).
M. cephalus recruits were found at all stations in the lagoon (Figure 6). The highest number of juveniles was captured at station 5 (43.5%) followed by stations 7 (33.2%) and 1 (15.5%).

Liza saliens
A small number of *L. saliens* YOY individuals were intermittently collected during the study period with SL ranging from 11.2 to 30.9 mm. Recruitment of small individuals (<20 mm) occurred in September and October. Recruitment of *L. saliens* juveniles was very low in the Schiapparo Channel (Figure 3). In the Acquarotta Channel, two recruitment peaks were recorded: the

first in October and the second in March and April. It was not possible to construct length-frequency distributions due to the small number of juveniles caught in the tidal channels. It is likely that the observations in the tidal channels started after the maximum recruitment period for this species.

In contrast, a large number of *L. saliens* YOY individuals were captured in the lagoon basin. They were found at all stations (Figure 4). The highest number of juveniles was captured at station 5 (77.1%) followed by station 2 (8.7%).

Chelon labrosus

A low number of *C. labrosus* YOY individuals were collected in April 2007 in the Acquarotta Channel with SL ranging from 18.0 to 22.0 mm (Figure 3). In the lagoon basin, *C. labrosus* recruits were found only at stations 1, 2 and 9, with the highest abundance occurring in the latter (88.0%) (Figure 6).

Sparus aurata

S. aurata YOY individuals were collected from December 2006 to April 2007 with SL ranging from 15 to 27.8 mm. The highest number of individuals was captured in the Acquarotta Channel in late March - early April (Figure 3). Small individuals (<20 mm) occurred in the catch throughout the recruitment period. The length-frequency distributions showed the progression of a single cohort (Figure 5).

A small number of *S. aurata* recruits were captured in the lagoon basin at stations 3 and 7 (Figure 6).

Anguilla anguilla

Glass eels were collected from January to February 2007 with TL ranging from 56.2 to 72 mm in the Acquarotta Channel. The highest abundance was recorded in January (Figure 3). In January, the length-frequency distribution showed the presence of only one mode (Figure 5). In February, the length-frequency distribution became flatter. No glass eels were captured in the lagoon basin.

4 Discussion

From this study it emerges that Lesina Lagoon acts as a nursery mainly for species of the Mugilidae family, and to a lesser extent for Sparidae, in agreement with what has already been observed in previous studies conducted in the area [24]. Recruitment of economically valuable marine species continues to be modest, as evidenced previously by other authors [25, 24] although there does not appear to have been a significant reduction in the number of recruits.

The pattern observed here has been found to be similar to those recorded in the same region and in many other brackish areas of the Mediterranean, with *L. ramada*, *L. saliens*, and *L. aurata* or in some cases *M. cephalus* accounting for the largest number of recruits, together with smaller numbers of glass eels, sea-bass and Sparidae [26, 27, 28, 24, 29]. Migration of glass eels into Lesina Lagoon was found to be quantitatively limited, as along the entire Italian Adriatic coastline, though it is greater on the Tyrrhenian side [30, 25, 24, 31]. However, this reflects a marked decline in recruitment in Europe as a whole, not just the Mediterranean [32, 33].

Recruitment of juveniles to coastal lagoons is the result of complex interactions between spawning success, hydrodynamic processes, and pre-recruitment mortality. Moreover, the hydrographical regime near and within the seaward channels of these lagoons is a key factor in immigration of juveniles given their limited swimming ability during this phase of their life his-

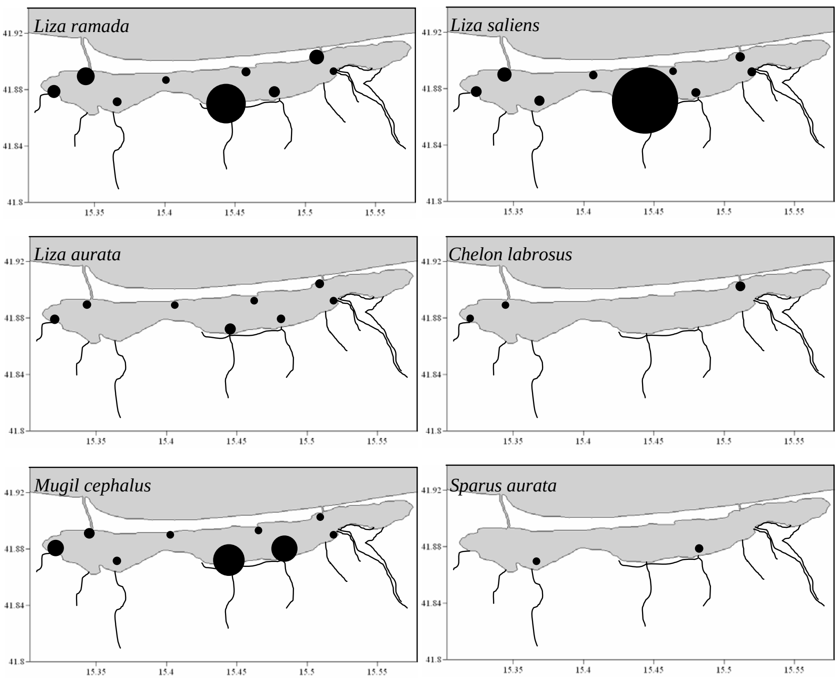

Figure 6: Spatial distribution of juveniles of six species in Lesina Lagoon. Circle size is proportional to abundance of species at each station.

tory [34]. The low numbers of recruits of euryhaline marine species found in the tidal channels of Lesina Lagoon are in the first instance probably due to the modest flows of these channels and to the limited tidal range of the marine waters off the lagoon (about 30 cm, [35].

In this study some differences in the total number of YOY marine fish captured and in the temporal dynamics of recruitment in the two tidal channels were observed. The discrepancy in the volume of recruitment and in the temporal trend of the catch for most of the marine species found may be due to the different morphometric features of the two tidal channels in terms of total length and mean width and depth. The Acquarotta Channel is about three times the length of the Schiapparo Channel. This fact certainly has an effect on the time taken to pass through the two tidal channels by YOY marine fish, which may by itself have caused the different temporal recruitment trends observed in the two tidal channels. Further studies on the timing of YOY marine fish recruitment to the tidal channels of Lesina Lagoon with closer sampling times (e.g. daily) are essential to understand whether the different morphometric characteristics of the two tidal channels, which influence the dynamics of water exchange between sea and lagoon, may also cause a different degree of attractiveness for marine juveniles in the

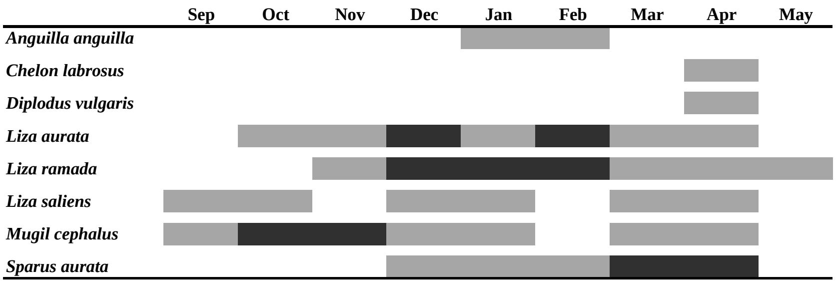

Figure 7: Recruitment calendar of marine species captured in tidal channels during study period. Present, grey; abundant, black.

open sea, thus affecting recruitment patterns through the two tidal channels.
In addition, the inadequate maintenance of the structures in the tidal channels (mechanical sluices often encrusted with fouling organisms and grilles occluded with weeds) clearly constitutes an obstacle to the entrance of recruits, preventing the proper exchange of waters between the sea and the lagoon basin and further reducing the "appeal" of the lagoon to juveniles of euryhaline marine species. The presence of the landing stage for fishing boats at the seaward end of the Acquarotta Channel, where the highest number of recruits was observed, may also constitute a factor of disturbance for the entry of fish juveniles into the lagoon.
This study enabled us to verify the migration calendar of economically important marine species entering Lesina Lagoon (Figure 7). The data obtained are comparable to those already available for the same study area [25, 24]. On the other hand, differences were observed with respect to brackish areas along the northern Adriatic coasts of Italy [27] and the coasts of Greece [29], where recruitment of *L. aurata* and *L. ramada* peaks in spring. This discrepancy may be due to differences in the distance between the coast and the reproductive areas and to local climatic factors [1].
All species except *L. aurata* showed one cohort, which is the result of a single spawning event. In contrast, the two cohorts of *L. aurata* may be the result of two spawning events of different intensities or of a single protracted spawning event.
In the lagoon basin, the distribution of YOY marine fish was not uniform across stations. *L. aurata*, *L. ramada*, *L. saliens* and *M. cephalus* juveniles showed a clear preference for the area near the mouth of the Pilla Channel (station 5). High abundances of *L. ramada* and *M. cephalus* juveniles were also found at stations 1 and 7, located near the mouths of the Cannelle Channel and the Zannella River, where effluent is discharged from buffalo and fish farms. These findings indicate that these areas function as nursery grounds for these two species. Moreover, high abundances of *L. ramada*, *L. saliens* and *M. cephalus* juveniles were found at stations 2 and 9, near the tidal channels, where they are assumed to have been in transit towards the nursery grounds. In a recent study on the spatial-temporal variations of chemical-physical parameters, nutrients and phytoplankton in Lesina Lagoon, Roselli et al. [23] observed

high concentrations of DIN, dissolved inorganic nutrients, silicate and ammonium, the latter probably originating from the decomposition of labile organic matter on the surface of the sediments in the southern-eastern part of the lagoon as a result of agricultural runoff. High phytoplankton and diatom biomass was also found at the stations in this part of the lagoon. The presence of high phytoplankton biomass and organic substance-rich sediments near freshwater inputs may constitute a source of food for mullets [36], explaining the highest abundance of Mugilidae in these areas. In addition, the highest abundances of YOY *L. ramada* and *M. cephalus* specimens near the freshwater inputs may be related to a preference of these species for fresh or oligohaline waters as described by Cardona [37], who studied habitat selection by juveniles of Mugilidae species in Mediterranean estuaries.

5 Conclusions

The conservation of lagoon fish populations requires active management by human beings. Specifically, the nursery function of coastal lagoons for euryhaline marine species, many of which are economically valuable and thus often overexploited by commercial fishing both inside and outside these systems, needs to be continuously monitored and possibly enhanced.

Successful management of lagoon fish populations requires measures to ensure a high number of recruits of euryhaline marine species. The magnitude of YOY marine fish recruitment to Mediterranean coastal lagoons is heavily dependent on the efficiency of the tidal channels. The connections between Mediterranean coastal lagoons and the sea are typically unstable and inefficient due to their morphology and meagre tidal regime. In addition, the presence in the tidal channels of poorly managed sluices and *lavorieri*, as in Lesina Lagoon, can make this situation even worse, with repercussions for the hydrological, physical and chemical characteristics of the lagoon basin, especially salinity. The correct management of the tidal channels, protecting them from silting up, and the recovery or improvement of their flow regime are fundamental to an effective fish production strategy, which at the same time would assist in the conservation of the lagoons.

Furthermore, the identification of the areas in the lagoons with an abundant presence of juvenile fish, which in Lesina Lagoon coincide with the areas near the freshwater inputs, can certainly contribute to the improvement of the nursery function of the lagoon promoting the growth and survival of juveniles. Indeed, these areas need to be preserved by acting to halt processes associated with the deterioration and disappearance of nursery habitats, and by excluding them from non-selective lagoon fishing so as to reduce the bycatch.

6 Aknowledgements

This research was supported by Puglia Regional Administration as part of its "Policy for rural development; Hunting and Fishing" (POR 2000/06 - 4.13E) grant n° 1939/P to R. D'Adamo. The authors would like to thank fishermen Mr Antonio D'Addetta and Mr Michele Giovanditti of the Lesina Lagoon management company (Società di Gestione della Laguna di Lesina) for their assistance in field collection.

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Revaluation of DEPM Biomass Estimation of European Anchovy (*Engraulis encrasicolus*) in Central Mediterranean Sea with a New Post-Ovulatory Follicles Age Method.

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Abstract

European anchovy (*Engraulis encrasicolus*) represents the most caught species in the Mediterranean Sea. It's very important to monitor the biomass stock abundance to ensure sustainable exploitation of this resource. Daily Egg Production Method (DEPM), a direct stock assessment methodology, was applied to estimate the anchovy biomass in the Strait of Sicily since 1998. The DEPM was specially developed for anchovy populations inhabiting areas where temperatures generally do not exceed 22°C (Oceanic waters). In Mediterranean Sea during the anchovy spawning period, the sea surface temperature may reaches 28 -30°C and the average temperature in the upper water column reaches 24°C in the Strait Sicily. To carry out the spawning biomass (B) evaluation it's necessary to compute a reproductive parameter, the daily spawning fraction (S) which in other words would be the proportion of mature females which spawn in a determinate day. The S was calculated by the postovulatory follicles method (POF). The POF are cellular residues remained in the ovary after deposition. The follicles resorption rate is influenced by temperature. Recent studies showed a most rapid POFs resorption at Mediterranean temperature. Therefore within the first 24 hours after the deposition there are 2 different stage of POFs, not only one as in Ocean wares. An overestimation of the POFs duration leads to underestimation of S and a subsequent overestimation of B. So it was deemed appropriate to make a new estimate of B and S calculated previously by the method developed for the ocean. A sensitivity analysis was also performed to evaluate the variability of biomass estimation in relation with spawning fraction estimates. The results show an underestimation of spawning fraction and an overestimation of stock biomass, as expected. This overestimation is even more significant considering the anchovy low biomass levels in recent years.

1 Introduction

The small pelagic species, especially sardines and anchovies, represent an important resource worldwide. Particularly, the European anchovy (*Engraulis encrasicolus*) is widely distributed along the European Atlantic coast from South Africa to North Atlantic and all over the Mediterranean and Black Sea. Moreover, this is the species more fishing by principal Sicilian fleet (Mazara del Vallo and Sciacca) into last years [1].

These resources undergo wide interannual biomass fluctuation mainly due to environmental variability effects on the recruitment. In recent decades, the refinement of knowledge on growth and development of fish oocytes [2] and reproductive biology [3] permitted more precise definitions of fish fecundity. The Daily Egg Production Method (DEPM) was developed in the late 1970s by the Coastal Division of the Southwest Fisheries Center, La Jolla, CA, USA [4] as a response to the growing need to devise a suitable direct method for the assessment of northern anchovy (Engraulis mordax), an indeterminate spawner with pelagic eggs [3]. The DEPM is based on the number of oocytes released per fish in each spawning event (batch fecundity) and the proportion of females reproducing daily (spawning fraction). Estimation of spawning fraction for anchovy is possible because of the identification of Post-Ovulatory Follicles (POFs).

The POFs represent cellular residuals in ovary reabsorbing after deposition [3] and the resorption time is species specific and changes accordingly to the residence temperature. In literature most studies which report anchovy POF duration vs. temperature were performed within different temperature ranges but under 22°C [5, 6, 7]. In Mediterranean Sea, especially during summer season when anchovy spawning takes place, the SST reaches 28°C and the water column temperature 24°C.

The official methodology foresee to calculate the spawning fraction by means of POFs 24 hours old (POF 0), but these are generally overrepresented in the samples, so Piquelle and Stauffer [8] suggest to replace POF 0 with an average of POF1 + POF2. So far, the POFs aging and S estimate methods used in Mediterranean was the same produced for lower temperature, but recent studies show a most rapid resorption at Sicilian temperatures than in Atlantic water [9]. In the Strait of Sicily both POF 0 and POF 1 are in one day. An overestimation of the POFs duration leads to an underestimation of S and consequently an overestimation of B. It was deemed appropriate to reconsider previous estimates of biomass and spawning fraction, because only the POFs of first day (24h) after spawning should be used to estimate the spawning fraction. The evaluations, obtained by old and new method of POFs aging, were compared and they were showed significant differences. These results cannot be neglected to the stock sustainable management.

2 Materials and methods.

Anchovy adults specimens were caught during the peak spawning season (June and August) in the Strait of Sicily (Figure 1), from 1998 to 2006 (in a non-continuous), aboard a research vessel equipped to DEPM surveys. During the catches, the water temperature was measured by a CTD probe. In 2005, the sampling was increased by specimens from commercial vessels. The ovaries were preserved in buffer formalin at 4% and analysed by traditional histological techniques and Haematoxylin and Eosin Method for colouring. Sexual maturity stages (ICES 2004, modified by authors) and presence and age (POF 0, 1 and 2; Figure 2) of post-ovulatory follicles were estimated for each ovaries. For 2005 and 2006, the histological sections were observed twice: according to new findings about the POFs duration in the Mediter-

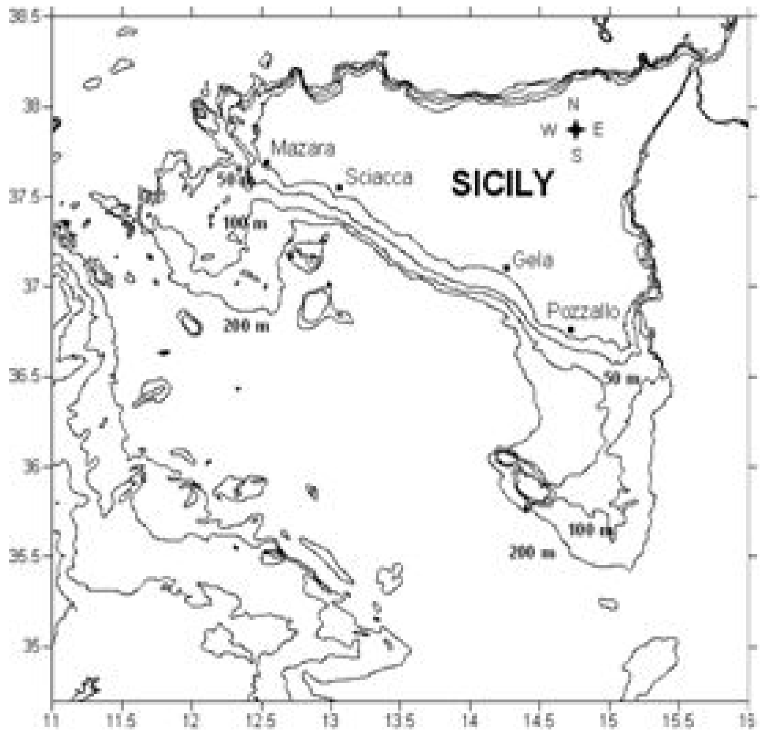

Figure 1: The study area: the Strait of Sicily.

ranean, it was considered appropriate to re-check previous readings. To assess the relative difference in the POFs classification with the old and the new method was used the Receiver Operating Characteristic (ROC) plot and the Area Under the Curve (AUC) [10, 11] for both examined years. ROC and AUC estimation was made with the presence/absence library of the R statistical software. The values of AUC vary between 0 to 1, where a score of 1 indicates perfect discrimination, a score of 0.5 implies a random guess and values lower than 0.5 indicate performance worse than random [12, 13].

S and B for each year were calculated using the old and the new POFs aging method. The biomass and spawning fraction estimates have to be considered preliminary results of an early application of DEPM procedures in the study area and are here used only to provide reference points to evaluate the extent of the variability within realistic variations range for the observed two variables. Both estimates of these parameters were considered by one day old POFs. According the old method each age class of follicles lasts 24 hours, an average of POFs 1 and 2 (equivalent at the number of POFs 0) were used to avoid over- or underestimation of one of the age classes [8]. According to new method, both POFs 0 and POFs 1 are within first 24 h. The anchovy spawning occurs at 0:00 (GMT) and

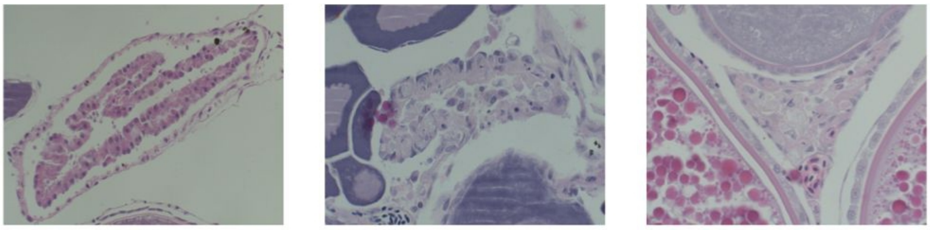

Figure 2: Three different age of Post-Ovulatory Follicles (POFs).

Year	POF0	POF1	POF2
1998	41	40	53
1999	55	34	70
2001	9	4	17
2005	0	22	104
2006	1	18	38

Table 1: Total number of each age POF.

the POF 0 remain in the ovary about six hours after deposition. To reduce the mistake, with new method S was evaluated by POFs 2 which should be equivalent to the sum of POFs 0+POFs 1, because the POF 0 are always subsampled and because the POF 2 should correspond in number to the sum of the other two classes. The results obtained by old aging method were compared with the results by new aging system.

3 Results

Almost all ovaries collected are in pre-, post- or spawning, confirming that the sampling were conducted during the anchovy reproduction season. The abundant presence of POFs in each year attests that the spawning occurred before the capture. The total of postovulatory follicles of each age class is showed in Table 1.
The values of AUC for the ROC curve, the estimates of sensitivity and specificity for each examined year (2005 and 2006) show a high percentage of correct classification with the old method for both years. The sensitivity, that represents probability of correct classification of POF0-1, is 0.91 in 2005 and 0.81 in 2006 and the specificity, that represent probability of correct classification of POF2, is 0.75 in 2005 and 0.88 in 2006. The proportion of correctly classified units (kappa) is 0.62 in 2005 and 0.61 in 2006. The estimation of area under ROC curve (AUC) is equal to 83.4% in 2005 and 85% in 2006 (Table 2; Figure 3). So it was considered unnecessary to continue with a second observation of histological sections for other years.
Excluding 1998, in the study period the re-estimation of the spawning fraction increases and consequently the stock biomass decreases, when we used the new aging method (Table 3; Figures 4 and 5).

	2005	2006
Sensitivity	0.91 (0.035)	0.81 (0.07)
Specificity	0.75 (0.13)	0.88 (0.11)
Kappa	0.62 (0.12)	0.61 (0.13)
AUC	83.4% (0.067)	85% (0.06)

Table 2: Values of sensitivity, specificity and Area Under the Curve (AUC).

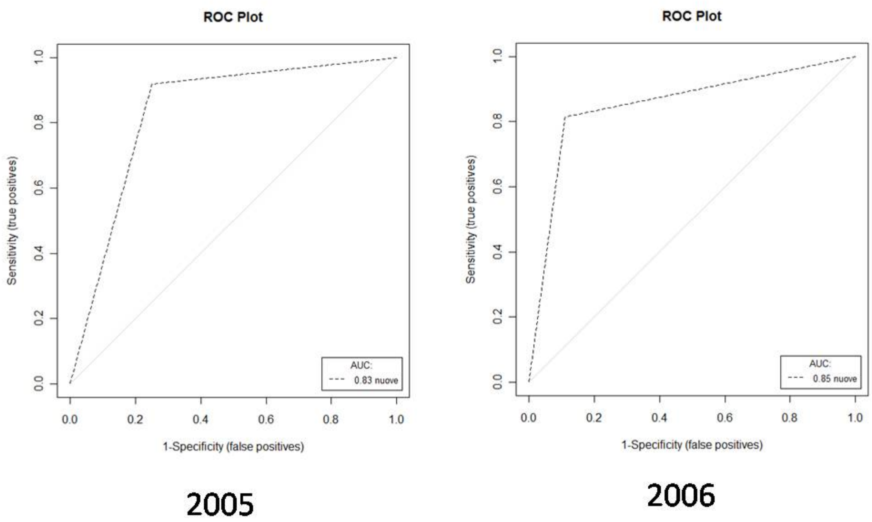

Figure 3: 2005 and 2006 Receiver Operating Characteristic (ROC) plot.

Variations of S and B are related to the number of POF 0; the lower the number of POF youngest class, the better the variations of S and B calculated using the two methods (Tables 1 and 3). The lower S estimations are in 1998 (both method); the higher in 2001 (both method; Table 3). The stock biomass shows a large variability among the study years (Table 3), but it is a peculiarity of small pelagic fish. The higher B values are in 1998: about 13.000 t with both method; the lower in 1999: 2404 t with the new method and 3231 t with the old one (Table 3).

The proportion of variation in biomass abundance, except 1998, is about 30%, (Figure 6). About 1998, the discrepancy percentage is not significant both for S and B: 0.16% (Figure 6). The greater difference between the first estimates and the second re-evaluation has been recorded in 2005 with S variation of 55% and values changing from 0.218 to 0.339 (Table 3; Figure 4). Biomass variability was also relevant (35.6%) with values from 12729 to 8200 (Table 3; Figure 5). In this year there aren't POF 0 (Table 1): this confirms that if there aren't samples immediately after the

Year	S1	S2	B1	B2
1998	0.14	0.14	13,003.63	12,982.65
1999	0.21	0.16	2,404	3,231.49
2001	0.39	0.27	4,650.07	6,711.43
2005	0.34	0.22	8,200.96	12,729.20
2006	0.30	0.20	5,897.96	9035.79

Table 3: Values of Spawning fraction and Biomass calculated by new (S1 and B1) and old (S2 and B2) methods.

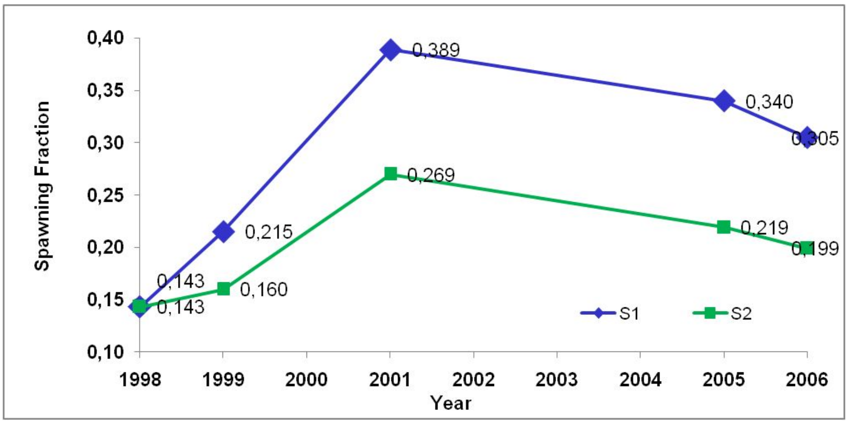

Figure 4: Variations of Spawning Fraction between new (S1) and old (S2) evaluation methods.

spawning it is almost impossible to find the first age class POF. In 2005 the catches start at 07:30 a.m. and the POFs 0 disappear at 6:00 a.m. about.

4 Discussion

The importance of DEPM application to suitable management of commercially important fish resource, as sardines and anchovy, is confirmed worldwide since 1980s. Knowledge of species reproductive biology and a survey design adapted to local population dynamics are required to optimize DEPM performance in terms of bias, precision and cost. The method seems better adapted to the life history of anchovies than of sardines, leading to more precise estimates of anchovy spawning biomass and facilitating extensions of the method to estimate total biomass [14]. Spawning fraction (S) is the proportion of mature females in the target population that spawn daily, and often constitutes the most difficult adult DEPM parameter to estimate [15]. Detailed knowledge of the reproductive biology of the species may allow to obtain much more accurate estimations. Several studies revealed the ephemeral life of POFs, lasting usually between 15 to 72

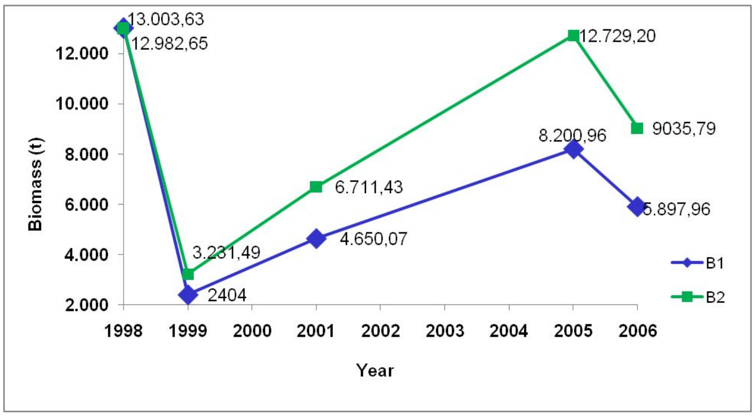

Figure 5: Variations of stock Biomass between new (B1) and old (B2) evaluation methods.

h, according to species and spawning temperatures [5, 16, 17]. POFs degeneration is a continuous process that has to be categorized into an ordinal classification key of stages, with the additional complexity that POF characteristics become less clear with time and their size is reduced until they may be confused with late atretic stages [5]. The increasing awareness of the effect that different water temperatures might have on the rates of degeneration of POFs [17] made it necessary to examine the potential effect that changes in the average temperature of the sea water on different cruises might have on the degeneration of POFs and on the ageing procedures.

The very low presence of POF 0, especially in 2001, 2005 and 2006 (Table 1), confirms the difficult to caught specimen immediately after the spawning, because unfortunately, due to aspect regarding the research vessel crew watches, the trawl sampling during night-time is not complete over the whole night.

The old and new histological slides comparison (Table 2; Figure 3) showed an high correspondence in the age assignment between the two estimates method. The last finding because the POF morphology classification criteria do not change respect to literature (ICES, 2004). The observed differences were mainly related to the POFs resorption rate.

The large interannual variability of biomass is typical for small pelagic fishes, as the European anchovy, however our results showed a greater variation and decrease in stock abundance when the new ageing criteria were applied. This finding is also important for fishery management purposes, if we take in account the very low values of anchovy biomass in the Strait of Sicily. The evaluations of stock biomass and spawning fraction have to be considered preliminary results of an early application of DEPM procedures in the study area and here represent a reference points to evaluate the extent of the variability within realistic fluctuations range for the observed two parameters. The evaluation of precise and accurate spawning fraction and biomass estimates are beyond the scope

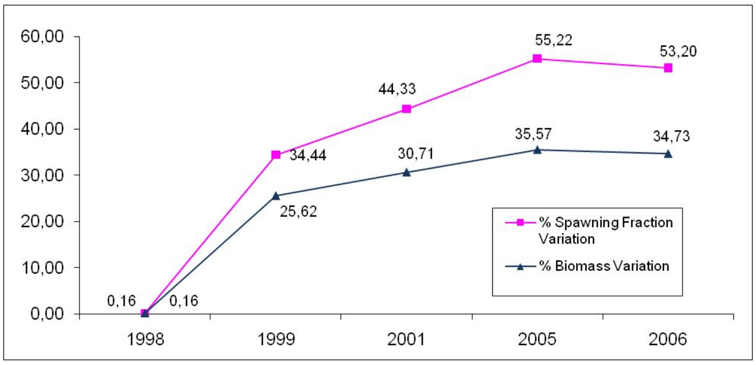

Figure 6: % of Variations of Spawning Fraction and Biomass between new and old evaluation methods.

of the present paper that is more devoted to analyze the effect of spawning fraction computation method on the total biomass variability. Generally when applied in new areas or to new species DEPM estimates have to be re-evaluated after several years period application, as it is also suggested by the rather long time lag existing between the DEPM worldwide applications and their publication [14].

Using the system applied in the Ocean, the resorption rate of postovulatory follicles is overestimated. Consequently, the daily spawning fraction was underestimated, thus overestimating the stock biomass.

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The Issue of Non-Indigenous Species in Italian Coastal Waters: the Case of Taranto Seas (Ionian Sea)

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Abstract

A non-indigenous species (NIS), is any species extraneous to the ecosystem where it is found. Its translocation could be either human-mediated via different vectors (e.g. import of commercial species, ballast waters) or could be the consequence of natural active dispersal. This issue has a great relevance on a global scale: NIS now represent one of the four greatest threats to the integrity of world's oceans. NIS can rapidly become invasive, replacing native species and changing communities' structure. Besides this, effects on human activities and health can be of outstanding relevance. NIS can damage fishing and aquaculture and, in case of toxin producers, can cause human intoxication via consumption of contaminated seafood.

In Italy this problem has been investigated in few areas particularly subject to both introduction and spreading of NIS. At present, we surveyed more than thirty exotic species in Taranto seas, but this number is constantly increasing, probably due to the expansion of the harbour and also because the Italian laws are quite vague and there is little control. Only the introduction of species for commercial or farming purposes are ruled and placed under a sanitary control, but the discharge of ballast waters is a "common" procedure in harbour waters.

On the base of the results of a monitoring project on planktonic and benthic communities, we propose a set of rules to regulate introduction of NIS in Taranto Seas as well as in other Mediterranean sites.

1 The non-indigenous species (NIS)

During the last 10-15 years, a new type of pollution, the "biological" pollution [1], joined the others that are more or less linked to human activities in harming the marine environment.

The biological pollution consists in the presence in an area of organisms not native of that area, i.e. non-indigenous.

These organisms, both animal and vegetal but also viruses and bacteria, once arrived in the new environment can settle, becoming a stable component of the ecosystem. This could lead to consequences that are generally unpredictable with changes of the structure and functioning of the receiving ecosystem [2].

Recently, many studies have highlighted an increase on a global scale of this phenomenon, so that we refer to it as "biological invasions" [3], to indicate a massive colonization as in the case of some algae

Figure 1: *Caulerpa taxifolia* (Vahl) C. Agardh is a green alga of tropical origin, commonly utilized in aquaria. After an accidental discharge, this species is now widespread along French, Spanish and Italian Tyrrhenian coasts. (Photo Michelagnoli Foundation Archive)

and molluscs. The most well-known example is that of *Caulerpa taxifolia* (Figure 1) that, due to its very high invasiveness has been defined the "killer alga" [4].

The intentional or accidental transport of NIS to new regions now represents one of the four greatest threats to the integrity of world's oceans, together with the chemical pollution of continental origin, the over-exploiting of resources and the loss of habitats [5].

NIS can cause severe economic damages in that they impact activities such as aquaculture, fishing and industry. Paradigmatic examples are the effects caused by the bivalve mollusc *Dreissena polymorpha* (the so-called zebra mussel) (Figure 2) and the European crab *Carcinus moenas* in the U.S. [6] or by the ctenophore *Mnemiopsis leidyi* in the Black Sea [7]. Beside this, not always the addition of non-native species negatively affects the native biota. According to Reise et al. [8], in many cases such species expand the functioning and services of the recipient ecosystem, being complementary to the natives, e.g. in the better use of resources and the increasing of functional redundancy.

In Italy, the issue of NIS introduction has become evident with the appearance of easily detectable exotic organisms, such as the above mentioned green alga *Caulerpa taxifolia* and the Japanese clam *Tapes semidecussatus*, which was intentionally introduced for aquaculture purposes.

The incoming of globalization in the markets directly affects the amplification of this phenomenon that, however, follows also natural pathways with dispersion dynamics known since hundreds of years. Indeed, recently, in the Mediterranean a northward expansion of the distribution of tropical species arrived through Gibraltar Strait or Suez Canal is occurring, [9] even in Taranto (Southern Italy, Ionian Sea) where subtropical molluscs and fishes are more and more frequently observed (Figure 3).

Figure 2: *Dreissena polymorpha* (Pallas), commonly known as “Zebra mussel”, is an Euro-Asiatic bivalve mollusc that was introduced into North American Great Lakes in the early 80s. It causes huge damages both to industries, since it grows in the aspiration pipes of cooling plants, and to the ecosystem, competing with local fish species. (Photo www.wildlifedepartment.com/ images/mussel2.jpg)

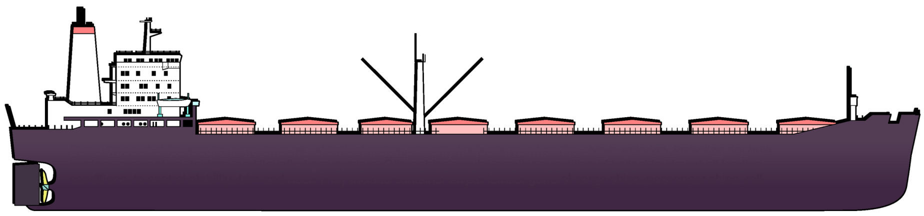

Figure 3: Ballast waters of cargo ships are a very efficient vector for the introduction of many exotic species of viruses, bacteria, algae and animals. (Picture www.imo.org)

2 The arrival and spreading of NIS

The most common way of intentional introduction of exotic species is the import of organisms to be farmed [10]. In this case the carrier is man. Anyway, in many cases the arrival of NIS is accidental or unintentional. In particular, for the marine environment, one of the main vectors for accidental arrivals are just these imported organisms that can “transport” individuals of other species as parasites, epibionts or symbionts. For example, even if a EU Decision of the 14th November 2003 expressly forbids this procedure, imported molluscs are often maintained in the sea water until they can be sold, releasing in this way possible “guests” [11]. (Table 1)

Moreover, merchant cargos transport exotic species both on their hulls and in ballast waters, that are charged in specific tanks to ensure the stability and the structural integrity of the ship during the navigation [12, 13] (Figure 4).

This latter vector is particularly important

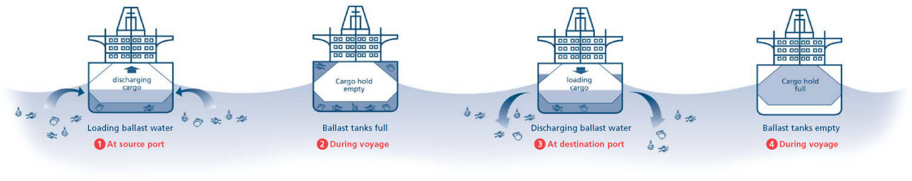

Figure 4: 1. cargo ships board ballast water in specific tanks at the departure, especially when they must sail with low charge, to achieve best stability during the voyage; 2. many organisms, boarded with ballast water die during the crossing, but many other survive, mainly in dormant forms; 3. at the arrival, ballast water is discharged in port and with it the survived organisms too; 4. after the loading of the cargo, the ship sails toward the next destination. (Picture by F. Rubino)

Intentional	Accidental
Aquaculture	Organisms associated to: Aquaculture Selling & Commercialization
Repopulation	Aquaria
Biological Control	Ballast Waters

Table 1: Types and Vectors of NIS introduction

for planktonic species whose resting stages can easily overcome the hard conditions in the ballast tanks (e.g. lack of light, very low or high values of temperature and salinity) [14] (Figure 6).

Finally, NIS can be introduced also by activities linked to aquaria, both private and public.

Once arrived in a new area, a NIS can spread for a long distance from the arrival point and cover even surfaces of many square kilometres. This can more easily happen for clonal organisms and macroalgae that can be dispersed by recreational and fishing boats as fragments trapped in nets and anchors (Figure 5). Then, a species can become invasive because in the new environment:

- it finds optimal conditions to its growth and reproduction;
- its natural enemies (parasites, competitors, predators) that in the native area control its expansion are lacking;
- its presence modifies the structure of the recipient ecosystem and this gives rise to one of the previous conditions [1].

3 The consequences

The introduction of non-native organisms in any region has often unpredictable consequences. Usually, their arrival is undetected, but if they become invasive the effects of their presence can unbalance the ecosystem equilibrium with damages to the environment but also to the human health

Figure 5: For many macroalgal species, thallus fragmentation is an effective way of reproduction and dispersion. (Photo by E. Cecere)

[15] (Figure 7). They can affect:

- **the environment**
• substituting the native species and modifying the community structure and trophic chains;
• altering the local biodiversity and the functioning of the ecosystem;
- **the human activities**
• damaging fishing, directly or indirectly as a result of the replacement of species of commercial value, or making dirty and heavier the nets;
• damaging aquaculture, either causing the death of reared organisms in case of both toxic phytoplanktonic species and pathogens or competing with the farmed species for the substrate colonization;
- **the human health**
• toxins produced by phytoplanktonic species can accumulate along the trophic chain reaching the last consumer, i.e. the humans and causing different types of syndromes, even very serious such as the gastro-intestinal and neurologic ones (Figure 8).

4 Taranto, an area at risk of NIS introduction

4.1 Vectors

1. Port activities (Figure 9)
   Taranto houses the moorage of the region's fishing fleet, the most important base of the Italian Navy, a commercial port and a new container terminal. The increase of the harbour activities, specially along the transcontinental course connecting the Far East with Europe through Suez Canal, makes Taranto the third vertex of the motorway-maritime triangle made by Trieste-Taranto-Genoa.
2. Aquaculture activities (Figure 10)
   Taranto is an important area for mus-

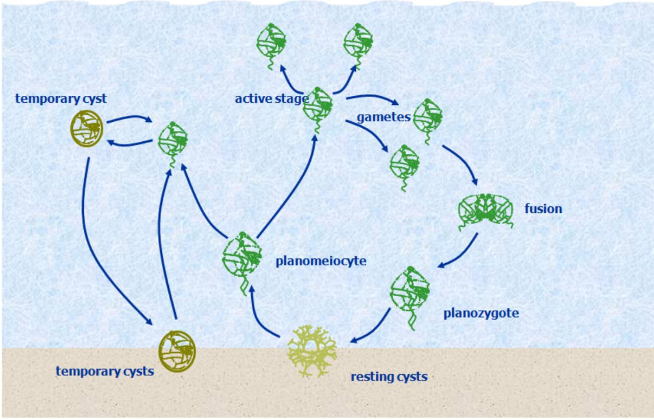

Figure 6: The life cycle of a dinoflagellate (planktonic microalga) consists of an asexuate phase, with the active stage in the water, and a sexuate phase that leads to the production of the zygote that encysts, becoming not motile, and falls to the bottom where it remains dormant until the environmental conditions return favourable for the species. Then it germinates (excysts) refuelling the planktonic population in the water. (Scheme by F. Rubino)

sel farming, with more than 40,000 tons of mussels produced per year from both Mar Grande and Mar Piccolo basins. This activity represents one of the main employer at Taranto, with nearly 1,000 employees and an annual product value of about 13 MLN of Euros that makes this the most important mussel production at national scale.

The transit of many species of bivalve molluscs, such as mussels, cockles, oysters and clams, is linked to the aquaculture activities. These organisms, coming from both EU and extra-European countries, arrive to the expedition points, to the depuration plants and directly to the sellers.

Due to this, it is evident that Taranto is seriously subject to the introduction of NIS. Therefore, it is very important to enhance the study of this phenomenon at local scale, to identify all the possible vectors, predict the possible consequences and play out adequate prevention tools.

In the framework of the Research Project IMSAT funded by the Italian Ministry of University and Scientific Research between 2002 and 2005, we surveyed more than thirty exotic algal and animal species in Taranto seas (Table 2), but this number is constantly increasing, probably due to the expansion of the harbour and also because the Italian laws are quite vague and there is little control.

4.2 Types of NIS

1. Alien algae (Figures 11-18). Numerous macro- and micro-algal non-native species have been discovered in Taranto seas since the 80s. They are species of both tropical/sub-tropical and cold-

Figure 7: *Caulerpa racemosa* (Forsskål) J. Agardh var. *cylindracea* (Sonder) Verlaque, Huisman *et* Boudouresque and *Caulerpa prolifera* (Forsskål) J.V. Lamouroux, exotic species and native species for the Mediterranean, respectively; these two macroalgae can coexist, but the exotic is gradually substituting the native species in Taranto seas. (Photo by G. Fanelli)

temperate affinity. The formers entered the Mediterranean after the opening of Suez Canal or through Gibraltar Strait. About the other ones, more probably, their introduction has been mediated by human activities. For none of them, presently, the way of introduction in Taranto seas has been certainly ascertained.

2. Alien animals (Figures 19-26). The recent so-called tropicalization of the Mediterranean, with the arrival of many tropical species even in its northern region, is strictly linked to the Global Change. This phenomenon affects both the organisms able to swim, like fishes, and those that during their life cycle produce stages (eggs, larvae) that can be transported by currents or by other organisms, even if the adult lives settled on the sea bottom, as in the case of some molluscs, annelids and other invertebrates. In Taranto seas, many animal species of tropical and sub-tropical affinity have been observed since many years.

5 The regulations

The existing regulations for the introduction of NIS are obviously very important in those countries, such as US, Australia, New Zealand and northern Europe where negative effects hardly impacted their economy. In the Mediterranean area, included Italy, at the moment, only "aims" exist as replies to official papers of EU, ICES (International Council for the Exploration of the Sea) and IMO (International Maritime Organization).

In particular, about the intentional intro-

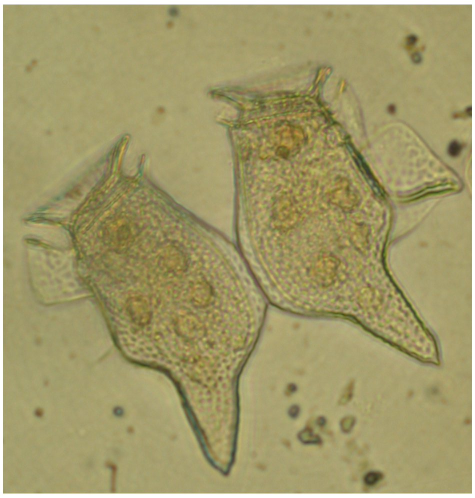

Figure 8: *Dinophysis caudata* Saville-Kent is a toxic marine dinoflagellate (planktonic microalga) that causes huge fish-kills and even severe gastro-intestinal syndromes in humans. (Photo by O.D. Saracino)

duction of NIS, ICES elaborated a Procedure Codex directed both to private and public operators, with suggestions and advices to manage the transfer of exotic species and to prevent the illegal and not authorized introductions (Figure 27).
Some of these exhortations have been received from EU and in November 2003 a regulation concerning the sanitary police and the veterinary certification has been introduced for the import of molluscs, in order to avoid the contamination of sea water by pathogens for the humans and for the locally reared molluscs too.
Italy has formally received this regulation also in its executive form (UE Reg. 708/07), but at present no specific set of rules exists (Figure 28).
About the introduction of NIS through ballast waters, in 2004 IMO, the main agency for the control of the international maritime traffic, has published the "Ballast Water Management" Convention, that was updated in 2008 and adopted by all the member countries, Italy included [16].
But, the current Italian law protects the marine environment only by the effects of hydrocarbons traffic, contemplating a series of measures to prevent accidental flows in the sea water. Even the decree n° 73/99 of the Port Authority of Taranto, dated 04.08.1999, forces the incoming cargos without separated ballasts only to have a logbook of the ballast water destination (Figure 29).
This decree has been recently integrated by the duty, for the incoming vessels, to present to the Port Authority of Taranto a ballast water logbook reporting data on ballast water on board (source, amount, exchange practices) (Ballast Water Convention, 04/03/2009).

Figure 9: The merchant harbour of Taranto is in expansion, with commercial activities that are joining the industrial and oil ones. Many ships arrive from extra-Mediterranean ports with ballast on board. (Photo by F. Rubino)

Groups	N° of Species
Macroalgae	14
Microalgae (planktonic)	4
Sponges	1
Molluscs	3
Polychaetes Annelids	3
Crustaceans	1
Ascidians (sea squirts)	3
Osteichthyes (bony fishes)	4

Table 2: Number of NIS found in Taranto seas, reported for the main taxonomic groups

6 The prevention . . . in practice

As seen before, the Italian regulations are not aligned with those of other countries that regularly experience problems linked to the introduction of NIS. Even the scientific research in Italy is at an early stage, especially for that concerning ballast waters. Anyway, the few studies point out that the Mediterranean is an at risk area, both for its natural dynamics and the constant increase of the maritime traffic with the opening of new routes.

Certainly, in order to avoid big problems in the future, the way to be undertaken is that of prevention, the only one really effective and the least expensive too (Figure 30).

To put preventive measures into practice, a careful and exhaustive risk valuation strategy is needed to ascertain all the possible way of introduction of NIS both at local and national scale.

Taranto, as highlighted before, is an area

Figure 10: In Mar Piccolo of Taranto, mussel farms extend for 17 km^2, i.e. 61% of the surface of the entire basin. (Photo by G. Squitieri)

where different risky factors exist. For Taranto as well as for other towns, the economic activities of which strictly depend on the sea, the prevention of the occurrence of big problems has a fundamental importance. With this aim, all the Institutions must be involved and have to cooperate, i.e. the Municipalities, Provinces, Regions, Port Authorities with the support of the Scientific Research and the Local Public Health Units (Figure 31).

The main objectives to be achieved in order to prevent accidental introductions should be:

- to make controls on "risky" activities;
- to identify the possible vectors of introduction;
- to process and realize a set of regulations at local scale;
- to promote an "ecologic awareness" into all the citizens and the "sea-workers" (Figure 32).

People employed in the different activities linked to the sea must attempt to:

- strictly follow the rules that regulate the introduction of non-native species for rearing purposes;
- do not release into the sea garbage and wastes coming from imported stock of organisms to be reared or sold;
- avoid the rearing of potentially harmful organisms in aquaria.

Finally, the cooperation of all the citizens is needed to:

- avoid the discharge into the sea of the material from old aquaria;
- regularly clean the hulls of recreational boats;
- signal to the qualified Institutions the sighting and finding of "strange" organisms.

Figure 11: *Caulerpa racemosa* var. *cylindracea*. It is a green alga of Indo-Pacific origin. Its first record in the Gulf of Taranto, near the Cheradi Islands, dates back to 1996 [17, 18]. Since some years, it seems to have become highly invasive, forming very large meadows in the Mar Grande and Mar Piccolo basins, and also along the eastern and western coast of the Gulf of Taranto. (Photo by G. Squitieri)

Figure 12: *Undaria pinnatifida* (Harvey) Suringar. It is a brown alga, native of the Japan Sea, reported from Mar Piccolo since 1998 for a small area, very close the fishing fleet moorage. It is a species typical of cold-temperate waters and, probably for this reason, its population has remained confined in the first signalling area, shortly yearly widening its settlement until 2006. Starting from 2007, the species is receding [17, 18]. (Photo by E. Cecere)

Figure 13: *Hypnea cornuta* (Kützing) J. Agardh. This red alga, of tropical affinity, has been discovered in 2000 in the eastern region of Mar Piccolo. Now it is widespread in the whole basin with attached and floating assemblages. Both tetrasporic and propagule-bearing thalli are present from late spring to early winter [17, 18]. (Photo by E. Cerere)

Figure 14: *Grateloupia turu-turu* Yamada. This is a red alga collected for the first time in Mar Piccolo in February 2007, near the fish market of the Old Town. Sterile and tetrasporic thalli are present from early autumn to early summer [17, 18]. The recruitment is continuous in this period. (Photo by E. Cecere)

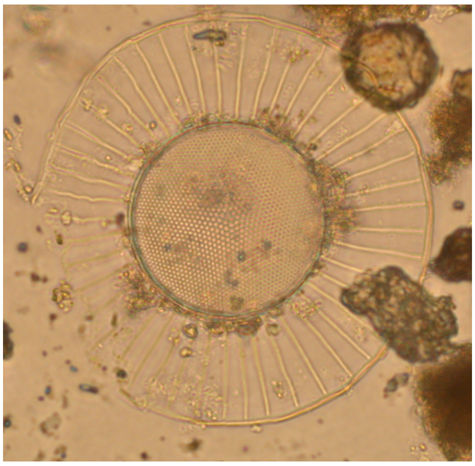

Figure 15: *Planktoniella sol* (Wallich) Schütt. It is a marine diatom (planktonic microalga) native of North Atlantic. Some specimens of this species have been collected in the ballast waters of some cargos arrived at Taranto in 2003. (Photo by O.D. Saracino)

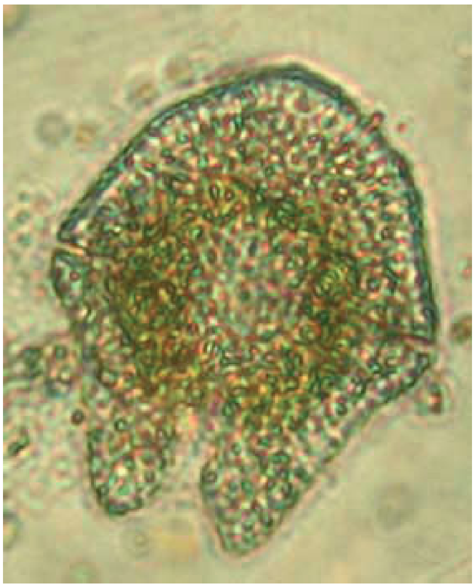

Figure 16: *Akashiwo sanguinea* (Hirasaka) G. Hansen *et* Moestrup. This marine dinoflagellate (planktonic microalga) has been observed in the ballast waters of ships arrived at Taranto in 2003. It is a toxin producer, commonly found also in Mar Piccolo since few years. So, this species could have been recently introduced into the area through ballast waters. (Photo by O.D. Saracino)

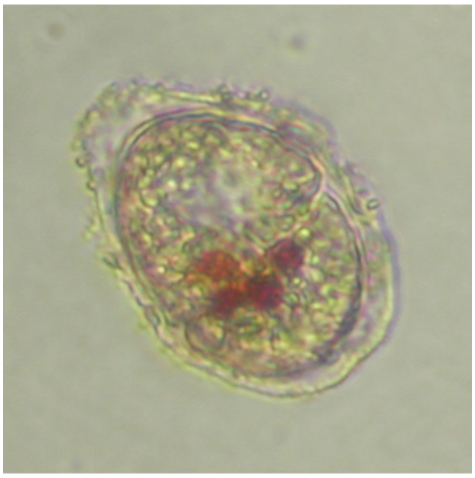

Figure 17: *Gyrodinium instriatum* Freudenthal *et* Lee. Even this resting stage of the dinoflagellate *Gyrodinium instriatum* (planktonic microalga) has been found in the sediments of ballast tanks of ships arrived at Taranto in 2003. (Photo by F. Rubino)

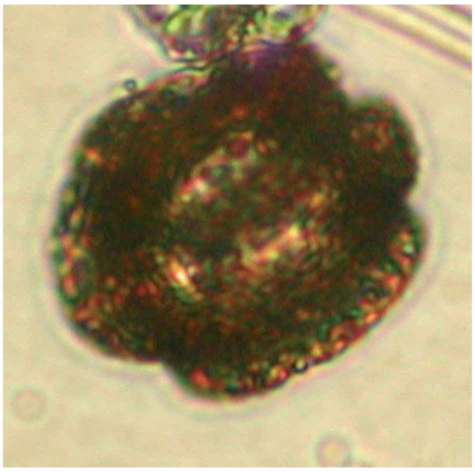

Figure 18: *Bicarinellum tricarinelloides* Versteegh. It is a dinoflagellate resting stage. As the species mentioned before, it has been collected from the sediments of ballast tanks of some ships that arrived at Taranto in 2003. For this species the paleontological name is still used because the active form that lives in the water is unknown. (Photo by F. Rubino)

Figure 19: *Musculista senhousia* (Benson). It is a bivalve mollusc, native of Indo-Pacific. It has been collected for the first time in 2003 in Mar Piccolo sediments where it is present mostly in areas where also unattached macroalgae are abundant. (Photo by F. Mastrototaro)

Figure 20: *Callinectes sapidus* Rathbun. This crustacean known as “blue crab” is native of the North Atlantic American coasts. It has been found in 2004 along the coast at West of Taranto. (Photo by M. Pastore)

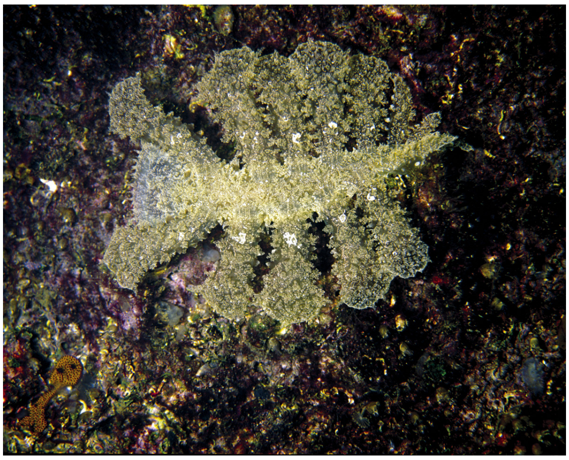

Figure 21: *Melibe fimbriata* Alder *et* Hancock. This gastropod mollusc of Indo-Pacific origin, has been signalled from Mar Piccolo since 2001. It is widespread also in Mar Grande and lives on muddy bottoms near mussel farm plants [19]. (Photo Michelagnoli Foundation Archive)

Figure 22: *Bursatella leachii* De Blainville. A gastropod native of the Red Sea, present at Taranto since 1968. (Photo Michelagnoli Foundation Archive)

Figure 23: *Balistes carolinensis* Gmelin. This "trigger fish" is native of the sub-tropical Atlantic. It is present at Taranto since 1965 even though its findings are occasional. (Photo Michelagnoli Foundation Archive)

Figure 24: *Sphyraena viridensis* Cuvier. The "Mediterranean barracuda" is native of Cabo Verde Islands and recently it has become a common presence in Taranto seas, even in numerous groups. (Photo Michelagnoli Foundation Archive)

Figure 25: *Sparisoma cretense* (Linnaeus). Numerous specimens of this “parrot fish” native to Eastern Atlantic, have been observed near San Pietro Island, but this species is widespread along the eastern coast of Taranto littoral. (Photo Michelagnoli Foundation Archive)

Figure 26: *Diodon hystrix* Linnaeus. This species of Indo-Pacific origin, has been occasionally observed by local fishermen. A specimen captured in 1963 near San Pietro Island is kept in the collection of the IAMC Talassografico of Taranto. (Photo by F. Rubino)

Figure 27: The Japanese clam *Tapes semidecussatus* Reeve, introduced in Italian waters for aquaculture and repopulation purposes has almost completely replaced the native *Tapes decussatus* (Linnaeus). (Photo Michelagnoli Foundation Archive)

Figure 28: Also the imported bivalves are placed in depuration plants before selling. (Photo by M. Pastore)

Figure 29: A cargo ship is discharging ballast water in Taranto harbour (July 2005). To reduce the risk of introduction of NIS in Taranto coastal area, ships entering the port should exchange the ballast water in open sea at a depth of at least 200 m, and then deballast "clean" water in the port. (Photo by Ecotaras spa)

Figure 30: Even a rope immersed in the sea water is enough for the growing of organism, the so called "fouling organisms", which form a typical community. This type of community, that can offer protection and food to fishes and invertebrates, when develops on ships' hulls, can be easily transported through the oceans. (Photo Michelagnoli Foundation Archive)

Figure 31: *Undaria pinnatifida* probably has been introduced into Mar Piccolo of Taranto simply by throwing into the sea water fragments covering imported molluscs. (Photo Michelagnoli Foundation Archive)

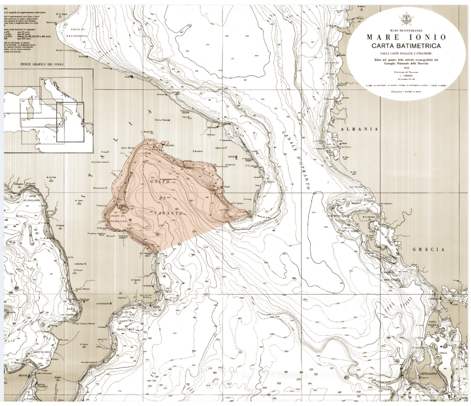

Figure 32: The IMO Ballast Water Management Convention rules the discharge of ballast waters that must be done either in special areas in the ports or 200 nautical miles far from the coast and never closer than 50 nautical miles at least at 200 m depth. For ships entering the Port of Taranto, this means that they must exchange the ballast water outside the red area.

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Welfare Status of Cage Farmed European Sea Bass *Dicentrarchus labrax*: a Comparison Between Submerged and Surface Cages

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Abstract

As alternative farming methods, we investigate growth, haematological, biochemical and immunological parameters of European sea bass (*Dicentrarchus labrax*) farmed in submersible cages in order to compare physiological status under different rearing conditions and to validate the efficacy of submersible technology in solving several of the substantial problems that exist in surface-based fish farming as heavy storms, algal and jellyfish blooms and attacks by predators.
The study was conducted in 2008 in the Gulf of Castellammare (NW Sicily, Mediterranean Sea) in two submerged and two surface cages filled with 75.000 *D. labrax* (initial weight: 28.2 $\pm$ 4.3 gr), for each cage.
No significant differences were shown in specific growth rate between the two groups, even if, fish cultured in submerged cage reached the largest size.
Results from biochemical and haematological parameters examined indicated higher welfare state in fish cultured in submerged cages. Fish reared in surface cages showed a significantly higher blood cortisol and glucose levels and haematocrit value than those of submerged cages.
Also lysozme and haemolytic activity, used as indicators of immunocompetence in fish exposed to stress, lead us to suppose that submergence could have a positive effect on some components of innate immune system of cultured fish.
Results of this study suggest mariculture in submerged net cages a promising system that allows to minimize stress and therefore that favours animal welfare.

1 Introduction

Offshore submersible fish farms may play an important part in the expanding fish farming industry as inshore sites reach full capacity and offshore farming will open an unknown potential for aquaculture [1].
Submergence may solve several of the substantial operational challenges that exist in surface-based fish farming, including those related to heavy storms, algal and jellyfish blooms, unsuitable temperatures, and biofouling of net cages [2, 3].
Moreover, the high concentration of fish confined in surface net cages systems attracts predators [4], primarily sea birds (i.e., cormorants) and aquatic mammals (i.e., dolphins).
Predation on fish farms results in death and injury to fish, resulting in a economic damage to the farmers [5, 4].
The stress on cultured fish subjected to repeated attacks by predators shows itself in poor feed conversion efficiency and hence

the weight at harvest is not maximized [5]. The negative effects of stress in fish do not occur only on growth but affect many body functions.

Fish respond to stressful conditions with a typical stress response consisting in the release of catecholamines and activation of the hypothalamus-pituitary-interenal (HPI) axis which implies the synthesis of cortisol hormone [6]. Both catecholamines and cortisol initiate secondary and terziary stress responses which translate in maladaptive effects of the organism.

Where fish cannot escape a stressor, or where the stressful stimulus is episodic or intermittent, prolonged activation of the stress response has deleterious consequences. These include loss of appetite, impaired growth and muscle wasting, immunosuppression and suppressed reproduction.

In particular, prolonged activation of HPI axis, reduces growth, indirectly, through a negative effect on energy balance and, directly, through a reduced secretion of growth hormone [7, 8]. Since growth and reproduction are functionally linked, [9] stress-induced impairment of growth may indirectly interfere with maturation. Additionally, reproductive activity is suppressed directly during period of stress *via* a wide range of mechanisms [10]. Finally chronic stress has a generally immunosuppressive effect in fish, mediated in particular by the action of cortisol [11].

On the basis of the above scientific evidences, aquatic producers known that controlling animal welfare is absolutely essential for their economic success and that the development of specific stress management protocols is essential for animal health and survival.

Moreover severe stress conditions can heavily influence the expression of cultured fish quality because of changes during storage of the final product [12].

Reducing stress and maintaining fish welfare is, therefore, an important issue for aquaculture industry for production efficiency, quantity and product quality and recently is also becoming important for both consumer perception and marketing [13, 14, 15].

Submersible cages, also for Mediterranean aquaculture, could improve production conditions and reduce environmental effects as escapes due to storms, sea lice infestations, visual impacts sea birds attacks [16] and may also have a positive effect on fish welfare.

Subsurface technologies have been tested in several production experiments e.g., in farming of yellowtail (*Seriola spp.*) in Japan [17] and Italy [18], Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) in the U.S.A [19], cobia (*Rachycentron canadum*) in the Caribbean [20] and recently Atlantic salmon (*Salmo salar*) in Norway [21].

The growth and behaviour of fish in submerged cages relative to standard surface systems is however largely unknown; objective comparisons of fish performance in commercial-scale submerged cages vs. surface cages have only been undertaken for short-term, shallow submergences [16, 3], or for long-term in Atlantic salmon [21].

The aim of this study is to investigate haematological, biochemical and immunological parameters of European sea bass (*Dicentrarchus labrax*) farmed in submersible cages in order to compare physiological status under different rearing conditions and to validate the efficacy of submersible technology.

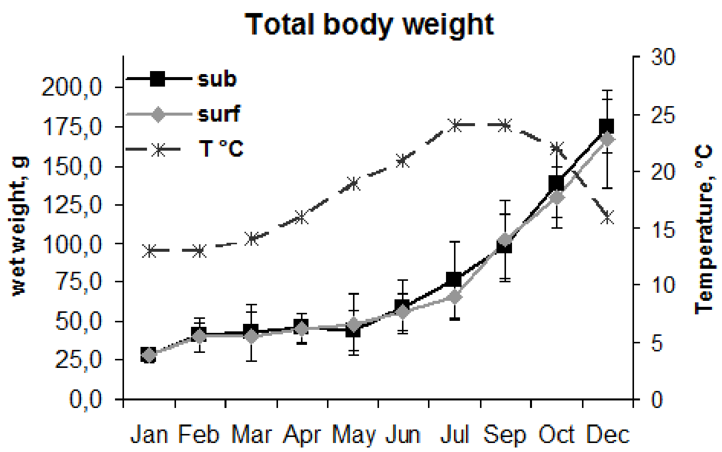

Figure 1: Monthly trends of wet weight of *Dicentrarchus labrax* cultured in submerged (sub) and surface (surf) cages, relative to water temperature changes during farming.

2 Materials and Methods

The study was conducted from January to December 2008 in the Castellammare Gulf (NW-Mediterranean Sea) in four fish cages (two submerged and two surface cage, respectively). The fish cages under investigation (2000 m^3) were filled on January 2008 with 75.000 specimens of European sea bass (*Dicentrarchus labrax* initial weight: 28.2 $\pm$ 4.3 gr), for each cage.
The fish were manually fed with a commercial diet twice a day according to the estimated live weight and water temperature.
During the trial, fish were randomly collected on a monthly basis, and total and standard length and wet weight were immediately recorded for each specimen, to test growth performance, by calculation of the daily specific growth rate (SGRW %).
The relative condition factor (Kn, [22]) was also calculated by using the follow relationship: $Kn = W_{means} \overline{|aL^b|}$ where W represented our measured weights and $|aL_b|$ represented weight estimated by means allometric equations.

2.1 Experimental design and sample collection

At 3 samples time (February, June and September), samples of fish (n=40) were taken to investigate haematological, biochemical and immunological parameters.
Individuals were taken from the cages and immediately anaesthetized using tricaine methanesulfonate, MS222 ($0.1gl^{-1}$). Careful netting and handling were implemented to minimize stress. Blood samples were withdrawn separately from the caudal vein of each individual and then divided into different test tubes depending on the parameter to be analyzed. Part of the blood collected in heparinized tubes was used for the immediate determination of haematocrit value. The remaining fraction was centrifuged and the obtained plasma was stored at -80°C. In order to extract

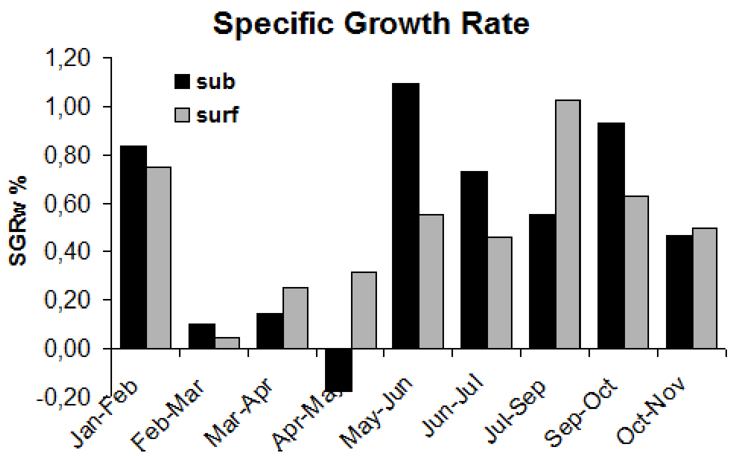

Figure 2: Specific daily growth rates in wet weight of *Dicentrarchus labrax* cultured in submerged (sub) and surface (surf) cages.

serum, blood samples not treated with heparin, were allowed to clot at 4°C, centrifuged at 1500g for 20 minutes and stored at -80°C until analysis.

2.2 Analytical assays

Haematological and biochemical parameters: all assays were carried out using commercial diagnostic kits.

Serum cortisol concentration was determined by a enzyme-linked (ELISA) immunoassay kit (*Alpha Diagnostic International*, USA).

Serum glucose and total protein levels were determined by kits respectively based on the colorimetric Glucose Oxidase-Peroxidase (GOD-POD) and chemical biuret-tartrate methods (*Sclavo Diagnostics*, Italy).

Plasma lactate was assayed by a kit that utilize the enzymatic colorimetric method of Lactate Oxidase-Peroxidase (LOD-POD) (*Roche Diagnostics*, Italy).

Haematocrit value (% red blood cell) was determined in heparinized capillary tubes after centrifugation in a standard micro-haematocrit centrifuge at 12000 g for 10 minutes and comparison of the capillary tubes with a reference scale.

2.3 Immunological parameters

Haemolytic activity against sheep erythrocytes were determined in serum samples, according to Caruso et al. [23].

Non specific haemolytic activity (SH50) was assayed using a 2.5% suspension of sheep red blood cells (SRBC) in phosphate buffered saline (PBS) containing 10 mM Mg^{2+} and 2 mM Ca^{2+} added to serially diluted serum in PBS-Ca-Mg buffer. After incubation at 22°C for 1 h, unlysed erythrocytes were removed by centrifugation and the content of haemoglobin in the supernatants measured at 540 nm [24]. The SH50 units were obtained from the concentration of serum giving 50% haemolysis of

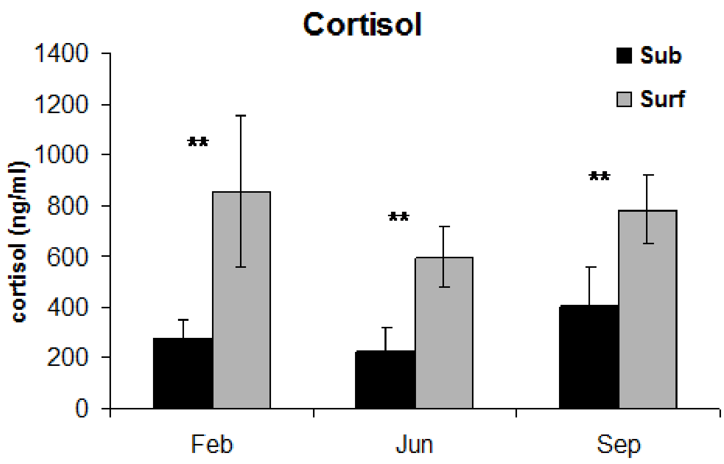

Figure 3: Cortisol concentration in *Dicentrarchus labrax* cultured in submerged (sub) and surface (surf) cages. Reported are the result of the Student t-test: $* = p < 0.05$, $** = p < 0.01$, no indications is reported when difference is not significant.

SRBC, where 100% haemolysis was calculated by incubation with distilled water of the SRBC suspension.

Lysozyme content was measured on samples of mucus, serum and kidney, according to the plate diffusion method [25, 26]. Lysozyme content was evaluated by measuring the diameter of lysis produced into 1% agar plates added with 0.05% *Micrococcus lysodeikticus* cells, incubated at 33°C for 22°C.

3 Results and Discussion

3.1 Growth parameters

Figure 1 shows the monthly trend of total wet weight of the two groups of *D. labrax* during the experimentation period. Although there were no significant differences in wet weight, fish cultured in submerged cage reached the largest size.

No significant differences were shown in specific growth rate between the two groups, even though group sub showed a peak in May-Jun and Sept-Oct. The two populations showed a positive trend, except for the month of Apr to May (Figure 2).

The overlapping of the growth curves for the fish cultured in surface cage and those cultured in submerged cage was also confirmed by the relative condition factor K. The relative condition factor showed that increases in weight and length did not lead to a corresponding increase in this factor.

Under the environmental conditions present during the trial, submerged and control European sea bass, experienced broadly similar light and temperature regimes, as control fish preferred to swim at depths in the cage which were similar to those at which submerged fish swam.

The results from this trial contrast with all previous commercial scale sea-cage

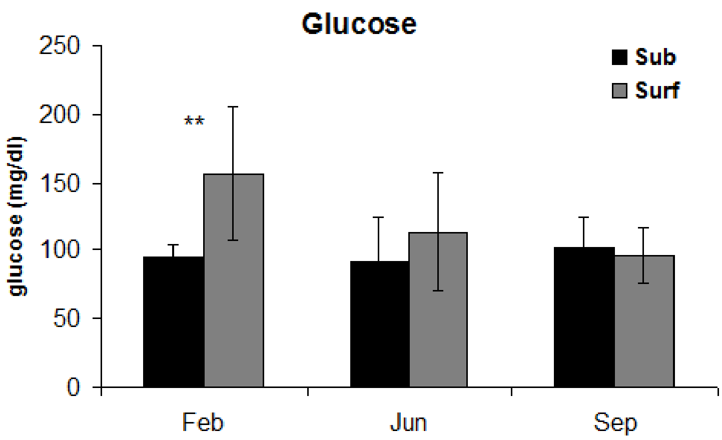

Figure 4: Glucose concentration in *Dicentrarchus labrax* cultured in submerged (sub) and surface (surf) cages. Reported are the result of the Student t-test: $* = p < 0.05$, $** = p < 0.01$, no indications is reported when difference is not significant.

submergence experiments which have invariably documented negative effects of submergence upon growth or condition [27, 16].

3.2 Haematological and biochemical parameters

As in other vertebrates, fish experiencing stress show several physiological changes that are expressed through a number of particular indicators [6].
Principal results of our study underline a clear relation between observed changes in physiological parameters and the farming type (submerged or surface cage). All biochemical and haematological parameters examined were significantly higher in sea bass cultured in surface indicating a lower state of welfare than fish in submerged cages.
Fish reared in surface cages showed a significantly higher cortisol levels ($p \leq 0.01$) than those of submerged cages (Figure 3).
Blood cortisol levels are widely used as an indicator of stress condition in fish [6] because the extreme sensitivity of the HPI axis that is activated in response to most forms of stress in teleost fish.
An increase in cortisol levels means that fish are experiencing a stress and they are reacting with a typical “stress response”, of adaptive value characterized by an immediate release of catecholamines and cortisol into the blood stream.
As shown in Figure 4, overall lower values of glucose are observed in submerged cage but the difference is significant (p<0.01) only in the February sampling.
Blood glucose levels have been also described to be affected by chronic and acute stress [28]. Indeed stress is typically associated with increased metabolic rate that is positively correlated to plasma glucose levels [29]. Therefore increased glucose concentrations are commonly explained by

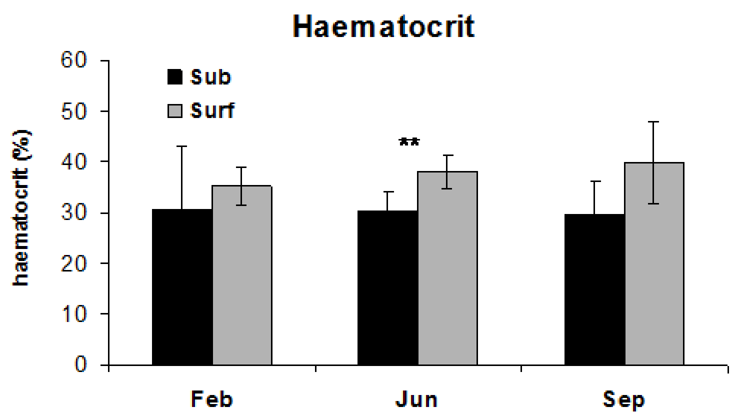

Figure 5: Haematocrit values in *Dicentrarchus labrax* cultured in submerged (sub) and surface (surf) cages. Reported are the result of the Student t-test: $* = p < 0.05$, $** = p < 0.01$, no indications is reported when difference is not significant.

the increased requirement of energy occurring during stress conditions.
Haematocrit value is also consistent with other parameters, showing significant higher value ($p \leq 0.01$) in surface cages in June (Figure 5).
In general haematocrit value rises in fish exposed to stress as a direct consequence of the increase in hearth-beat and the need of higher oxygen uptake [12].
The increase of lactate is used as a stress index and is related to glycolysis anaerobic that occurs when muscular activity is intense [30, 31], but statistical differences were not observed between cages.
It is also important underline that in *D. labrax* reared in submerged cages all considered parameters are maintained in the range of basal values reported in literature on the contrary fish of surface cages often exceed this range.

3.3 Immunological parameters

The non-specific or innate immune system is the most important resistance mechanism in fish [32, 33] and some of its components (e.g., lysozme, haemolytic and haemagglutinating activity) are used as indicators of immunocompetence in fish exposed to stress [34, 35].
As shown in Figure 6, fish of submerged cages showed significant higher values ($p \leq 0.01$) in non specific haemolytic activity (SH50), in June and September.
Lysozyme content was measured in different tissues or organs (mucus, plasma and kidney) but higher and significant difference ($p \leq 0.05$) was found in the mucus of seabass cultured in submerged cage only in September (Figure 7).
Mucus lysozyme represents the first defence mechanism against foreign agents [32, 26].
The detection of different immunological defence ability lead us to suppose that subemergence could have a positive effect

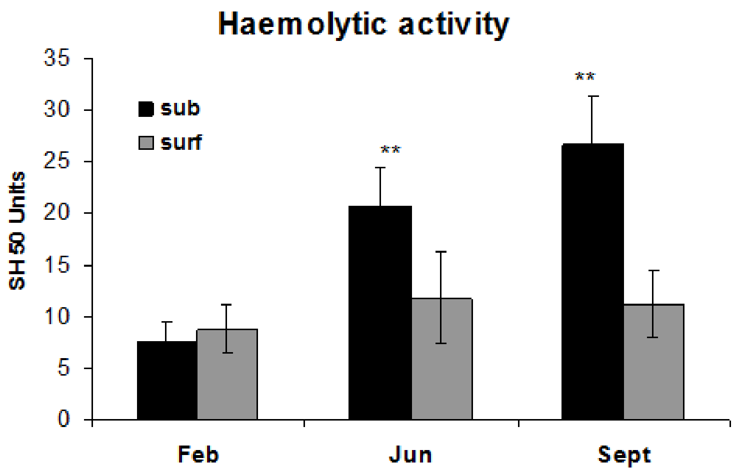

Figure 6: Haemolytic activity in *Dicentrarchus labrax* cultured in submerged (sub) and surface (surf) cages. Reported are the result of the Student t-test: $* = p < 0.05$, $** = p < 0.01$, no indications is reported when difference is not significant.

on some components of innate immune system of cultured fish.
Moreover results of immunological parameters are in agreement with those haematological and biochemical and seem to further confirm the hypotesis that submergence promotes sea bass welfare.

4 Conclusions and future perspectives

The research provides for the first time data on stress assessment in submerged cages, a particular rearing condition. In off-shore cages, where handling or other stressors currently acting in intensive practices do not affect the quality of the final products, the main negative factor that may influence the specimens reared is mainly referable to chronic stress condition with a continuous disturbance due to the growth itself and consequent increase of the specimen size.
Submergence seems to be a favourable condition for sea bass culturing, as evidenced by growth, haematological, biochemical and immunological parameters.
One reason for this could be linked to stress response from predation. In fact, due to predation activity (i.e. birds attacks), surface-based fish farming can become an environment favouring high mortalities, injury and stress; in a research on dynamics of predation, Dieperink [36] refers that a flock of cormorants are able to empty the pound net in about 30 minutes and consume 110 fish weighing a total of approximately 50 kg.
This hypothesis is, also, supported by studies on neuroendocrine perception of stress in fish where is demonstrated that prey/predator encounter is a stressful situation and induce a stress response with energy cost for escape [37].

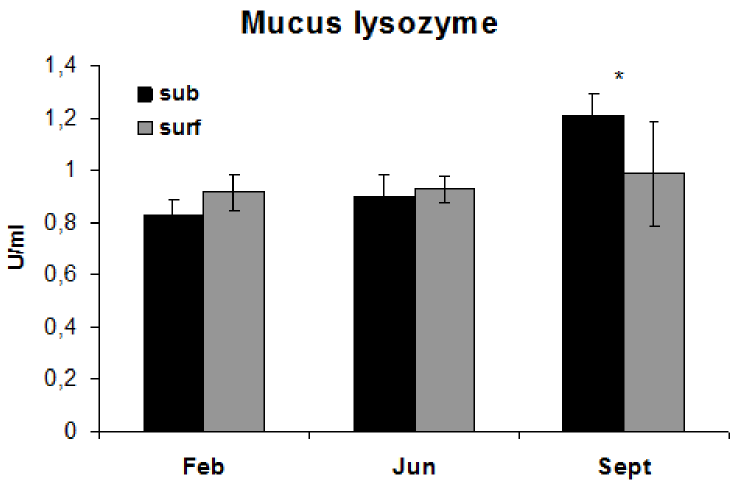

Figure 7: Lysozyme content in the mucus of *Dicentrarchus labrax* cultured in submerged (sub) and surface (surf) cages. Reported are the result of the Student t-test: $* = p < 0.05$, $** = p < 0.01$, no indications is reported when difference is not significant.

Rehnberg and Schreck [38] report in coho salmon (*Oncorhynchus kisutch*) an increase in plasma cortisol following chemosensory detection of a predator Woodley and Peterson [39] refer in long-nose killifish (*Fundulus majalis*) elevated plasma cortisol when exposed to visual stimulus of a predator similarly, Jarvi [40] reports that Atlantic salmon (*Salmo salar*) displayed a physiological response following predator exposure in the form of elevated blood glucose, lactate concentrations and chloride concentration.

Moreover literature reports a relationship between high plasma cortisol levels and immunosuppressive effect producing alterations in some components of immune system e.g., phagocytic activity [41], lysozyme activity [42], or serum haemolytic and agglutinating activity [34, 43, 44].

Among the parameters used in our experiment to monitor the response to stress induced by the different culture conditions, cortisol and glucose proved to be the most sensitive and therefore their determination is suggested in the early warning of metabolic alterations following sea-cage farming.

All these results lead us to conclude that the conditions in which fish are cultured do influence their welfare. Haematological and biochemical parameters suggest that sea bass reared in submersible cages show the best welfare condition compared to fish kept in surface cages. The high immunological reactivity displayed by fish reared in submersible cage also supports this conclusion.

This study lead us to suggest mariculture in submergible cages as a rearing practise that allows to minimize stress and therefore that favours animal welfare.

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Meiofauna as Indicator for Assessing the Impact of Fish Farming in Coastal Marine Sediments

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Abstract

The present study aimed to detect the impact of organic loads due to the biodeposition of a fish farm in a coastal area of the Tyrrhenian Sea (Western Mediterranean). Sediment chemistry and meiofaunal assemblages were investigated from December 2006 to October 2007 on seasonally basis at four stations: two were located under the fish farm, while the others were about 1-km distance, and served as controls. Variations in the biochemical composition of the sedimentary organic matter and meiofaunal community structure were also related to changes in reared fish biomass. The presence of the cages induced changes in the benthic compartment: the sediments showed reducing conditions. A significant accumulation of biopolymeric carbon was observed beneath the cages during the entire investigated period, resulting in a reduction of the meiofaunal density in the impacted stations, a higher proportion of meiofauna was observed in the top 2 cm of the sediment. Changes were evident also in terms of community structure as meiofauna under the cages, when compared to the Controls, was characterised by increased importance of polychaetes and copepods vs. a much lower significance of kynorhynchs and gastrotrichs that we proposed ad indicator of organic enrichment. Finally, biodiversity decreased beneath the cages due to the disappearance of taxa more sensible to organic accumulation.

1 Introduction

The rapid expansion of marine aquaculture is a potential solution to the problem of overfishing and fisheries depletion worldwide, but also a major threat to marine ecosystems reducing the value of the natural capital and benefits that humans gather from the exploitation of coastal areas' resources [1]. One of the most widely cited but poorly quantified impacts of open netpen aquaculture is its release of nutrients and other wastes to the surrounding environment [2].

Aquaculture is a fast-growing industry which, through the release of organic and inorganic N and P contributes to the progressive eutrophication of coastal areas [3, 4]. Aquaculture activities are relevant at local and regional scales, and recent estimates indicate that, in Mediterranean coastal areas, the release of nutrients from fish farming contributes for up to 7 and 10% of N and P total discharge, respectively [5].

Changes induced by aquaculture installations can have a negative impact on the natural environment, threating the quality of coastal zones, and generating conflicts between aquaculture and the conservation of marine habitats [6].

The organic enrichment of the sediments

immediately beneath the sea cages is a direct result of the sedimentation of particulate waste products from the fish farm and a decreasing concentration with the increasing distance from the point source is typically observed [6, 7]. The continuous flow of faeces and food pellets from fish cages alters the quantity and the biochemical composition of sediment organic matter, and the progressive transformation of the substrate into an anoxic environment [8, 7]. These environmental changes may lead to a reduction in benthic species richness, abundance and biomass, mainly due to oxygen depletion associated with biochemical oxygen demand and the formation of toxic products in anoxic sediments [9].
Monitoring of biological parameters rather than chemical ones is important as it gives the bioavailability rather than the absolute concentration of pollutants. The close association of meiofauna with the sediment matrix means that changes in interstitial water chemistry will soon lead to changes in meiofaunal abundance and diversity [10]. Since meiofauna organisms play a crucial role in the decomposition of detritus, as well as in nutrient cycling and in energy flow, they proved to be one of the fauna among the benthic components that are appropriate for detecting biodeposition impact [11, 12, 13].
Metazoan meiofauna, for their ecological importance in the benthic ecosystems and the lack of larval dispersion, are becoming a popular tool for investigating structural and functional changes of natural and anthropogenically-impacted ecosystems [13, 14, 15].
We hypothesized that the presence of fish farms influences the meiofaunal assemblages in terms of abundance, community structure and diversity (i.e. richness of taxa).

2 Materials and Methods

2.1 Study Site and Sampling

This study was conducted from December 2006 to October 2007 in an coastal area along the South-West of Sicily (Sicily Channel, Mediterranean Sea, Lat. 37°32'079" N; Long. 12°57'202" E). The fish farm is composed by 6 floating cages (n=2, 6500 m^3, n=4, 2100 m^3) that produces about 500 t $year^{-1}$ of European sea bass (*Dicentrarchus labrax*).
Salinity and bottom temperature ranged from 37.4‰ to 37.8‰ and from 14°C to 25.3°C respectively, during the entire sampling period. The area has microtidal regime and dominant currents flow in SE-NW or WNW-ESE directions, following the main circulations of the Sicily channel. The study area is sheltered and has sandy-muddy sediments.
Fish are fed using automatic distributors supplying an amount of pellets equivalent to about 3% (daily) of the total biomass contained in the cage. Food supplied is composed of proteins (46-51% of dry weight, DW), carbohydrates (18-20%), lipids (14-17%) and the remaining fraction is accounted by ash, vitamins and pigments.
A preliminary survey was carried out to determine the spatial extent of the fish-farm influence and to identify the control station. Sediment samples were collected on a seasonally basis (December 2006, April, May and October 2007), manually by SCUBA divers at four stations: the two Impacted stations under the net pens (in the farm-sediments) and the two Control stations, about 1 km far from the fish farms, in a area

not affected by the aquaculture plants). All stations were located at a depth of 30-35 m, and displayed a very similar sediment texture as the silt-clay fraction accounted for 19.2% at the Control sites and 21.9% at the Impacted sites. The remaining fraction was accounted by sand containing a carbonate fraction averaging 30%. Visual census and preliminary analyses, performed on a grid of stations, revealed that the Control area was not affected by fish farming and could be considered as representative of the average conditions of the area at similar depths. Meiofaunal samples were collected in replicate plexiglas cores (n=3, diam. 3.7 cm, 10.7 cm^2 surface area) down to a depth of 10 cm This depth appeared to be adequate for quantitative analysis as the evaluation of the top 15-cm of the sediment core revealed that $<1\%$ of the total meiofaunal density was present in the 10-15 cm sediment horizon. Sediment cores were sectioned into layers (0-2, 2-5 and 5-10 cm) to evaluating vertical distribution of meiofauna. The sediment layer of three additional cores (diam. 3.7 cm) was sectioned into layers (0-2, 2-5 and 5-10 cm). Each layer was mixed and frozen at -20°C for the analysis of photosynthetic pigments and organic matter variables.

2.2 Environmental parameters

Organic matter content and composition analyses (in terms of lipid, protein and carbohydrate) were carried out according to Fabiano and Danovaro, [16]. For each analysis about 0.5 g of sediment was used and blanks were made using the same previously calcinated sediments (450°C, 2 h). All analyses were carried out in three replicates. Carbohydrate, protein and lipid concentrations were converted to carbon equivalent assuming a conversion factor 0.40, 0.49 and 0.75 respectively, and the sum of the protein, carbohydrate and lipid carbon is referred as the biopolymeric carbon (BPC).
Only the results of the top 2-cm layer are reported in this study.

2.3 Meiofaunal analysis

Samples were fixed with 4% buffered formaldehyde in 0.4 μm prefiltered seawater solution. Sediments were sieved through 1000 and 37 μm mesh nets. The fraction remaining on the 37 μm sieve was centrifuged three times with Ludox HS (density 1.18 g$\cdot$$cm^3$) as described by Heip et al. [17]. After staining with Rose Bengal (0.5 $\cdot g \cdot l^{-1}$), all meiobenthic animals were counted and classified per taxon under a stereo microscope.

2.4 Statistical analysis and ecological indexes

Differences in benthic variables between Control and Impacted stations were tested using a non-parametric analysis (Kruskal-Wallis). The use of this simple statistical method was justified by the small sample size and by the non-homoscedasticity of the data.

3 Results

3.1 Organic matter concentrations in the sediment

Protein, carbohydrate, lipid and biopolymeric carbon (BPC) concentrations are shown in Table 1.
Proteins and lipids were the two main biochemical classes of organic compounds. The proteins ranged from 2325.9 $\pm$ 420.2

		December		April		July		October	
		Avg.	**±s.d.**	**Avg.**	**±s.d.**	**Avg.**	**±s.d.**	**Avg.**	**±s.d.**
PRT ($\mu g\ g^{-1}$)	**Control 1**	1060,8	127,7	1749,8	313,1	1436,7	116,2	1744,1	282,4
	Control 2	1204,3	335,9	1806,2	248,7	1489,4	272,8	1809,2	254,2
	Impact 1	2735,9	275,0	3068,3	228,9	3405,0	533,2	3390,2	270,7
	Impact 2	2325,9	420,2	2877,8	212,8	2928,7	555,8	3205,4	860,5
LIP ($\mu g\ g^{-1}$)	**Control 1**	1579,8	391,2	2353,4	1247,0	1842,9	419,0	2296,3	337,7
	Control 2	2093,4	137,1	2937,1	412,1	2274,9	245,2	1784,5	614,8
	Impact 1	3920,0	498,6	5274,5	252,8	4777,4	525,1	3431,8	258,5
	Impact 2	3313,4	395,1	4995,9	716,7	4124,8	270,4	3262,5	358,5
CHO ($\mu g\ g^{-1}$)	**Control 1**	800,5	66,9	729,0	125,0	1272,9	493,5	892,5	296,1
	Control 2	826,7	142,3	882,8	196,9	1297,2	434,5	1067,9	147,7
	Impact 1	1288,6	375,2	1134,3	215,3	1522,1	322,9	1292,0	325,6
	Impact 2	1027,3	272,6	1049,4	246,9	1734,5	232,0	1117,3	330,4
BPC ($\mu gC\ g^{-1}$)	**Control 1**	1752,1	185,1	2345,5	635,5	2395,8	248,7	2442,4	320,7
	Control 2	2047,5	227,6	2721,9	247,6	2612,7	529,6	2401,2	399,6
	Impact 1	3875,1	198,2	4464,0	69,4	4721,0	169,2	4002,9	338,2
	Impact 2	3235,5	279,5	4195,5	422,3	4385,9	536,3	3713,6	614,8

Table 1: Environmental parameters: PRT= Proteins, LIP= Lipids, CHO= Carbohydrates and BPC=Biopolymeric carbon in the top 2-cm sediment layer.

to 3405.0 $\pm$ 533,2 μgg^{-1} in the impacted stations (in December and July, respectively), and from 1060.8 $\pm$ 127.7 to 1809.3 $\pm$ 254.2 μgg^{-1} in the control stations (in December and October, respectively). The total carbohydrate ranged from 1027.3 $\pm$ 272.6 to 1734.5 $\pm$ 232.0 μgg^{-1} in the impacted stations (both at Impacted 2 station in December and July, respectively), and from 800.54 $\pm$ 66.9 to 1297.2 $\pm$ 434.5 μgg^{-1} in the control stations (in December and July, respectively). The lipid ranged from 3262.5 $\pm$ 358.5 to 5274.5 $\pm$ 252.8 μgg^{-1} in the impacted stations (in October and may, respectively), and from 1579.8 $\pm$ 391.8 to 2937.1 $\pm$ 412.1 μgg^{-1} in the control stations (in December and October, respectively). Among the biochemical variables investigated, lipid and protein displayed significant differences between the impacted and control stations at each sampling period, (K-Wallis test, $p<0.05$).

The BPC concentrations (expressed as the sum of the protein, carbohydrate and lipid carbon) ranged from 3235.5 $\pm$ 279.5 to 4721.0 $\pm$ 169.4 μgCg^{-1} in the impacted stations (in December and July, respectively), and from 1752.1 $\pm$ 185.1 to 2721.9 $\pm$ 247.6 μgCg^{-1} in the control stations (in December and May, respectively). Significant higher values were in observed for BPC, in the impacted stations respect the control stations, in the four sampling periods (K-Wallis test, $p<0.01$ and K-Wallis test, $p<0.05$, for Impact 1 and Impact 2 station, respectively).

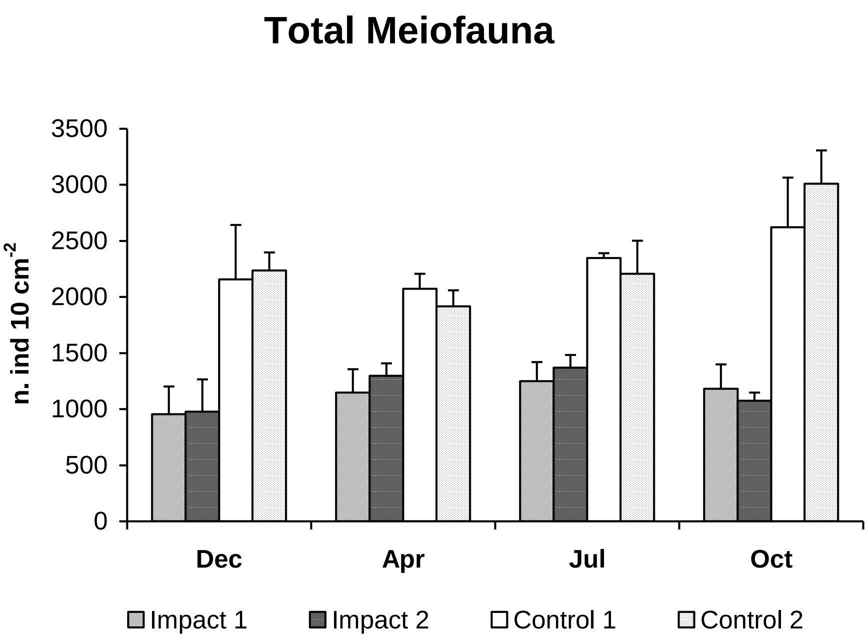

Figure 1: Temporal variations of total meiofaunal density at the Control and Impacted stations. Data are expressed as ind. 10 cm^{-2} $\pm$ SD.

3.2 Meiofaunal abundance and community structure

The meiofaunal densities in the top 2 cm of the sediments ranged from 955.2 $\pm$ 247.1 to 1369.7 $\pm$ 113.2 ind. 10 cm^{-2} in the impacted stations (in December and July, respectively, Figure 1), while in the control stations they ranged from 1975.7 $\pm$ 144.5 to 3009.8 $\pm$ 297.4 ind. 10 cm^{-2} (in April and October, respectively, Figure 1). Significant differences were observed between the impacted and the control stations at each sampling time (K-Wallis test, $p<0.01$).

Temporal changes in the meiofaunal community structure in the impacted and the control stations are illustrated in Figure 2. In the impacted stations, nematodes accounted for 47.5 to 67.9% of the total meiofaunal abundance (in October and April, respectively). They were followed by copepods, accounting for 16.1% to 32.8% (in April and October, respectively), polychaetes (range: 8.9 to 14.7%, at Impact 2 station, in April and December, respectively) and oligochaetes (range: 0.3 to 1.8%). Among the minor taxa, gastrotrichs accounted for 0.62 to 1.55% of the total meiofaunal abundance (at impacted 2 station, in December and October, respectively), turbellarians accounted for 0.52 to 2.07% (in October and December, respectively) and ostracods accounted for 0.32 to 3.82% (at impacted 1 station, in April and October, respectively).The contribution of

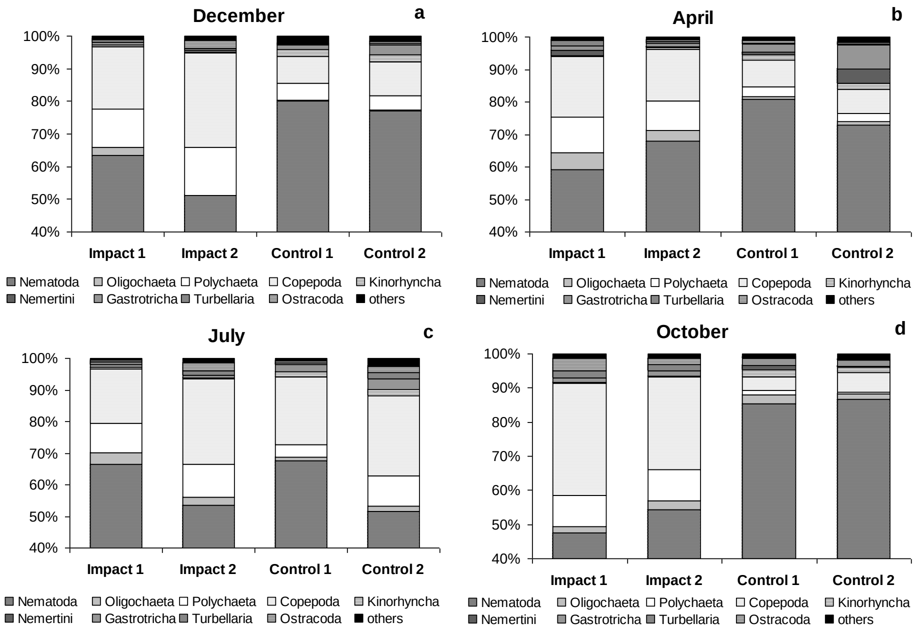

Figure 2: Meiofaunal community structures in the four sampling periods: (a) December, (b) April, (c) July and (d) October.

all of the other remaining taxa was lower than 1%.

In the control stations, the meiofaunal community structure was dominated by nematodes (accounting for 51.4 to 86.8% of total meiofaunal abundance at Control 2 station, in July and October, respectively), followed by copepods (range: 4.0 to 25.4%), polychaetes (range 0.6 to 9.4%, at Impact 2 station, in October and July, respectively), gastrotrichs (range: 1.3 to 7.4%, in December and April, respectively), kynorhinchs (range: 1.7 to 2.1%, in April and December, respectively) and oligochaetes (range: 0.2 to 2.5%, at Impact 1 station, in December and October, respectively). The contribution of all of the other remaining taxa was lower than 1%.

4 Discussion

The potential spatial impact of fish-farm industries Fish farms generally release large amounts of soluble and particulate organic matter (POM) into the surroundings [18] and may thereby exert bottom-up pressure on the benthic community [19]. We found considerably higher organic matter concentrations under the cages compared to control sites, during the entire study.

Downward fluxes derived from fish-farming activities contribute to the accumulation of organic matter in fish-farm sediments and have the potential to change the distribution patterns of benthic assemblages [20, 21].

Fish farm sediments have been observed

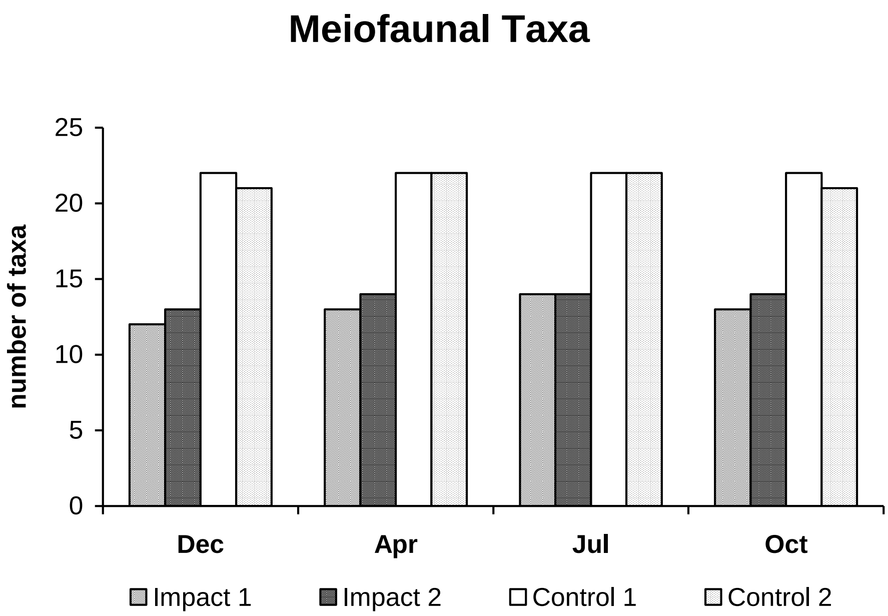

Figure 3: Temporal variations of number of meiofaunal taxa at the Control and Impacted stations.

to be organic enriched environments relative to adjacent areas outside of the region of direct impact of increased sediment accumulation [22, 23], see [24] for comparison), and differences between Impacted and Control sites were evident and significant, in this study, in all sampling periods, in terms of proteins, lipids and biopolymeric carbon concentrations.

The presence of the fish farming had a considerable impact on the biochemical composition of the sedimentary organic matter, as in the impacted stations protein and lipid concentration were about 2 times higher than in the Controls.

The increase in proteins and lipids in fish-farm sediments is likely to be related to the composition of the food pellets provided to the fish being reared [6]. Indeed, the food pellets were typically composed of 48% to 52% protein and of 20% to 22% lipids, which when not consumed by the fish, accumulates in the surface sediments beneath the cages [23].

This study highlights that changes in quantity and biochemical composition of sediment organic matter caused by intensive aquaculture are critical for assessing the presence and levels of impact induced by fish-farm activities, confirming that intensive aquaculture can significantly contribute to benthic eutrophication processes, although the extent of the spatial effects of fish-farm effluent is potentially limited.

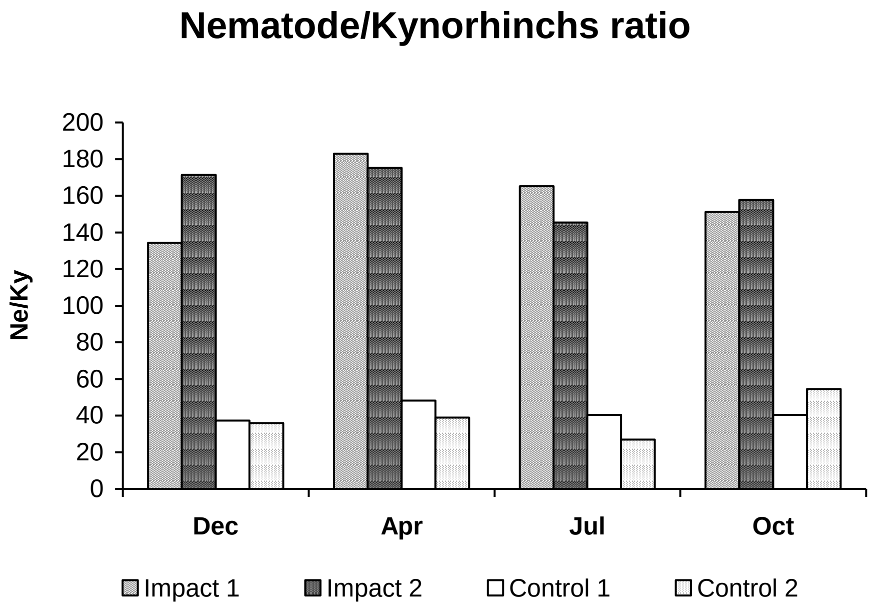

Figure 4: Temporal variations of Nematode/Kynorhinchs ratio at the Control and Impacted stations.

4.1 Meiofaunal assemblages

Meiofauna are becoming good tool to reflect changes of environmental disturbance both spatially and temporally [15, 25]. The general outcome is that anthropogenic disturbance alters meiofaunal abundance, diversity, biomass, and species composition.
Meiofaunal abundance in this study was comparable to values reported from other farm sediments, but we observed a strong reduction in density at the impacted stations when compared to the Control sites (for about 40%, Figure 1).
In a similar study on fish-cage impact, Mirto et al. [26] pointed out a clear reduction of the meiofaunal abundance in systems subjected to high organic load, and the drastic reduction (50-70%) in meiofauna abundances has been reported from other fish farm areas in the Mediterranean [23, 13, 27, 28].
Biodeposition influenced also the meiofaunal assemblages inhabiting farm-sediments, in terms of organisms abundance, and each taxon showed a different sensitivity to the presence of fish-farms (Figure 2).
The sediment organic enrichment, determined altered conditions under the cages. This caused a modification of the meiobenthic community structure in comparison to the Control sites with increased significance of copepods (from 11.3 to 23.4% of the total density), polychaetes (from 3.8 to 10.5%), oligochaetes (from 1.2 to 3.7%),

ostracods (from 0.6 to 1.55%) and turbellarians (from 0.54 to 1.18%). By contrast we observed reduced abundance of nematodes (from 75.2 to 57.9%), gastrotrichs, (from 2.9 to 1.0%), kinorhynchs (from 1.9 to 0.4%) and nemertins (from 0.9 to 0.3%), suggesting their higher sensitivity to the impact of the farms' effluents.

The sensitivity of certain taxa, such as kinorhynchs to organic pollution is in agreement with previous studies on the effects of hydrocarbons on meiofaunal assemblages [29], Previous studies reported that kynorhynchs are among the most sensitive taxa to organic enrichment [27, 30, 13], but the sensitivity of nematodes to increased organic loads is in contrast with most studies in this field [31, 27]. Other taxa profited from the organic enrichment and among them polychaetes were present in high densities, thus displaying opportunistic behaviour. Therefore, it is possible to conclude that the farm-sediments determined evident quantitative and structural changes in meiofaunal assemblages.

Fish farm impact was evident also utilising general biological descriptors such as the number of taxa, which strongly decreased under the cages, due to the disappearance of the more sensitive taxa (Figure 3), suggesting that Classes or Phyla can disappear in areas subjected to the biodeposition. Thus presumably having profound implications on the benthic ecosystem functioning, the functional role of species production and their energy transfer to higher trophic levels.

Since total meiofauna density showed spatial and temporal fluctuations and is known, in general, as inconsistent throughout sampling sites [4], densities of selected taxa should be considered as more stable indicators of fish farm pollution. Copepods, polychaetes and kinorhynchs contributed the most to the differences in community structure between impacted and control sites and, in the present study, were the most sensitive taxa.

In this study, an attempt was made to examine the behaviour of the Ne/Ky (nematode/kynorhinchs) ratio that we propose to verify in the future as a possible, potentially interesting indicator of organic enrichment (i.e. biodeposition) in specific areas. In fact we found strong differences between impacted and control stations in the Ne/Ky ratio (Figure 4), with significant higher values in the impacted stations in all sampling times, suggesting his potential as indicators of organic loading associated with aquaculture operations.

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Fishery as Collective Resource. Review on Italian Legislation: Italian Unity – Present

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Abstract

The essay offers a new analysis of the Italian fisheries legislation since the 19th century.
It shows that the Italian Parliament felt the need to meet the criteria for the protection of the marine ecosystem, increase the sustainability of fishing practices and the adoption of rules guided by the precautionary principle in times in which this principle was completely unknown, at least in the modern sense.
Through regulation of practices that have an impact on the environmental balance, the attention for environment is an ancient concept that we find today in any assessment and legal ethics relating to environmental policy, bolstered by recourse to the precautionary principle.

1 Introduction

As Aristotle remarked, "what is collective is barely looked after, since men have great respect for what belongs to them and very little for what they own in communion with the others". The consequence of all this is that the management of the environmental resources, (of the fish ones, on which we will focus), proves a rather complex activity. The issue gets more and more significant if we consider that every individual lives its life surrounded by environmental resources shared and exploited together with other human beings, with permanent management difficulties.
For their intrinsic features, environmental resources are goods which are available for everyone, since it is not possible nor opportune to exclude some from the benefits deriving from the use of the goods. Yet the consume in favour of one individual affects the capacity of utilization of all other individuals. This condition originates a negative externality: with the increase of the number of people using the asset, the utility that these obtain from the asset tends to a constant decrease and those specificities which make the asset precious risk to be cancelled and to provoke a damage not only to the present community, (which cannot use it) but also to the future generations, which cannot claim rights on it. As a matter of fact, in presence of externalities, the market cannot harness all the costs and benefits bound to the production and use of the asset, with the consequence that the market balance diverges from the social optimum. In presence of externalities, the market fails in its role of efficient allocator, thus exposing the natural resources to overexploitation.
To correct the market inefficiency, the com-

munity can try and engage a joint strategy which leads to the creation of shared informal rules, or it can entrust the management of the natural resource to the public decision-maker. This survey will focus on those decisions taken by the Italian public legislator, analyzing the evolution of the specific fishery law from the Italian unity to present. In particular, we will try and examine the parliamentary discussions, analyzing the contents of the legislative acts approved by the Parliament and contextualize such legislative acts based on the main economic theories which prevailed in the different historical periods.

2 The first fishery law of Italian Unity

In 1877 Italy was united since a bit more than ten years. The competence for the fishery sector belonged to the Ministry of Agriculture, Industry and Trade, in charge of "determining the bonds and guarantees for the preservation and multiplication of fish as well as the more profitable exercise of the fishery industry" ([1]). In an attempt to give more homogeneity to the issue of fishery (ruled by an incredible number of legislative provisions and enforcements, often conflicting), Minister Majorana Calatabiano presented - in November 1876 – a bill titled "Fishery provisions". According to the Minister's intentions "it is necessary to determine to which extent the general interest for the preservation of the animals living in the waters justifies the restrictions to the exercise of a profession, limiting the legal provisions to those which prove strictly necessary for the preservation and multiplication of the fishes" ([2]). This position was heavily influenced by the liberal beliefs of the 19th century political economy, which had inherited from the politics of the Enlightenment, an anthropocentric vision of the reality which focused on the human needs, rather than on the environment. Even though first the physiocratic school, and then the classical school came to the conclusion that the laws of nature and the availability of resources were insurmountable limits to the human capacity to produce wealth, the trust in the progress overcame any existing fear about the possibility of an unstoppable growth. According to liberal orthodoxy, there were no considerable problems relevant to the availability of human capital. Thus, since the economic system was held free from any bond with the external environment, to impose conditionings on the human activities in order to regulate the exploitation of territory and resources appeared not justifiable.

Although the minister's positions interpreted the liberal thought of the time, inside the Parliamentary Committee a whole different discussion originated. Particularly emblematic is the introductive report of the bill, i.e. "provided the sea beds are respected and the eggs incubation is not disturbed and the species are not destroyed, the fishermen will always - and in any time - harvest their abundant production .... Yet if men, with their means, destroy the animal species, they nullify the benefits of nature thus making it sterile: and this presently occurs in our waters", and besides "our fishermen, with unconceivable carelessness, slaughter little swordfishes which are sold for very little money. ... Anchovies and sardines which travel from coast to coast for the most part of the year, are also cruelly slaughtered by the fishermen who catch the newborns, thus carelessly destroying the bread and butter of their families" (Parliamentary Committee Acts – 14th February 1877, page 2). The strong contents of the introductive report generated a lively debate, whose contents, one century later, are still particularly up-to-date. MP Di Rudinì, for instance, underlined how fishery had to be meant not only as industry but also as an activity aimed at the management and future preservation of fish resources. "Since the actions of the single individuals develop consequences which affect everyone, it is common interest to protect renewable yet unlimited resources such as forest and fish resources " (Parliamentary Committee Acts – 14th February 1877, page 1272).

This modern vision which looked into the long term period collided, during the discussion of the law project, with a short term vision which underlined even the necessity of thick mesh trawling, justified by the insufficiency of the fish resource. The use of trawling was considered the only option to poverty for the fishermen families who, without the use of such tools, could not fish with other means obtaining the same results. According to MP De Saint Bon, "we should know to which extent we can limit the fishermen's freedom as well as dispute their bread and butter in favour of a theoretic vision to perpetuate some or all fish species" (Parliamentary Committee Acts – 15th February 1877, page 1316). Furthermore, MP Randaccio's remarks added that the reason for the lack of fish was due to the same fishes and "in this great organisation of productive and destructive forces, constantly struggling, a struggle which is commendably and appropriately ruled by nature, mankind with its nets matters very little" (Parliamentary Committee Acts – 16th February 1877, page 1332).

Analyzing the Parliamentary Committee discussion, emerges the opposition between the two tendencies: one aimed at preserving the community's interests with an eye to the future, while the other aims at the short term protection of the fishermen's class, even at the expense of the total depletion of the marine resources. This opposition reflects two different visions of nature: anthropocentric and bio centric. The former considers nature as a res thus as an asset which mankind dominates in terms of propriety rights; whereas the latter vision regards it no longer as a simple way to satisfy the human needs but rather as a means (together with mankind) to ensure the mankind survival as well as the improvement of the quality of life [3]. In a holistic vision of the relationship between mankind and nature, the mutual respect represents the necessary condition for the survival of all the living beings on earth.

The broad discussion generated in the Parliamentary Committee produced (on 4th March 1877) the first Italian law on fish resources, i.e. law n. 3706. Although the law was in line with the liberal spirit of the time, it introduced bonds of times, places, means and species which could be fished and sold. Furthermore, the law had a wide recourse to decentralisation, entrusting peripheral administrations the task to establish the contents of the prohibitions as well as their regulation and settlement. These choices collected practices which were consolidated in many Italian maritime communities and, for some aspects, underlined the worries for the capacity of the regeneration of the marine environment to the extent of compensating the growing human consumptions ([4]). A concern we could define as "modern", since it did not belong to the heritage shared by the economic culture of the time nor to the theses which were largely spread by natural sciences.

3 The fishery law between the two world wars

At the end of WW1, Italy had to face the high unemployment rate which threatened the social and politic stability of the Country. In this context, the rigid liberalism makes way to an increasing trust in the State intervention also in the fields of natural assets and territory management. The consequence is an ever increasing commitment in public investments by the state finances as well as the creation of administrations specifically in charge of the supervision of the country's natural heritage.

In July 1920, the bill titled "Provisions in favour of fishery and fishermen" appears within this new cultural setting. Main purpose of this bill was the reorganisation and modernisation of the fishery industry through tax privileges, economic aids, credits and any other necessary support to make the Italian fishery industry competitive in comparison to other European countries, especially France. Although the modernisation of the fleet was the specific purpose of the bill, the report issued by the central Office of the Senate of the Kingdom supplies a punctual analysis of the problems of fishery at the time, which – for many aspects – reconnect to the discussion which preceded the approval of law n. 3706/1877. Already in the foreword, the central Office of the Senate warned that "a profitable employment of capital in the fishery industry was possible, yet exceeding certain limits would represent a risky imprudence". Although supported by scientific evidences and by a minor number of naturalists, the central Office of the Senate clearly dissented from the "easy letting go", underlining how dangerous it was to allow the fishing of juvenile fish while tolerating abusive fishing. This circumstance anticipates the precaution principle which will be stated many years later, during the Rio de Janeiro International Conference on Environment and Development, in 1992. Furthermore, debating the environment preservation, the report by the Central Office of the Senate underlines the need to consider fish resources as an economic asset not only for the present generations, but also for the future ones, claiming that "our grandchildren should use them just like us" (Report issued by the Central Office of the Senate of the Kingdom dated 7th July 1920, page 6). Once again, quite a "modern" concern which recalls the principle of responsibility, formulated by the German philosopher Hans Jonas.

The report used by the central Office of the Senate was widely appreciated by all political parts, and this led - on 24th March 1921 – to the approval of law n. 312. Unlike the previous 1877 law, the new legal text is affected by the intervention logic of the time and is characterised by the public expenditure which the State commits to pay in two directions. The former meant to stimulate the growth of the Italian maritime industry by means of tax privileges and credit, whose expected result was the modernisation of the vessels, means and catch preservation techniques as well as their numeric growth. The latter and more innovative direction provided for an action aimed at supporting the biologic research as well as the vocational training of fishermen.

4 Fishery law in republican Italy

After more than 40 years from the law dated 24th March 1921, n. 312, the problems of Italian fishery pointed out by the report of the Central Office of the Senate were not solved at all. Therefore, on 24th October 1963 a new bill titled "Provisions concerning maritime fishery" was presented. The guiding principle of the bill was the preservation of the fish stock, in a cultural context which had overcome the belief that the living resources of the sea were unlimited. Both in economic literature and in jurisprudence raised the awareness that such resources were collective, instead of res nullius, thus needing protection [5]. Given this, in the Parliamentary Committee meeting of 30th October 1964, MP Amodio remarked that "the nature of res nullius commonly assigned to sea fishes, impedes any civil action, and it also eliminates any chance of compensation. The most advanced legislations remedied to this gap identifying the principle of the public interest for the protection of biologic resources of the sea" (Parliamentary Committee meeting, 30th October 1964, page 119).

After some partial amendments to the bill in implementation of International conventions (among which Geneva's, 1958), law n.963 was approved on 14th July 1965. Once again, the law presents the two main State commitment goals for the fish sector which can be summarized in the regulation of the activities and the promotion of the scientific research. The legal text also indicates a series of detailed administrative prescriptions to practise fishery, organizes central and peripheral supervisory authorities and bodies monitoring possible violations. The legislator ordered the Minister for Merchant Shipping to be active in didactic divulgation and scientific research, also realizing no-fishing biological protection areas.

This new legislative provision submits the freedom to conduct a business to limits set and defined by the state administration, appointed to pursue extra-economic goals, aimed at the protection of the ecosystems. Therefore, human activities could be carried out only to the extent that their impact did not impair the complete performance of the ecosystem.

5 The protection of the fish resources in the European context

From the '70s on, the European Community starts showing some interest for the fish production. The creation of one single market could not leave out the marine environment as well as its fauna which - for their own nature – are common goods par excellence. Since the waters constitute one of the spaces which the European coastal nations share, in the Mediterranean, Atlantic and Northern Seas, it proved essential to guarantee to the operators of each country fair access conditions as well as the birth of a common market for the alieutic products. Fishery thus gradually became an activity subject to the community management as well as the addresses of the fishery common politics.

In order to discipline the matter, the Council meeting of 20th December 2002 was approved the Regulation n. 2371/2002 titled "sustainable conservation and exploitation of the fishery within the common fishery policy". The regulation, like every community legislative measure, consists of many "whereas" which clarify the reasons at the base of the processing of the measure. Among those, the Council underlined that "given the progressive exhaustion of several fish stocks, it is essential to enhance the fisheries common policy in order to guarantee the long-term profitability of the fishery sector by means of a sustainable exploitation of the live water resources, based on reliable scientific opinions as well as on the precautionary approach grounded on the same considerations of the precautionary principle mentioned in art. 174 of the treaty establishing the European Community.

The sustainability principle is already present in the Maastricht Treaty. This is the result of a process started during the 1972 Stockholm Conference where the Earth was defined as a "capital" to be preserved, taking into account the critical relationship between growth of ecosystem and the irreversible process deriving from the exploitation of non-renewable resources. However, only in 1987 the concept of sustainability received an official acknowledgment with the publication of the report "Our Common Future" of the World Commission on Environment and Development created by the Bruntland Commission. This concept contains the idea that it is necessary to guarantee "the needs of the present without compromising the ability of future generations to meet their own needs". In 1992, the Rio de Janeiro Declaration affirmed this concept by making it a legal principle which is pervasive of every law at international, European and national level concerning the preservation of the environment and of its resources.

Consequently, through the resources intervention measures, such as the elaboration of plans for the management and the reconstitution of the fish stock (indicating the limits of their exploitation as well as the details of collection) or the assignment of capture and marketing shares, the Regulation n. 2371/2002 [6] adopts a turnover of the anthropocentric mentality. Quoting from Regulation - art. 2 "the target is to gradually implement a management of the fishery based on ecosystems". The human activities can thus be carried to such an extent which their impact does not affect nor impair the integrity of the ecosystem and, consequently, the downsizing and the modernisation of the fishing vessels fleets proves an essential measure to realize the intent of a sustainable exploitation, as well as the development of the common market of alieutic products, goals to be pursued thanks to the appropriation of structural funds.

6 Concluding remarks

This overview of rules and parliamentary discussions, allowed us to highlight the evolution of the public intervention in the regulation of fish resources which, well before 19th century, were perceived as a common good whose use could not be left to a free and indiscriminate access. The prevailing of this vision, a rather opposing trend in comparison with the economic, politic and scientific mores of the century, can be brought back to the customs shared by the different coastal communities active in the exploitation of the fish resources.

The attention for the fish resources will grow during the 20th century, in parallel with a growing intervention of the State in economy. The early years of '900 saw the diffusion of the idea of the existence of a wide field of interests which the central government can manage better than the market. This belief consolidates in the second half of '900, when the natural resources start being considered as strategic assets which need to be protected in nation's interest. Thus emerges the awareness that mankind is not free from those bonds with the planet's bio economic and physical law, but – on the contrary – it is plunged in a thick web of interdependences which influences its work and actions. However, such awareness leads to a significant increase of the public expenditure in the fishery sector. This element continues to characterize the present reference institutional frame work yet, it should be reconsidered based on the various neo-istitutionalists contributions by Elinor Ostrom (Nobel Prize winner in Economic Sciences, 2009).

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