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Spatial Distribution of Oceanographic Properties, Phytoplankton, Nutrients and Coloured Dissolved Organic Matter (CDOM) in the Boka Kotorska Bay (Adriatic Sea)

A. Campanelli[1], M. Cabrini[2], F. Grilli[1], Z. Kljajic[3], E. Paschini[1], P. Penna[1], M. Marini[1]
1, Institute of Marine Sciences, CNR, Ancona, Italy
2, National Institute of Oceanography and Experimental Geophysics, Trieste, Italy
3, Institute of Marine Biology Kotor Dobrota, Kotor, Montenegro
a.campanelli@ismar.cnr.it

Abstract

The temporal variations of temperature, salinity, fluorescence, dissolved oxygen concentration, Coloured Dissolved Organic Matter (CDOM) and of chemical (nutrients, chlorophyll a) and biological (phytoplankton composition) parameters in the Boka Kotorska Bay were observed during two periods (May and June 2008). CDOM regulates the penetration of UV light into the sea and therefore plays an important role in many hydrological and biogeochemical processes on the sea surface layer including primary productivity.

In the framework ADRICOSM-STAR it was possible to investigate the Boka Kotorska Bay during May and June 2008 in order to increase the understanding of optical and chemical characteristics and their evolution through these periods.

Owing to the Karst river inputs and the reduced water exchange with the open sea, in both periods station KO (located furthest from the open sea) presented different physical, chemical and biological characteristics with respect to the other stations inside the Boka Kotorska Bay.

A positive correlation was found between CDOM and chlorophyll a ($R=0.7$, $P<0.001$, $n=15$) and this implies that in this area, similarly to the open sea, the primary source of CDOM should be the biological production from phytoplankton. This is probably due to the fact that the rivers entering the Boka Kotorska Bay are not severely impacted by man.

1 Introduction

Light entering the ocean is absorbed by water, living and detrital particles, and dissolved materials (< 0.2 μm). Absorption by the latter component, also known as coloured dissolved organic matter (CDOM), is mostly attributable to humic substances.

It is well known, that the abundance and distribution of CDOM for many coastal waters is dominated by terrestrial inputs from rivers and runoff as decomposition of terrestrial organic matter yields lightabsorbing compounds such as, humic and fulvic acids [1, 2, 3]. In particular, CDOM is produced near the surface of the open ocean as a result of a heterotrophic process [4, 5, 6, 7] and is destroyed by solar bleaching in stratified waters [8, 9, 10, 11, 3]. The optical properties of CDOM are (almost) never completely eliminated by solar bleaching or other natural processes, suggesting a pool of CDOM that is at least partially resistant to solar bleaching and microbial degradation. CDOM regulates the penetration of UV light into the sea and mediates photochemical reactions, therefore playing an important role in many biogeochemical processes on the ocean surface including primary productivity and the air-sea exchange of radiatively important trace gases (e.g. [12, 13, 14, 15]). The absorption of blue light by CDOM overlaps the phytoplankton absorption peak near 440 nm, resulting in a competition between CDOM and phytoplankton for light in this region of visible spectrum [16, 17, 18]. To better characterize the relationship between phytoplankton biomass and the absorption by dissolved materials, CDOM absorption coefficients aCDOM (λ) have been compared with chlorophyll concentrations [19, 17]. Significant correlations between chlorophyll a and aCDOM (λ) have been observed in eutrophic waters [20]. Generally, however, aCDOM (λ) does not covary linearly with instantaneous estimates of pigment concentrations or phytoplankton productivity in coastal regions [4]. Bricaud et al. [19] hypothesized that such a covariation might exist if biological activity was averaged over a seasonal time period.

The Boka Kotorska bay is a semi-enclosed basin situated in the south-eastern Adriatic sea (Mediterrean Sea - Montenegro), sometimes called Europe's southernmost fjord. Boka Kotorska bay represents a drowned valley shaped during the Pliocene period continued later by tectonic downwarping. Kotor and Risan bays are characterized by karstic rivers and underground springs, which influence temperature, density and salinity of sea water ([21] Kljajic, personal data). The freshwater runoff from these rivers probably modifies the optical and biochemical properties of seawater during the different seasons.

The enrichment of water with nutrients primarily nitrogen, silicate and phosphorus might result in algal biomass growth. In particular, light and nutrient levels in the surface layer were sufficient to sustain active phytoplankton growth in similar basins, but if there is a short residence time of the surface water this means that most of this production can be exported to the outer basin [22]. The nutrients can come from land surface runoff within a watershed (rivers and underground water discharges) and from direct urban inputs (sewage treatment plant outflows, industrial and storm water drains).

Variations in the stratification regimes and mixing depths in basins of this type have interesting effect on phytoplankton growth. However, Mikee et al. [22] found that high CDOM content in fresh water runoff was the most obvious factor limiting production in a fjord situated on the west coast of Scotland. They found that if CDOM concentrations in the surface layer were reduced then the euphotic zone would extend to the bottom and conditions would be favourable for substantial growth of the phytoplankton population.

The aim of the present study is to understand and assess the optical biochemical and biological characteristics and their evolutions throughout May and June in the Boka Kotorska bay characterized by fresh

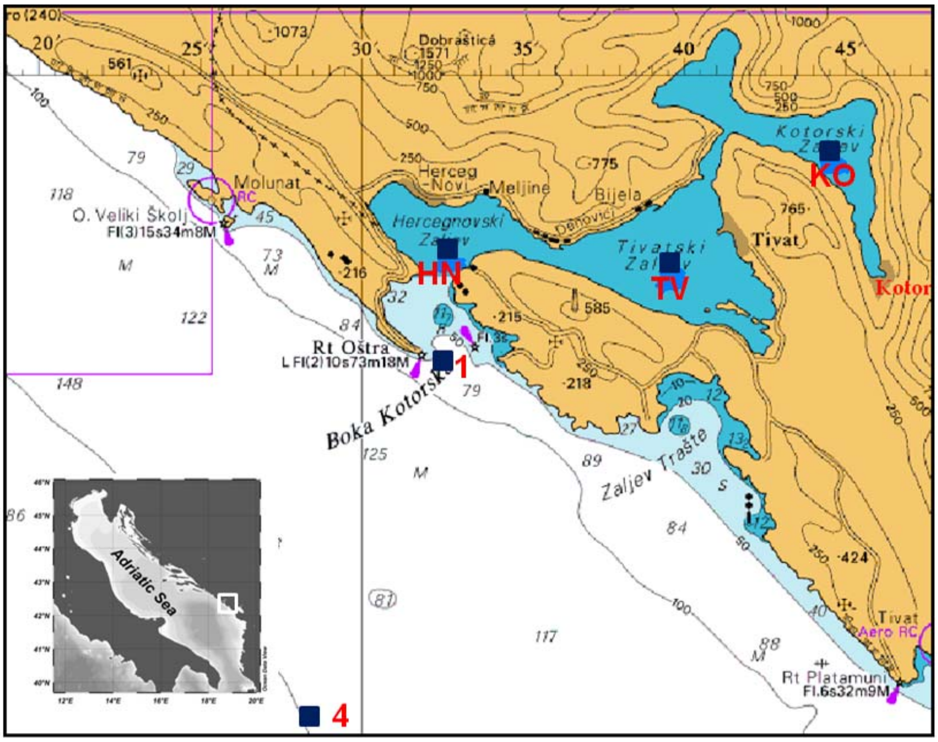

Figure 1: Geographical positions of stations in the study area (redrawn from [23]).

water inputs and large biomass growth.

2 Methods

Measurements were collected in the Boka Kotorska bay (Figure 1) during two cruises carried out aboard the R/V G. Dallaporta from May 10-17 and June 24-29, 2008.

The CTD (Conductivity-Temperature-Depth) data were collected at all the stations with a SeaBird Electronics SBE 911-plus CTD equipped with additional sensors for dissolved oxygen (SBE43) and in situ fluorescence + turbiditimetry (Turner-SCUFA). The 24 Hz CTD data were processed according to UNESCO (1988) standards, and pressure-averaged to 0.5 db intervals. Water samples were obtained by the upcasts with a SeaBird Carousel rosette water sampler equipped with 10-litre Niskin bottles.

Samples of CDOM, TSM (Total Suspended Matter), Chlorophyll a, nutrients and Phytoplankton at the surface and at 10 m depth were collected at all the stations.

The measure CDOM absorption water samples were filtered through 0.2 μm Nucleopore membrane filters, then stored in the dark under refrigeration (4° to 8° C) and analysed on board within 24 hours using a Perkin Elmer spectrophotometer 550A model (10 cm cuvette pathlength). Absorbance data were converted to absorption coefficient (aCDOM) according to Mitchell et al. [24]:

$$\mathrm{aCDOM}(\lambda) = (2.303 \cdot \mathrm{l}^{-1})\cdot$$
$$\cdot[\mathrm{ABs}(\lambda) - \mathrm{ABbs}(\lambda) - \mathrm{ABnull}(\lambda)],$$

where l is the cuvette pathlength, ABs(λ) is the optical density of the filtrate sample relative to purified water, ABbs(λ) is optical density of a purified water blank treated like a sample relative to purified water, and ABnull(λ) is the apparent residual optical density at a long visible or near infrared wavelength where absorption by dissolved material is assumed to be zero.

For TSM measurements, 1litre of the seawater sample was filtered into a preweighed 47 mm GF/F Whatman filter (0.7 μm). The filter was rinsed with 25-30 ml of deionised water to remove salt crystals, then dried at 60°C and weighed [25].

Chlorophyll a was measured by filtering 3l samples through 47 mm GF/F filters and immediately extracted with 5 ml of acetone at –22 °C. The analysis were made, in the ISMAR-CNR laboratory, with a Dionex HPLC equipped with a gradient pump GP50, Photodiode Array Detector PDA100 (wavelength range: 190–800 nm), C18 reversed phase column (4.6 mm x 250 mm, 5 μm particle size), AS50 Autosampler and 300 μl sample injection loop. Pigment concentrations were determined employing a modification of the procedure developed by Wrighit et al. [26].

Nutrient samples were filtered (GF/F Whatman), stored at -20 °C in polyethylene vials and analysed in the ISMAR-CNR laboratory. The nutrients (nitrate-NO_3, orthophosphate-PO_4 and silicic acid-$Si(OH)_4$) were analysed with a Bran+Luebbe Autoanalyzer QUAATRO system, and the resulting data processed with AACE 6.0 (Automated Analyzer Control and Evaluation) software. Nutrient concentrations were determined employing a modification of procedures developed by Strickland and Parsons [25].

Microphytoplankton samples (250 ml) were fixed with $Ca(HCO_3)_2$ buffered formaldehyde (4% final concentration). Samples were processed using sedi-mentation chambers according to Utermöhl [27, 28] and observed with a light inverted microscope in order to determine and count all the cells.

3 Results and Discussion

3.1 Hydrography

Figures 2 and 3 show profiles of the temperature, salinity, dissolved oxygen and fluorescence concentrations for the four stations inside the Boka Kotorska bay (KO, TV, HN and 1) and for one station (station 4) in the open sea during the two cruises. In May, temperature and salinity levels, in all four sites inside the bay, were comparable. However, the KO station compared to the other stations showed a higher temperature (18 °C) and less salinity water (28.5) in the first five metres. The KO station was influenced by river runoff, as the other stations inside the bay, but it was the station furthest from the open sea and this probably influenced mixing processes along the water column. Furthermore, the fluorescence and oxygen concentrations showed many more differences between KO station and all the other stations.

TV, HN and 1 stations showed nearly the same oxygen saturation and fluorescence concentration profiles. Fluorescence was homogeneous in the entire water column (0.7-0.8 A.U.) with a slight increase on the bottom layer (0.9-1 A.U.). However the oxygen saturation showed an opposite pattern by decreasing near the bottom.

KO station was the only one that showed high values of fluorescence at the surface (1.4 A.U.) in correspondence to the maximum of oxygen (101 %), and in the middle layer (1.2 A.U. at 22 m depth) coupled with the minimum oxygen values (89.4 % at 25 m depth). The minimum of oxygen saturation below the layer of the maximum of the fluorescence indicated a probable predominance of mineralization process rather than a primary production process and the absence of important vertical mixing.

The waters occurring in station 4 (Figure 3) showed the same charateristics of the Adriatic coastal waters. Salinity was constant enough from the surface to the bottom with a slight increase along the water column (from 38 to 38.8). A surface layer began warming as expected in this period [29, 30, 31]. Fluorescence and oxygen profiles showed the maximum values at 50-80 m where light conditions were good for the primary production [32, 33].

In June, the temperature increased in all the five stations in particular the 30 m layer from the surface. This increase in respect to previous period was around 7 °C (in HN and 1 station) and 9 °C (in TV and 4 station). The salinity increased at the surface by only 1-2 points in all the stations and this was probably due to the poor river runoff in the summer period.

The oxygen saturation presented lower values (less than 5-10 %) compared to May because during the summer period the vertical mixing was normally reduced [34].

The KO station showed some differences in respect to the other ones. Oxygen saturation increased to 7-8 % in respect to May. This increment was coupled with the fluorescence concentration decrease and it was not probably imputable to the primary production.

In respect of May, more changed were observed in fluorescence and oxygen saturation at the KO, TV and HN stations. In particular, the KO station showed, as observed in May, major differences in respect to the other stations.

3.2 CDOM, Biochemical and Biological characteristics

Table 1 shows the values of CDOM and biochemical parameters (nutrients, chlorophyll a) and phytoplankton (phytoflagellates, diatoms, dinoflagellates and others nanoflagellates) in the two months.

Microplankton community was dominated by diatoms, nanoflagellates both autotrophic and heterotrophic and dinoflagellates with lower abundances but high specific diversity, as previous observations documented by Vuksanovich and Krivokapic [35] in Boka Kotorska Bay.

The nutrient and phytoplankton concentrations showed a different distribution at the KO station compared to the others. In particular, orthosilicate concentrations and diatom abundances showed different patterns. In May, the silicate concentrations were about 5 μM at the KO station coupled with a diatom abundance of about 3000-3500 cell$\cdot$l^{-1}. In June an increment of cell number of diatoms at the 10 m depth (9040 cell$\cdot$l^{-1}) and an uptake of orthosilicates that reduced to 0.54 μM was observed. The other stations inside the bay and in open sea showed opposite behaviour. In these stations, the orthosilicate concentrations increased by about 1 μM in respect to May and the diatom concentrations reduced drastically (e.g. from 3900 cell$\cdot$l^{-1}to 380 cell$\cdot$l^{-1} at the surface of the TV station). In June the Kotor bay seems to have better physical and biochemical condition for diatoms growth.

Nitrates and orthophosphates showed a general increase from May to June in nearly all the stations and this is probably due to an enrichment of nutrient from river discharge. This was coupled with an increment of phytoflagellate populations and with a decrement of dinoflagellates. With the exception of station KO it was observed that the total phytoplanktonic populations remained sufficiently constant between the two periods of surveying. Furthermore, the chlorophyll a concentrations did not show relevant differences between May and June in nearly every station and the values ranged between 0.06 and 3.13 $\mu g \cdot l^{-1}$. At 10 m depth the maximum value (3.13 $\mu g \cdot l^{-1}$) was observed in June at the KO station and this was coupled with high diatoms concentration.

Absorbance of coloured dissolved organic matter at 440 nm ranged between 0.11 and 2.19 m^{-1}. In May the Boka Kotorska bay showed higher values of CDOM at the surface in respect to the 10 m depth. This feature was probably due to the major freshwater runoff that input organic matter into the bay as shown by surface salinity. In fact, rivers are the primary source of CDOM (mainly soil-derived) and the groundwater near the coastlines, but coastal waters may also contain plankton-derived CDOM which is produced in rivers and estuaries as well contained in man-made compounds [36]. A particular feature was observed at the KO station where surface absorbance was 0.18 m^{-1}. It could have happened that different physical conditions, observed at this station, improved the sink process of CDOM as photobleaching [37] and microbial decomposition [38, 39]. Furthermore, destruction of CDOM by exposure to sunlight released compounds used for organisms growth [40]; amongst these is nitrate that, in fact, was found to be higher (0.86 μM) at station KO than elsewhere [41].

Compared to May, June values of CDOM at the 10 m depth increased. More homogeneous CDOM values between the surface and the 10 m depths were found. In the KO station a high CDOM absorbance (2.19 m^{-1}, 10 m depth) was observed and this was coupled with high chlorophyll a and diatoms abundance and low nutrient concentrations (chl a=3.13 $\mu g \cdot l^{-1}$; $Si(OH)_4$=0.54 μM; NO_3=0.13 μM and PO4=0.09 μM).

CDOM versus salinity and chlorophyll a was correlated to try to understand its primary source. Salinity and CDOM did not show an inverse relationship through the two periods as often observed in coastal areas, instead there was a significant relationship between CDOM and chlorophyll a ($R=0.7$, $P<0.001$, $n=15$). These features support the hypothesis that the biological production was the primary source of CDOM in this area. A review by Coble [36] highlighted that, as demonstrated by numerous field studies, all the lower trophic groups (phytoplankton, grazers, viruses and bacteria) are involved in the production of CDOM, and in many locations a positive correlation has been found between CDOM and chlorophyll a. In this region the phytoplankton should appear as a primary source of CDOM instead of terrestrial input.

4 Conclusion

As there are few reference materials regarding this area this current work improves our knowledge of a previously area. The Boka kotorska Bay in respect to the open sea appeared to be characterized by a lower salinity surface layer and by a phytoplankton community dominated by phytoflagellates, diatoms and lower dinoflagellates abundance. Nutrient and chlorophyll a concentrations were not so different between the bay and the open sea. This feature may imply that the different phytoplankton populations found were attributable to different physical conditions, such as salinity, rather than to differences in biochemical characteristics.

In both periods the KO station located most farthest from the open sea presented different physical and biochemical characteristics in respect to the other stations inside the Boka Kotorska bay due to the Karsitc rivers input and lower exchange with the open sea. These features could improve phytoplankton growth during some periods, as found in June for Diatoms.

The positive correlation found between CDOM and chlorophyll a implies that in this area the primary source of CDOM should be the biological production from phytoplankton as happens in open sea. This feature was very interesting and supports the hypothesis that in the bay the terrestrial inputs were not so rich in organic matter. This was probably due to the fact that the rivers entering the Boka Kotorska bay are not severely impacted by man.

These results are important in order to value the role of the biological parameters respectively the anthropogenic impact and almost to characterize the hydrological properties of the entire Bay.

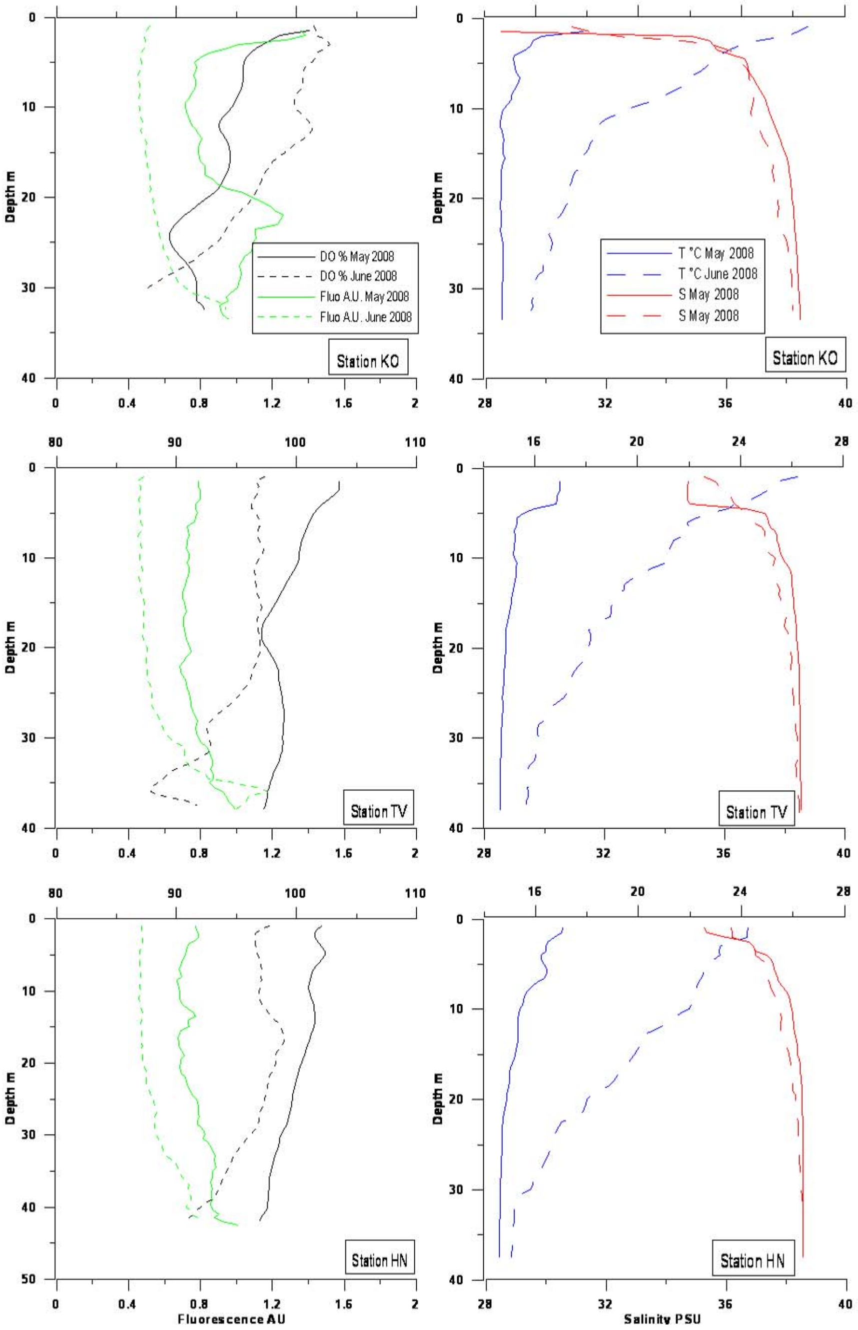

Figure 2: Profiles of Temperature (T, blu line), Salinity (S, red line), Dissolved Oxygen (DO, black line) and Fluorescence (Fluo, green line) in the KO, TV, HN stations during May (solid line) and June (dotted line) 2008 (redrawn from [23]).

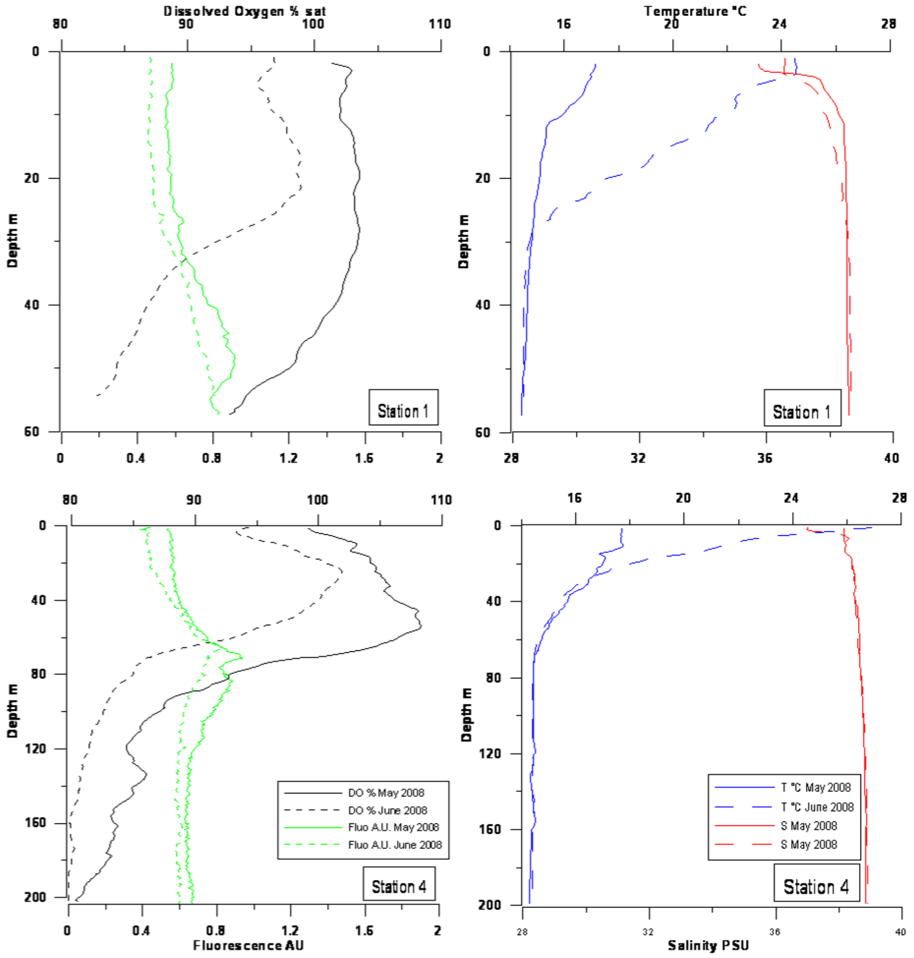

Figure 3: Profiles of Temperature (T, blu line), Salinity (S, red line), Dissolved Oxygen (DO, black line) and Fluorescence (Fluo, green line) in the 1 and 4 stations during May (solid line) and June (dotted line) 2008 (redrawn from [23]).

		St. KO		St. TV		St. HN		St. 1		St. 4	
		May	June	May	June	May	June	May	June	May	June
S	Surface	28.49	30.86	34.77	35.30	35.27	36.17	35.76	36.59	38.15	37.01
	10 m	37.44	36.90	37.92	37.39	38.17	37.71	38.25	37.80	38.17	38.18
	Bottom	38.45	38.18	38.53	38.49	38.55	38.58	38.59	38.67	38.85	38.89
T °C	Surface	17.83	26.55	16.96	26.21	17.04	24.25	17.13	24.50	17.69	26.90
	10 m	14.70	20.70	15.19	21.21	15.40	22.05	16.03	22.13	17.65	21.58
	Bottom	14.69	15.87	14.65	15.68	14.59	14.80	14.44	14.49	14.30	14.38
NO_3 µM	Surface	0.86	0.63	0.02	0.50	0.02	0.10	0.02	1.11	0.46	0.73
	10 m	0.02	0.13	0.02	0.39	0.02	1.63	0.02	0.55	0.81	3.17
	Bottom	0.35	0.02	0.02	0.20	0.02	1.69	1.29	3.24	6.71	3.48
PO_4 µM	Surface	0.03	0.32	0.04	0.19	0.02	0.03	0.03	0.03	0.04	0.12
	10 m	0.05	0.09	0.05	0.04	0.03	0.16	0.03	0.04	0.02	0.09
	Bottom	0.20	0.16	0.03	0.07	0.03	0.04	0.02	0.03	0.15	0.35
$Si[OH]_4$ µM	Surface	4.73	1.49	1.64	1.75	0.93	1.88	1.16	2.13	1.13	1.63
	10 m	5.17	0.54	0.91	1.37	0.52	1.58	0.81	1.55	1.12	2.96
	Bottom	2.43	3.36	1.04	2.64	1.22	1.74	1.59	4.05	5.40	4.81
Chl *a* µg l^{-1}	Surface	0.50	0.23	0.49	0.69	0.47	0.54	0.36	0.09	---	0.44
	10 m	0.17	3.13	0.06	0.21	0.07	0.08	1.96	0.16	---	0.51
a_{CDOM} 440 m^{-1}	Surface	0.18	0.99	1.01	1.13	1.33	1.31	0.99	0.55	---	0.87
	10 m	0.90	2.19	0.94	1.29	0.11	0.87	0.41	1.10	---	0.64
Diatoms cell l^{-1}	Surface	2980	160	3900	380	1120	400	1880	640	1260	180
	10 m	3515	9040	100	380	200	380	1340	40	500*	100*
Dinoflagellates Cell l^{-1}	Surface	1400	120	680	300	500	100	300	180	380	260
	10 m	280	220	420	40	300	140	100	0	540*	0*
Phytoflagellates Cell l^{-1}	Surface	1000	1200	1880	2600	1000	2800	1000	1600	800	2200
	10 m	160	2600	2200	2400	1800	2800	1600	2000	1400*	2400*
Others spp. Cell l^{-1}	Surface	0	0	160	160	0	0	0	20	1220	20
	10 m	20	0	720	1000	220	0	40	880	1620*	1060*

Table 1: Values of Temperature (T °C) , Salinity (S), Nitrate (NO_3 μM), Silicate (Si(OH)4 μM), Orthophosphate (PO4 μM), absorbance of CDOM at 440 nm (aCDOM 440 m^{-1}), chlorophyll a (chl a $\mu g{\cdot}l^{-1}$), Phytoflagellates (cell l^{-1}), Diatoms (cell$\cdot l^{-1}$), Dinoflagellates (cell l^{-1}) and Other spp (cell l^{-1}) in the five monitored stations (KO, TV, HN, 1 and 2) during May and June 2008.(

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Coupled Analytical-Numerical Model of Fish Response to Environmental Changes

A. Cucco[1], M. Sinerchia[1], C. Le Francois[2], P. Domenici[1], P. Magni[1], M. Ghezzo[3], G. Umgiesser[3], A. Perilli[1]
1, Institute for Coastal Marine Environment, CNR, Oristano, Italy
2, University of La Rochelle, La Rochelle, France
3, Institute of Marine Sciences, CNR, Venezia, Italy
andrea.cucco@cnr.it

Abstract

Eco-physiology studies, performed in laboratory under controlled conditions, provide an essential tool for quantifying the impact of environmental changes on the metabolism and behavior of individual fish. One way of quantifying such impact is by measuring the Metabolic Scope (MS) of a fish. Laboratory experiments were performed to calculate the MS of, textitMugil cephalus, under different environmental conditions (temperature and oxygen). The equations derived were introduced into an ecological model, which was then coupled to a high resolution hydrodynamic model. The model was calibrated for reproducing the environmental variability in the Cabras lagoon and Gulf of Oristano (Italy). We used the model to reproduce the temporal and spatial variation in MS of a textitM. cephalus fish population to investigate the relationship between changes in MS and the observed seasonal migration pattern between the gulf and lagoon. Results shows that during the spring-beginning of summer period Cabras lagoon provides a higher MS for textitM. cephalus than the Gulf of Oristano. During the rest of the year, apart from some transitional phases, the Gulf provides more suitable conditions (higher MS) for textitM. cephalus. Results were compared to fisheries data, showing that textitM. cephalus catches are highest during the end-July to August period. This period was characterized by fish were caught migrating from the lagoon into the Gulf and coincides with the reproduced drop of MS for textitM. cephalus in the lagoon.

1 Introduction

Several studies show the potential of changes in the physical and chemical environment in causing serious modifications in marine and coastal ecosystems [1]. Such changes influence directly the eco-physiological response of different levels of the ecosystem [2]. In particular, the progressive rise in temperature is thought to be a critical factor responsible for the migration of several fish species towards higher latitudes [3], for the changes in the ecosystem trophic structure due to the reduction or total lack of food, for the sub-optimal growth rate and lower reproductive potential [4]. In particular, the combined effect of the rise in temperature and reduction in dissolved oxygen has the potential to cause serious changes in the ecosystem dynamics. The effect of environmental changes on the ecosystem status and dynamics (e.g.

biodiversity, productivity, trophic cascade, etc.) is amplified in shallow water environments, such as coastal and lagoon areas, in particular those characterized by slow water renewal [5]. A typical example of such environments is represented by Mediterranean lagoons, rich in biodiversity and important nurseries for several fish species, many of which having commercial value and playing a fundamental role in the local economy [6]. The physical and chemical characteristics of the water in these environments fluctuate in an irregular fashion, due to sea storms, intense rainfall or river overflows [7]. Eco-physiology studies, performed in laboratory under controlled conditions, provide the perfect tool for quantifying the impact, in terms of energetic budgeting and fish behavior, of such changes. Quantification of the impact of environmental changes on the energetic budget of a fish was performed by measuring its Metabolic Scope, hereafter MS, [8]. The MS of a fish represents its energetic potential available to fuel all its activities (e.g. motion, digestion, reproduction, etc.), and provides a way of estimating the energetic cost fish have to pay for adapting to environmental changes. Results from studies on the MS of individual fish provided the bio-energetic basis for the construction of a numerical model projecting the impact observed at individual level to population level. In particular, the model was used to investigate how seasonal changes in temperature and oxygen concentration in two adjacent shallow water environments, the Cabras lagoon and Oristano gulf, affect textitmugil cephalus MS, which may explain the observed migration between the two areas.

2 Site description

The Cabras lagoon is a shallow water body (mean depth 1.7 m) located on the west coast of Sardinia, western Mediterranean Sea (39 57' N, 008 29' E), and is one of the largest brackish water basins in the Mediterranean region with a surface of 22 km^2 (Figure 1). It is connected to the Gulf of Oristano by a series of small creeks flowing into a main open channel, the Scolmatore. The lagoon is connected to two small rivers: Rio Mare Foghe, in the North representing the major source of freshwater, and Rio Tanui in the South. River discharge is rather limited due to a low rainfall regime in the region (about 10-100 mm from July to December, respectively). The lagoon salinity may drop to less than 10 psu during rainfall periods and rise to more than 30 psu, especially in summer. The tidal amplitude is less than 20 cm, making a very limited contribution to water exchange between the lagoon and the gulf. The lagoon is subdivided into 2 sub-basins, a northern one and a southern one, with a different water circulation pattern and water residence time. Cabras Lagoon interacts directly with the Gulf of Oristano, which in itself has a great economic value because is a nursery area for the most valuable fish species in the Lagoon [6].

3 Methods

In order to represent accurately the effects of environmental changes on the eco-physiological response of fish several tasks have been performed. First of all the relationship between changes in environmental variables, temperature and dissolved oxygen, and textitM. cephalus MS have been estimated by means of experimental activ-

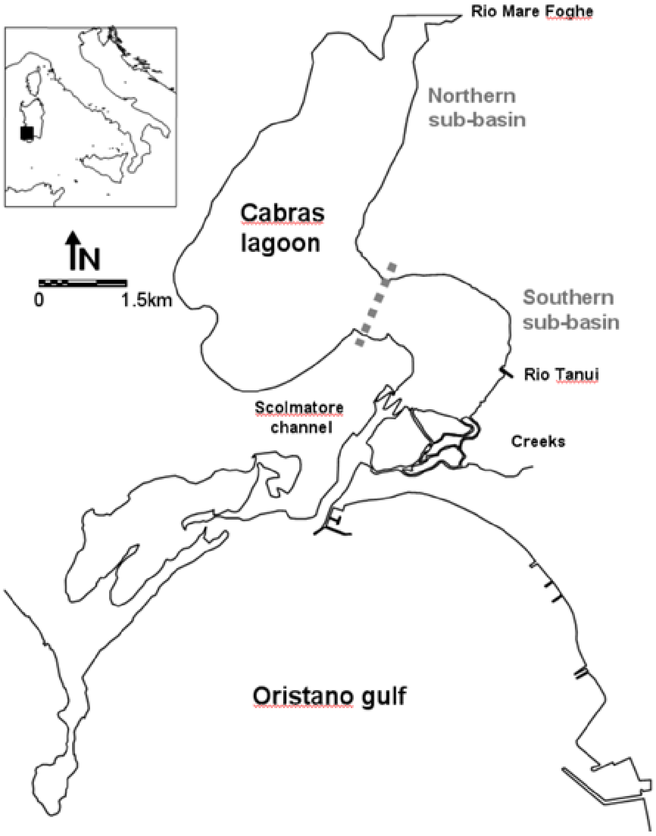

Figure 1: The Gulf of Oristano and Cabras lagoon.

ity. Then the environmental fluctuations in the Gulf of Oristano and Cabras lagoon has been reproduced by means of hydrodynamic and ecosystem model. Finally the physiological adaptation cost to such changes have been evaluated integrating results obtained by experimental and numerical activity in order to assess the onset of environmental conditions to trigger migration of the textitM. cephalus population between the Gulf and the Lagoon. The proposed approach is based on the used of two different modules consisting of empirical mathematical functions describing the relationship between environmental features (water temperature, salinity and oxygen concentration) and the physiologic response of a textitM. cephalus population, and a numerical model able to reproduce the time and space variability of the environmental features themselves. In the following, the method followed to compute the eco-physiological equations for textitM. cephalus population and the hydrodynamic and ecosystem model used are described.

3.1 Laboratory experiments

The equations describing the change of fish MS ($\mathrm{mgO_2 \cdot kg^{-1} \cdot h^{-1}}$) with environmental variables, such as temperature and dissolved oxygen, has been determined in laboratory experiments on 300 textitM. cephalus (weight 300 ± 25 g.) The Routine Metabolic Rate (fish under minimum level of activity), hereafter RMR, has been measured in a respirometer at five different temperatures (10, 15, 20, 25, 30C) and four oxygen concentrations (20, 30, 50, 85%). Standard or resting metabolic rate, SMR, which is difficult to measure directly, has been estimated as the minimal RMR measured during the night, when fish tends to remain motionless on the bottom of the respirometer. Experiments with increasing water current velocity have been performed in order to measure the maximum active metabolic rate before exhaustion for each combination of tested temperature and oxygen concentration. MS of a fish has been calculated as the difference between SMR and AMR at any given temperature and oxygen concentration and has been modeled using the equations:

$$\mathrm{MS = AMR - SMR}$$

$$\mathrm{SMR = a\left(1 - e^{bT^{c}}\right)}$$

$$\mathrm{AMR = \left(dT^{fT+h} + m\right) \cdot}$$

$$\mathrm{\cdot \left(1 - e^{nO_2+p}\right) - w\left(1 - e^{kT^{q}}\right),}$$

where MS, SMR and AMR were measured in mg of $\mathrm{O_2}$/(kg·h) T is temperature, $\mathrm{O_2}$ is dissolved oxygen concentration (%) and a, b, c, d, f, h, m, n ,p, w, k and q are constants.

3.2 Coupled hydrodynamic-ecosystem numerical model

The spatial and temporal variation in sea water temperature (T) and salinity (S) is controlled directly by physical processes such as transport, mixing, cooling and heating which are governed by the water circulation, evaporation and precipitation and by the heat fluxes at the sea surface. On the other hand, the concentration of dissolved oxygen (DO) in the water column, not only depends on physical aspects such as the water T itself and the degasification occurring at the sea surface, but depends strongly on biological processes such as the photosynthesis and the respiration of living aquatic organisms. Therefore, in order to numerically reproduce the time and space variability of water T, S and DO, both physical and ecological aspects have to be considered. With this purpose, a shallow water finite element hydrodynamic model and an ecological model have been coupled one each other and apply to reproduce the main processes affecting the water T, S, and DO distribution within the Cabras lagoon and coastal area and the Oristano gulf. In the following, the description of the hydrodynamic model, the ecological model and the coupling procedure adopted is reported.

The hydrodynamic model. A 2D hydrodynamic model (SHYFEM, [9]) based on the finite element method has been used. The model has been applied with success in other previous studies with the aim of investigating both the hydrodynamics and the flushing features of lagoon and coastal basin systems [10]. The model uses finite elements for horizontal spatial integration and a semi-implicit algorithm for integration in time. It resolves the vertically integrated shallow water equations in their formulations with levels and transports. The

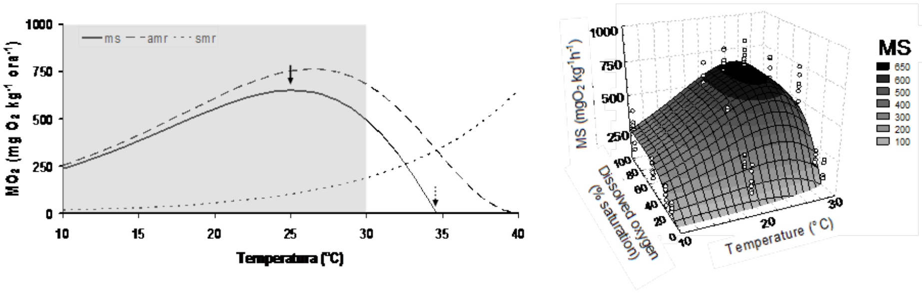

Figure 2: MS as a function of temperature (T) and dissolved oxygen concentration (DO). Open circles represent observations, while the surface of the plot represents MS predicted by the model.

horizontal diffusion, the baroclinic pressure gradient and the advective terms in the momentum equation are fully explicitly treated. The Coriolis force and the barotropic pressure gradient terms in the momentum equation and the divergence term in the continuity equation are semi-implicitly treated. The friction term is treated fully implicitly for stability reasons. The model is unconditionally stable what concerns the fast gravity waves, the bottom friction and the Coriolis acceleration [9].

The ecological model. The base ecological model used was the Biogeochemical Flux Model (hereafter BFM, [11], a biomass-based differential equation model. The model is built with respect to mass conservation following a top-down approach. The food web consists of functional groups. Each group is expressed by a set of state variables expressing chemical content (C, N, P and SI), or molecules (Chl-a). The food-web was constituted by primary producers (diatoms, 20-200 m ESD, picophytoplankton, 2 m ESD, flagellates, 2-20 m ESD, and dinoflagellates, 20-200 m ESD), zooplankton (microzooplankton, heterotrophic nanoflagellates, carnivorous mesozooplankton and omnivororus mesozooplankton), bacteria, nutrients and dissolved chemical species for oxygen and the carbonate system. Bacterioplankton represent free-living, non-colonial bacteria that can switch from aerobic to anaerobic metabolism according to the pelagic oxygen conditions. The nutrients included in the model were: nitrite, nitrate, ammonium, orthophosphate and silicate, dissolved bioavailable iron, oxygen, carbon dioxide, dissolved and particulate (non-living) organic matter. The model simulates the evolution of up to 44 state variables in the water column describing both the main metabolic processes within each functional group and the trophic interactions between functional groups. In particular, carbon assimilation, nutrient uptake, lysis of primary producers, grazing by and of secondary producers, respiration, mortality excretion and exudation of all organisms are modeled. We refer to Vichi [11] for a detailed description of the model structure and the adopted numerical method.

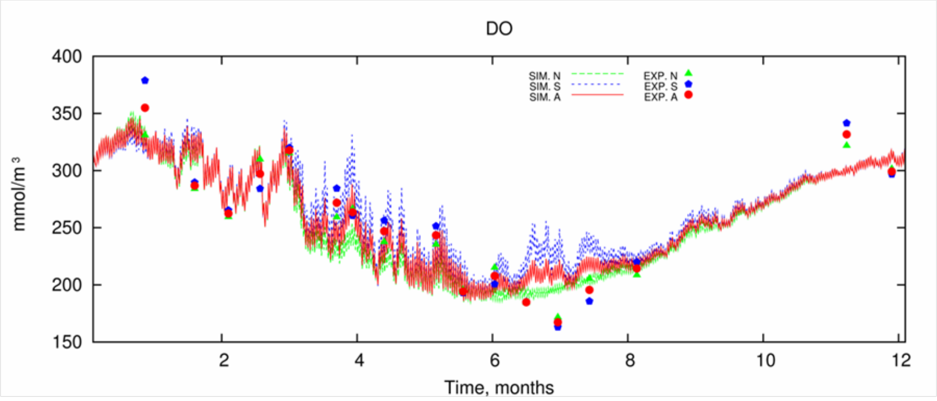

Figure 3: DO in the Cabras Lagoon during the year 2007. Comparison between model results (SIM. N, SIM. S. and SIM. A.) and measured data (EXP. N., EXP. S. and EXP. A).

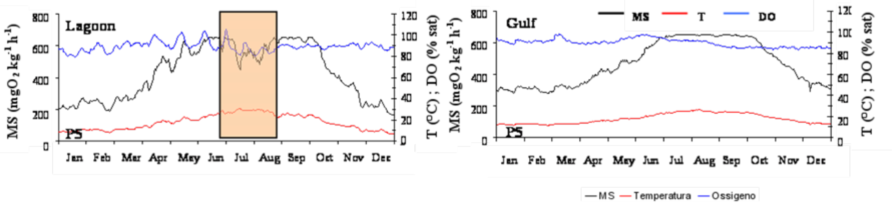

Figure 4: Variation in textitM. cephalus metabolic scope (MS) as a function of temperature (T) and dissolved oxygen (DO) during year 2007.

4 Results

4.1 Experimental energetic model

An experimental energetic model was used to predict the variation in MS (in $O_2/(kg \cdot h)$) of textitM. cephalus as a function of water temperature and oxygen concentration. Figure 2 illustrates the effect of temperature on fish metabolism and in particular shows that:

1. Standard Metabolic Rate (SMR) increases exponentially with temperature (Figure 2a).
2. Active Metabolic Rate (AMR) increases with temperature up to 760 $O_2/(kg \cdot h)$. When temperature is above 26.5 C, AMR becomes inversely proportional to temperature (Figure 2a).
3. When oxygen is not limiting (85%), Metabolic Scope (MS) reaches a maximum value of 651 $O_2/(kg \cdot h)$ at a temperature of 25C, which is defined as its optimal temperature, TMmax (Figures 2a and 2b).

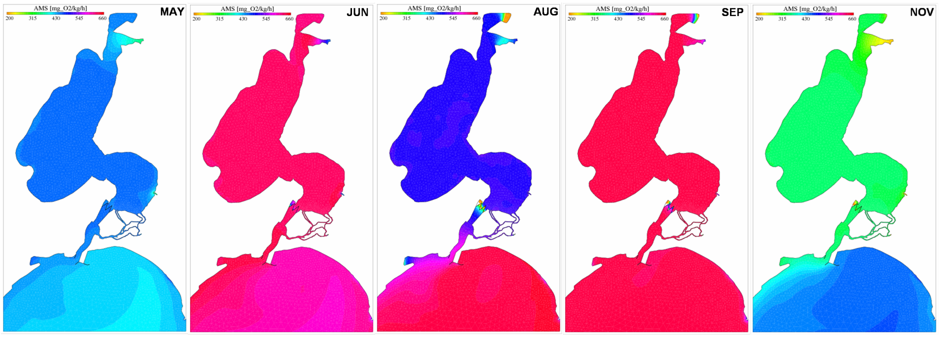

Figure 5: Spatial variation of the textitmugil cephalus MS in the Cabras lagoon and Gulf of Oristano. (-), (+) and (tilde) represent respectively: lower, higher or same MS in the lagoon compared to the gulf.

4.2 Temporal and spatial variation in the Cabras lagoon environmental conditions

A set of numerical simulations have been carried out to reproduce the time and space variability of water T , S and DO observed in the Cabras lagoon and part of the Oristano gulf during the period between December 2006 and December 2007 corresponding to a full meteorological year. Both the hydrodynamic and the ecological model are run over the same domain which corresponds to an extended version of the finite element grid adopted in Ferrarin et al., [12], including the whole Cabras lagoon and part of the coastal area in front of the Scolmatore channel. In order to reproduce the time and space variability of DO within the model domain, a calibration procedure based on the modification of the model zooplancton parameters, initial and boundary conditions has been carried out. In particular, model parameters, initial and boundary conditions have been modified by trial and error method, using the environmental data collected inside the lagoon basin in 4 stations for evaluating the model performances. The model accuracy has been established computing for water T , S, DO, Chla and nutrients the relative error RE and the coefficient of determination. The obtained results reveal an high accuracy of the model in reproducing DO, T and S with RE always lower than 40%. In Figure 3, the comparison between DO as computed by the model during the whole year and measured data is reported. In particular, the red line consists on the spatial average over the whole basin of DO as computed by the model, whereas the blue and green line consist on the spatial average over the southern and northern lagoon sub-basin respectively. Red, blue and green dots are the corresponding basin, northern sub-basin and southern sub-basin average values of DO measured data. As we can see the model results fit accurately the seasonal evolution of DO in Cabras lagoon.

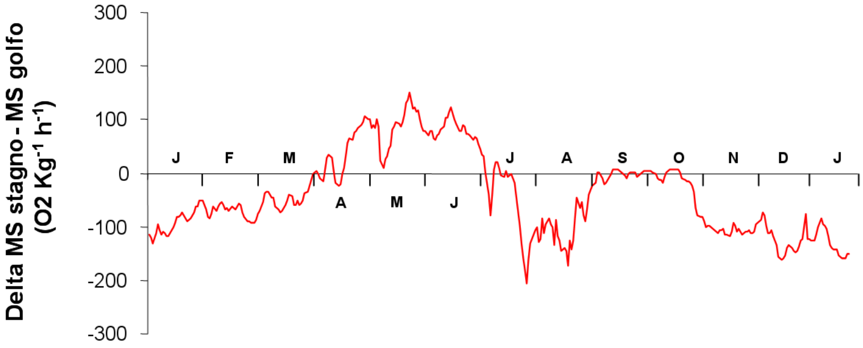

Figure 6: Differences between MS of *M. cephalus* computed in lagoon and gulf respectively.

4.3 M. cephalus MS in the Cabras Lagoon and Gulf of Oristano

In both environments, lagoon and gulf, temperature act as the critical factor for determining the seasonal variation in the MS, considering that the concentration of dissolved oxygen is always above 70%, thus never limiting (Figure 4). The seasonal variation in MS in the Cabras Lagoon occurs with a pattern consisting of four phases:

1. January to end of June: rise in MS.
2. Summer: MS drop due to high T.
3. September to October, no significant change (plateau).
4. From November, MS starts to decrease.

The seasonal change in MS in the Gulf of Oristano shows a similar trend to that of the Cabras lagoon, with the only difference that during the summer period, MS does not show any drop (Figure 4). Figures 5 and 6 show that, during April, May and June, the lagoon provides higher MS than the gulf, while, with the exception of the end of August and September, during which there was no difference in MS between the two, during the rest of the year the gulf provides an environment capable of supporting an higher MS.

5 Discussion

Changes in temperature seem to be the critical factor responsible for the fluctuation in MS. In particular, between mid-July and the end of August, water temperature in the Cabras Lagoon exceeds TMmax, causing a decrease in MS for textitM. cephalus. As a result, during that period, fish in the Gulf would have benefited from a higher MS compared to those in the Lagoon (“summer”, Figures 5 and 6). Temperatures exceeding TMmax are responsible for encouraging the occurrence of degradation phenomena affecting various physiological functions in fish limiting metabolism, feeding, growth and activity levels. Also during fall and winter, textitM. cephalus can benefit from higher MS in the Gulf (Figures 5 and 6). However, during these periods, the

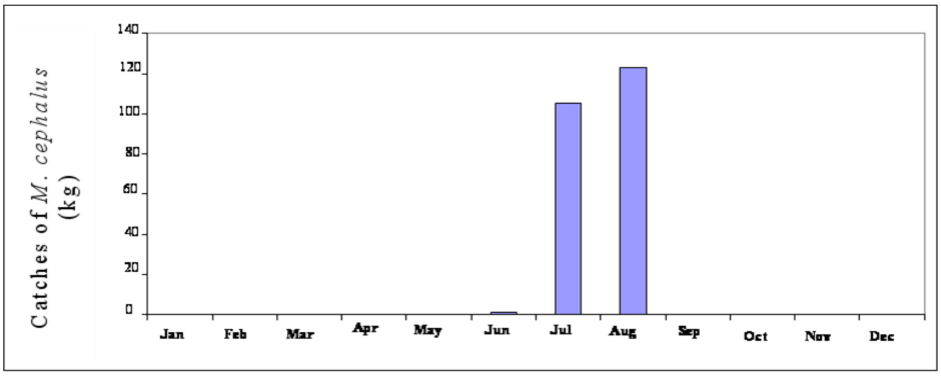

Figure 7: Annual textitM. cephalus catch (kg) in the Cabras lagoon between Jan. and Oct. 2007. Data from Cooperativa S. Vincenzo, Cabras

difference in MS between the two environments is determined by temperatures below TMmax, therefore not completely precluding the presence of individuals in the Lagoon. The opposite occurs during spring ("spring May") and the beginning of summer ("summer June", Figure 5) when the water of the Lagoon warms up before that of the Gulf, therefore providing a better MS to the fish in the lagoon. During the summer months the difference between the ambient temperature and the range of preferential temperatures is higher in the lagoon than in the Gulf. Such results can be compared to fisheries data on m .cephalus landings (tons) in the Cabras Lagoon for 2007. During the critical summer period (II, i.e. end-July to August), when MS gets lower in the lagoon and stays high in the Gulf (Figure 5), textitM. cephalus migrates out of the lagoon and into the Gulf. The onset of this migration mechanism is sustains by the high number of textitM. cephalus being caught exiting the lagoon through the Scolmatore channel at a time coinciding with the critical MS period (Figure 7).

6 Conclusions

We coupled modeling and laboratory experiments, in order to investigate the effect of variation in temperature and oxygen on the metabolic scope of textitM. cephalus and implication in the seasonal migration between the Cabras lagoon and the Gulf of Oristano. Results from the numerical simulations showed that:

- during spring and the beginning of summer Cabras lagoon provides a higher MS for textitM. cephalus than the Gulf of Oristano;
- during the rest of the year, apart from transitional phases ("Spring March", "Autumn September", Figures 4 and 5), the Gulf provides more suitable conditions and a higher MS for textitM. cephalus;
- although the metabolic scope in the Cabras lagoon is lower than in the Gulf during most of the year, it is only towards the end of summer, when water temperature exceeds TMmax, that the environmental conditions in the lagoon become physiologically particularly ad-

verse to fish;
- changes in MS related to the seasonal variation in temperature and oxygen could be a factor responsible for triggering the onset of seasonal migration between the two different environments.

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Mercury and Cadmium in Tissues and Organs of Two Cetacean Species (*Stenella Coeruleoalba* and *Tursiops Truncatus*) Stranded Along the Italian Coasts

A. Bellante[1], M. Sprovieri[1], G. Buscaino[1], D. Salvagio Manta[1], G. Buffa[1], V. Di Stefano[1], F. Filiciotto[1], A. Bonanno[1], M. Barra[2], B. Patti[1]
1, Institute for Coastal Marine Environment, CNR, Capo Granitola (TP), Italy
2, Institute for Coastal Marine Environment, CNR, Napoli, Italy
anto.bellante@gmail.com

Abstract

Distribution of mercury and cadmium in tissues and organs of two different cetacean species (*Stenella coeruleoalba* and *Tursiops truncatus*) stranded along the Italian coasts between 2000 and 2009 are here presented. According to previous authors mercury and cadmium accumulate preferentially in liver and kidney, respectively. All organs and tissues show a positive correlation of mercury and cadmium with length thus suggesting the effect of bioaccumulation processes over time. Specimens of *S. coeruleoalba* exhibit higher mercury and cadmium concentrations with respect to specimens of *T. truncatus*. This suggests the existence of different mechanisms of bioaccumulation, through different diet patterns and/or uniqueness in physiological and/or biological control of Hg and Cd incorporation, for the two groups of populations. Comparison among mercury concentration levels measured in liver samples of *S. coeruleoalba* from different Mediterranean and ocean areas, shows that southern Adriatic sea and Sicily channel are the areas at lowest risk of Hg pollution in the Mediterranean basin as a result of reduced industrial impact compared to the highly contaminated French marine areas.

1 Introduction

Large amounts of organic and inorganic chemicals enter estuarine and coastal marine environments from natural and anthropogenic sources. Human activities have increased the flux of many naturally occurring chemicals, such as metals and petroleum hydrocarbons, to the ocean [1]. Thus, marine ecosystems degradation and pollution are considered as a global problem [2]. Several metals, such as cadmium and mercury are considered highly toxic [1]. Those do not normally participate in metabolism and, at least in top predators, are accumulated throughout the entire life of an individual [1]. Due to their lengthy persistence and high mobility in the marine ecosystem, mercury and cadmium show a high level of bioaccumulation in the upper levels of the food web. Dolphins are at the top of the food chain and therefore accumulate high mercury and cadmium loads from their prey

[3]. A lot of study were carried out on trace metals concentrations in tissues of *Stenella coeruleoalba*. On the other side a limited number of data on trace metals is available for other species such as *Tursiops truncatus*. All of these studies point to the high variability found in mercury and cadmium levels. According to Thompson [4], the variation is likely to reflect both interspecies dietary differences with corresponding differing mercury and cadmium levels, and age-accumulation trends. In the present study, the distribution and the concentration of mercury and cadmium were examined in the organs and tissues of specimens of *S. coeruleoalba* and *T. truncatus* stranded along the Italian coasts. Moreover we compare our results with mercury concentrations previously reported by other authors in different marine areas within Mediterranean sea. The main aims of this research are at attempting to: (1) verify, on the basis of a new and larger dataset, previously reported mercury and cadmium distribution patterns in cetaceans, (2) investigate differential bioaccumulation mechanisms for different organs and species, (3) assess the importance of cetaceans tissues as indicators of marine pollution.

2 Materials and methods

2.1 Analytical methods

Samples of muscle, liver, lung, kidney and heart were collected from specimens of *S. coeruleoalba* (n=12) and *T. truncatus* (n=12) stranded along Italian coasts during the period 2000-2009. Samples were stored at -20°C after collection. Samples were dried at 60 °C for 48h and homogenised in a agate mortar. About 0.25g of each air-dried and homogenised sample were digested under pressure in 10ml of ultra-grade HNO_3 in Teflon liners using a microwave equipment (CEM MARS-5), for 4h at 200W and at T=160±5°C. Metal concentrations were measured by ICP-AES Varian Vista MPX.

2.2 Statistical methods

Mercury concentration in livers and muscles of *S. coeruleoalba* from the French Mediterranean coasts [5], Israeli coasts [6], Ligurian sea [3], Adriatic sea [7] are here considered and compared to our dataset from the Sicily Channel. Because variables were not normally distributed, nonparametric tests were used to compare different groups in the present study. Spearman's rank correlation coefficient was used to measure the proportionality between length and metals concentration in the studied specimens. Krustal Wallis test was used to compare metal concentrations between the different tissues analyzed. Analysis of covariance (ANCOVA) was used to compare the concentrations of metals between different species and sampling areas. In this study we use length as covariate.

3 Results

3.1 Mercury distribution

Mercury shows a wide range of concentrations, especially in liver samples (from 8,48 to 1752 $\text{mg}\cdot\text{kg}^{-1}$ dw for *S. coeruleoalba* and from 9,6 to 1404 for *T. truncatus*; Table 1). As shown in Figure 1, liver shows the highest concentrations of Hg in all specimens analysed, followed by samples of kidney and lung. The lowest values were found in samples of muscle and heart. Based on ANCOVA test, no

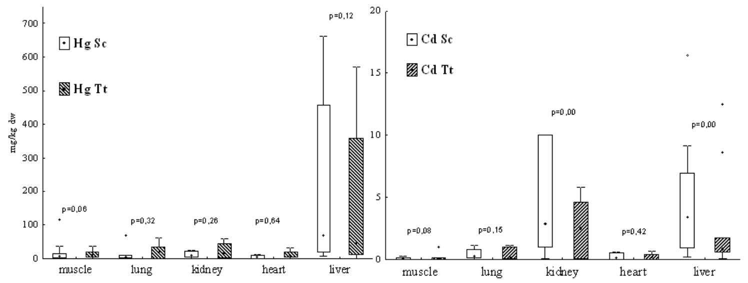

Figure 1: Mean and S.D. of Hg and Cd concentrations in tissues of *S. coeruleoalba* (Sc) and *T. truncatus* (Tt). P values for interspecific difference (ANCOVA test) are shown for all tissues.

significant differences were found between Hg concentrations in the different tissues for S. coreulaoalba. As regard specimens of *T. truncatus*, significant differences were found between Hg concentrations in liver and in the other tissues (except for lung samples). As shown in Table 2, positive significant correlations emerge between Hg concentrations and length in muscle and liver samples for *S. coeruleoalba* and in liver, kidney and muscle samples for *T. truncatus*.

3.2 Cadmium distribution

Cadmium shows a wide range of concentrations in kidney samples (from 0,08 to 84,77 mg·kg^{-1} dw for *S. coeruleoalba* and from 0 to 54,2 for *T. truncatus*; Table 1). Narrower range of concentrations were found in the other tissues. As shown in Figure 1, kidney shows the highest concentrations of Cd in all specimens analysed followed by liver. Low values of Cd were found in heart, lung and muscle samples. Based on analysis of covariance, significant differences were found between Cd concentrations in the different tissues of *S. coeruleoalba*. As regard specimens of *T. truncatus* no significant differences were found between Cd concentrations in the different tissues. As shown in Table 2, positive significant correlation was found between Cd concentrations and length in liver, kidney, lung and muscle samples of *S. coeruleoalba*. As regard specimens of *T. truncatus*, positive correlation was found between Cd concentrations and length in liver, kidney and muscle samples. However the coefficients correlation were not statistically significant.

3.3 Inter-specific differences

In Figure 1 are shown the differences of toxic metals concentrations between *S. coeruleoalba* and *T. truncatus*. Hg and Cd trends shows higher levels of these metals in tissues of the species *S. coeruleoalba*. However, based on analysis of covariance no significant differences in Hg concentrations were found between these species in all the analysed tissues. On the other side, Cd concentrations were significantly higher in the specie *S. coeruleoalba* in kidney and liver samples.

	Length (cm)	Site of sampling	muscle		lung		kidney		heart		liver	
Stenella coeruleoalba			**Hg**	**Cd**	**Hg**	**Cd**	**Hg**	**Cd**	**Hg**	**Cd**	**Hg**	**Cd**
Sc 1	85	Mazara del Vallo (Sicily Channel)	0.94	0.00	2.69	0.00	0.37	0.09				
Sc 2	190	Mazara del Vallo (Sicily Channel)	16.6	0.07	71.0	1.08		0.57	5.5		455.6	4.47
Sc 3	182	Mazara del Vallo (Sicily Channel)	13.58	0.12	5.64	1.13	24.59	84.77	9.85	0.12	97.38	16.45
Sc 4	131	Mazara del Vallo (Sicily Channel)	4.99	0.00	1.79	0.10	10.02	10.02	0.00	0.00	22.50	1.75
Sc 5	98	Mazara del Vallo (Sicily Channel)	0.00	0.00	2.42	0.27		1.79	1.12	0.00	15.30	0.25
Sc 6	103	Mazara del Vallo (Sicily Channel)	1.52	0.06	3.04	0.13	4.47	0.95	4.86	0.08	8.48	0.23
Sc 7	173	Mazara del Vallo (Sicily Channel)	11.75	0.17	11.29	0.48	23.66	49.54	11.12	0.19	51.43	6.93
Sc 8	110	Mazara del Vallo (Sicily Channel)			7.62	0.32	22.48	7.98	11.94	0.62	89.99	2.31
Sc 9	131	Monte Argentario (Thyrrenian sea)					13.08	0.08				
Sc 10	110	Viareggio - (Thyrrenian sea)	4.45	0.12			8.04	2.84			21.20	0.90
Sc 11	192	Viareggio - (Thyrrenian sea)	36.62	0.11							661.55	6.04
Sc 12	91	Orbetello - (Thyrrenian sea)	116.54	0.28							1752.18	9.16
Tursiops truncatus												
Tt 1	100	Mazara del Vallo (Sicily Channel)			0.0	0.00	0.0	0.00	0.07	0.00		
Tt 2	225	Mazara del Vallo (Sicily Channel)	19.97	0.00	62.56	0.95			2.47	0.68	181.00	12.47
Tt 3	180	Mazara del Vallo (Sicily Channel)	10.70	0.10	34.4	0.20	29.0	5.86		0.19	70.13	1.49
Tt 4	270	Mazara del Vallo (Sicily Channel)	17.09	1.01	20.87	1.09	21.46	54.26	13.99	0.41	261.68	8.63
Tt 5	130	Mazara del Vallo (Sicily Channel)	0.0	0.00	0.0	0.07	3.6	4.27	0.00	0.00	9.6	0.66
Tt 6	285	Jesolo (Adriatic sea)	35.92	0.07			60.03	4.61			570.49	0.82
Tt 7	136	Ravenna (Adriatic sea)	5.26	0.05			3.25	0.10				
Tt 8	276	Monte Argentario (Thyrrenian sea)	14.35	0.12			15.38	0.31				
Tt 9	213	Napoli - (Thyrrenian sea)	1.21	0.02			9.37	0.04			17.48	0.13
Tt 10	150	Marciana Marina - (Thyrrenian sea)	25.56	0.12			30.73	2.82			453.49	1.76
Tt 11	265	Viareggio - (Thyrrenian sea)	4.75	0.02			10.98	0.03			23.03	0.04
Tt 12	150	Jesolo (Adriatic sea)					56.66	2.50			1404.92	0.73

Table 1: Hg and Cd concentrations (mg·kg^{-1} dw) in tissues of *S. coeruleoalba* and T. truncates, length and sites of sampling for each specimens.

4 Discussions

4.1 Mercury and cadmium distribution

The highest concentrations of Hg were found in the liver samples of all the specimens analysed. This suggests that demethylation, namely the transformation of organic mercury into the less toxic inorganic form, occurs in this organ. There were significant correlations of Hg concentrations in both liver, muscle and kidney with length for both species analysed. Thus, a relatively constant input of mercury over time can be assumed. As shown in Figure 1, Cd accumulated preferentially in the kidney of all species analysed. This is in accordance with results of previous authors [5, 8] and suggests a storage of Cd in this organ possibly associated to effects of complexation with metallothioneins (MT) and an excretory function of this organ. Also, significant correlations were found between Cd concentrations and length in all tissues of *S. coeruleoalba* (except for heart samples). This may confirm the existence of bioaccumulation process of Cd with age for this species. Positive correlation with age were found also in tissues of *T. truncatus* although at not statistically significant levels. This can be explained by a lower accumulation of Cd in tissues of this species. These hypothesis seems to be confirmed by Figure 1.

4.2 Inter-specific differences

No significant differences in Hg concentrations were found between the two species in all the analysed tissues. However, as shown in Figure 1, it seems to be evident that *S. coeruleoalba* exhibits higher Hg and Cd concentrations than *T. truncatus* (in liver and kidney samples, respectively). Dolphins accumulate mercury and cadmium mainly through the diet. *T. truncatus* can feed on fishes and squids, which make up 60 and 20% of its diet, respectively, while *S. coeruleoalba* have a great proportion of squids in her diet [9]. Squids are well-known toxic metal accumulators and a source of these metals to their predators [10, 11, 12]. As a result, species or populations that consume an important proportion of cephalopods can be expected to exhibit higher Cd and Hg levels in their tissues than piscivorous predators [13]. Moreover the analysed species have a similar trophic level [9] (4.2 for *T. truncatus* and *S. coeruleoalba*). Thus, the higher Hg and Cd concentrations found in liver and kidney of *S. coeruleoalba* can be explained by a great proportion of squids in the diet composition of both groups of organisms.

4.3 Cetaceans as indicators of mercury marine pollution

A large number of data is available for mercury concentrations in liver samples of striped dolphin. The datasets of mercury concentrations in liver of striped dolphin from Mediterranean French coasts [5], Israel coasts [6], Ligurian sea [3], Adriatic sea [7] and Sicily Channel (this study) are here compared. Based on results of ANCOVA test, significant differences were found between Hg concentrations in liver samples of specimens from different marine areas. The highest Hg concentrations were found in liver of specimens of *S. coeruleoalba* from the French coasts [5] followed by liver of specimens from Ligurian sea [3], Israel coasts [6], Sicily channel (this study) and Adriatic sea

	Hg/Length		**Cd/Length**	
	Sc	**Tt**	**Sc**	**Tt**
liver	**0,67**	**0,96**	**0,75**	0,49
kidney	0,63	**0,94**	**0,93**	0,58
muscle	**0,8**	**0,92**	**0,75**	0,57
lung	0,64		**0,8**	
heart	0,32		0,35	

Table 2: Spearman correlation coefficients between Hg, Cd and length in tissues of *S. coeruleoalba* (Sc) and *T. truncatus* (Tt). In red: significant statistical differences ($p < 0{,}05$).

[7]. These results could be explained if we consider that Hg is delivered to marine environment mainly from atmospheric sources and river inputs [14]. Areas most at risk of Hg pollution include, in fact, bays and semi-closed sea, especially those influenced by large rivers flowing through heavily industrialised sites [15]. Data published by Pirrone [16] concerning the total anthropogenic mercury emissions in the Mediterranean region shown that France is the leading Hg emitter in the region with 21.4% of the regional total. These data could explain the highest Hg concentrations found in tissues of striped dolphins stranded along the Mediterranean French coasts. Moreover this area could be heavily contaminated with toxic metals from the nearby Rhone estuary [17]. As discussed by Pirrone [16], also Italy represent an important Hg emitter in the Mediterranean basin (10.8% of the regional total). This could explain the relative high Hg concentrations found in striped dolphins from Ligurian sea. Moreover, the North Tyrrhenian sea could be affected by significant inputs of Hg from rivers flowing through the Mt. Amiata area (one of the richest natural reserves of cinnabar [18]). In the Adriatic sea, a semi-closed sea, several industrial sites located on the coastal zone and contaminated rivers such as Po and Isonzo have cause important mercury pollution levels [15, 19]. However Ferrara [20] demonstrates a decreasing trend of mercury contamination from the northern to southern Adriatic regions. Thus, the fluvial contributions and the presence of localized anthropogenic mercury sources not influence the southern Adriatic basin, such as the Apulian coasts. This could explain the lower Hg concentrations found by Cardellicchio [7] in dolphins tissues from the Apulian coasts. Based on our results Sicily channel seems to be an area at lower risk of Hg pollution. We assume that the lower Hg concentrations found in dolphin's tissues from this area as representative of a lower industrialized area not significantly influenced by contaminated rivers. Finally,

as reported by Pirrone [16], Israel represents a minor Hg emitter in Mediterranean basin (0,8% of the regional total) with reduced impact from contaminated rivers. These data well reflect the lower Hg concentrations in tissues of striped dolphin found in this area by Roditi-Elasar [6].

5 Conclusions

Concentration and distribution ranges of Hg and Cd in the reported dataset are substantially comparable with those reported by other authors worldwide. In particular, the liver systematically shows the highest concentrations of Hg while the kidney shows the highest concentrations of Cd with evident high potential for toxic elements accumulation of these two organs. According with previous authors we report the existence of bioaccumulation process over time of these toxic elements. The reported dataset well documents the existence of different mechanisms of bioaccumulation, through different diet patterns and/or uniqueness in physiological and/or biological control of Hg and Cd incorporation for *S. coeruleoalba* and *T. truncatus*. Particularly seems to be evident the bioaccumulation of an higher amounts of Hg and Cd in target organs of *S. coeruleoalba*. Last but not least, Hg concentrations in tissues of striped dolphin show clear difference of concentrations of this toxic element within Mediterranean seawater. Particularly, it seems to be clear that differences of environment Hg contaminations can occur at regional scale due to differences of anthropogenic impacts in different Mediterranean areas. These differences may reflect the existence of different and separated populations of *S. coeruleoalba* in Mediterranean basin with different feeding habitats and exposition to different anthropogenic impact. This implies that migratory movements of the studied groups of cetaceans in the Mediterranean sea are decidedly limited.

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Food Web Reconstruction Gives Evidence of Increased Trophic Levels in No-Trawl Areas: the Red Mullet, *Mullus barbatus* L. Case in Northern Sicily

F. Badalamenti[1], F. Andaloro[2], D. Campo[3], M. Coppola[1], G. D'Anna[1], G. Di Stefano[1], A. Mazzola[4], C. Pipitone[1], C. Romano[1], M. Sinopoli[1], S. Vizzini[4]
1, Institute for Coastal Marine Environment, CNR, Castellammare del Golfo (TP), Italy
2, Institute for Environmental Protection and Research, Palermo, Italy
3, CEA LABTER Ragusa, Ragusa, Italy
4, Department of Ecology, University of Palermo, Italy
fabio.badalamenti@cnr.it

Abstract

This study was aimed at identifying the food web of the red mullet, *Mullus barbatus* in order to understand how it is affected by trawling disturbance. To achieve this objective: a) the main features of the red mullet habitat were investigated; b) the food web of this habitat was studied in two no-trawl areas and in two areas open to trawling.

The working hypothesis is that trawling affects the biochemistry of the sediment and the trophic structure of the benthic assemblage. It was predicted: a) less biomass, smaller size and higher production rate in the benthic assemblages of protected gulfs; b) higher average trophic level for both the red mullet and its predators in protected gulfs; c) a diet shift driven by the mechanical disturbance of trawling.

The results achieved confirmed our hypotheses and allowed us to characterize for the first time the trophic web structure in the red mullet habitat in the study area. Results also allowed us to compare the trophic level of red mullet of different sizes using two independent techniques, stable isotopes of nitrogen and gut contents. The average trophic level of the red mullet was higher and the energy consumption per unit of biomass larger in the protected gulfs. Species with a trophic level higher than the red mullet and therefore its potential predators were few and the most important were the white grouper, *Epinephelus aeneus*, the pandora, *Pagellus erythrinus* and the common torpedo, *Torpedo torpedo*.

1 Introduction

The Gulfs of Castellammare and Patti can be considered marine protected areas due to a trawl ban imposed in 1990 (Regional Act n. 25/1990). Studies carried out in the two gulfs [1, 2, 3, 4] showed a strong increase of groundfish biomass as a result of the ban. The red mullet, *Mullus barbatus* had one of the highest increase rates among commercial finfish species. This species, which accounts for about 25% of the total groundfish biomass in both gulfs, has an adult diet based on benthic organisms like polychaetes, molluscs and crustaceans [5]. Such huge "new" red mullet biomass most likely has a strong impact on the benthic food web - and also on the pelagic food web on which pre-benthic juveniles rely - which is poorly known. Badalamenti et al. [6] showed that the red mullet has a very high trophic level and speculated that bottom food webs are longer than hitherto expected, because many invertebrates could be actually omnivorous instead of simply deposit feeders or carnivorous.

In 2005 the Italian Ministry for Agriculture and Forest Policy funded the coordinated project "Evaluation of the efficacy of no-trawl areas through the study of the predators and prey of the red mullet, *Mullus barbatus* L.". This project evaluated the effect of the establishment of a trawling exclusion zone on the coastal marine ecosystem and in particular on the benthic food web. The study was aimed at identifying the predators and prey of the red mullet, *M. barbatus* to better understand how a disturbance, in this case the trawling ban, may affect the trophic structure. To achieve this objective (a) the main biological and sediment features of the red mullet habitat (i.e., muddy bottoms between 40 and 80 m depth) were investigated, and (b) the food web of this habitat was studied and the food web of the red mullet reconstructed. The study area included four gulfs along the northern Sicily coast, two under a no-trawl regime (Castellammare and Patti) and two open to trawl fishing (Termini Imerese and Sant'Agata).

The working hypothesis was that besides the well documented difference in fish biomass between protected and unprotected gulfs there were also differences in the benthic assemblage structure and production, in the biochemistry of the sediment and in the food web structure. It was predicted: (a) less biomass, smaller size and higher production rate in the benthic assemblages of protected gulfs because of larger groundfish biomass and hence higher feeding pressure on the benthos; (b) higher average trophic level for both the red mullet and its predators due to the extension of the food web and the increase in the number of omnivorous organisms at the base of the food web, and (c) a diet shift driven by the mechanical disturbance of trawling.

The study was carried out by a coordinated group that included three teams: CNR-IAMC (team 1 and project coordinator), ISPRA (team 2) and the Department of Ecology (formerly: Animal Biology) of the University of Palermo (team 3).

2 Materials and methods

The study area (Figures 1-3) included four locations off the northern Sicily coast: two no-trawl areas (Gulf of Castellammare, GCAST and Gulf of Patti, GPATT), and two trawled areas (Gulf of Termini Imerese, GTERM and Gulf of S. Agata, GSANT).

An experimental design based on protected and unprotected areas, each with two replicate locations (i.e., the gulfs), was adopted. Each location was divided in three equivalent surface sectors: West (W), Centre (C) and East (E). The design included three factors: Protection (fixed with two levels: protected PR and unprotected UPR), Gulf (random and nested in the Protection factor with two levels: GCAST and GPATT for PR and GTERM and GSANT for UPR),

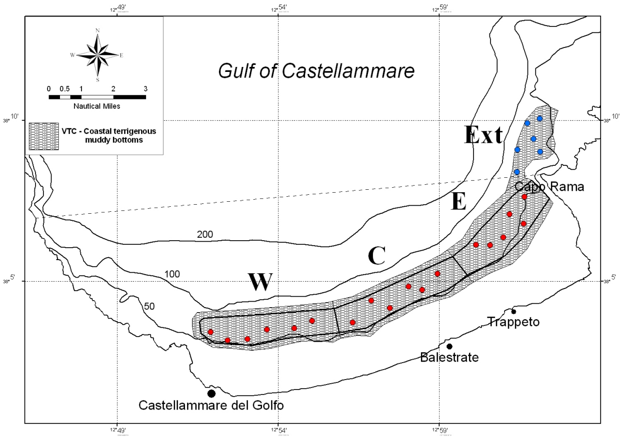

Figure 1: Gulf of Castellammare. The red dots indicate the single benthic samples. The shaded area indicates the coastal terrigenous mud assemblage. W, C, E: west, centre and east sectors.

and Sector (fixed with three levels: W, C and E). Factor Sector was not considered in the case of red mullet collected for stomach content analysis, potential predators and mesozooplankton.

Sampling sites were randomly chosen between 40 m and 80 m depth on sandy-muddy bottoms inside the coastal terrigenous mud assemblage [7], which hosts the preferential habitat of red mullet [4]. All samples were collected in spring 2005. Thirty red mullets were collected for each of the following size classes (total length) for stomach content analysis: I <7 cm; II 8-12 cm; III 13-17 cm; IV >17 cm. Six fish (potential predators) and six plankton samples were collected in each gulf.

Sediment samples were collected with a 0.15 m^3 grab. The minimum sample size for each replicate was 0.35 m^2 for taxa density estimate. Six replicates were collected for each sector. Samples were sieved over a 0.5 mm mesh. Infaunal organisms were counted and identified to the lowest practical taxonomical level. Polychaetes were identified at the family level and crustaceans at species level when possible.

The stable isotopes of nitrogen (δ^{15}N) and carbon (δ^{13}C) were used to assess the trophic level and the source of organic matter, respectively in the red mullet and its preys and predators. Trophic level estimates based on the value of δ^{15}N in the red mullet muscle tissue were compared to those obtained from stomach content analysis to validate the consistency of results among the two methods. Two reference materials were used in the calculation of δ^{15}N: a pelagic reference (mesozooplankton) and a benthic reference which included three deposit feeding crustaceans (the decapods *Alpheus glaber* and *Goneplax rhomboides* and the tanaid *Apseudes spinosus*). The mean δ^{15}N value of each gulf and the mean of all gulfs were used because δ^{15}N values did not differ sig-

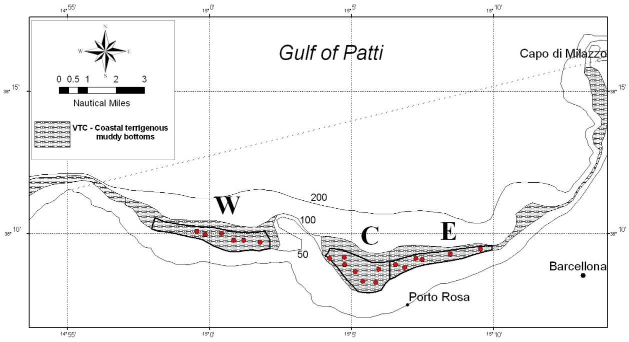

Figure 2: Gulf of Patti. The red dots indicate the single benthic samples. The shaded area indicates the coastal terrigenous mud assemblage. W, C, E: west, centre and east sectors.

nificantly between protected and unprotected gulfs neither for the mesozooplankton ($F_{1,2}$ =0.05; P=0.84) nor for the deposit feeders ($F_{1,2}$ =0.049; P=0.91).

Dietary preferences of red mullet in each size class in the four gulfs were assessed with the Ivlev electivity index.

The food web of the red mullet was graphically represented in a diagram using the mean values of δ^{13}C and δ^{15}N of all the taxa included in the study. Fish species that resulted enriched by half trophic level (i.e., +1.75 δ^{15}N and +0.5 δ^{13}C) were considered potential predators of the red mullet. Such enrichment was calculated over the actual isotopic values of juvenile red mullet (size class I) and over the smallest values for the cumulated adult red mullet size classes in each gulf.

3 Results

Lower biomass and higher abundance and P:B ratio of benthos were found in the protected gulfs. Abundance of benthic organisms and organic matter content in the sediment were correlated (r=0.86). The trophic levels of red mullet obtained from the isotopic analysis and from the stomach content analysis were non significantly correlated in the four gulfs for all size classes. Trophic levels calculated from stomach contents ranged between 3.22 ± 0.39 s.e. (size class II in GCAST) and 3.66 ± 0.61 s.e. (size class II in GSANT). Trophic levels obtained from δ^{15}N data ranged between 2.22 ± 0.14 s.e. (size class I in GTERM) and 4.06 ± 0.10 s.e. (size class III in GCAST). The lowest values (<3.0) were obtained always in size class I with δ^{15}N data. Trophic levels from isotopic data were always higher in GCAST than in the other gulfs with the exception of size class I. Among isotope-based trophic levels, those referenced to mesozooplankton were higher than those referenced to deposit feeders.

All the taxa identified in the benthic samples and in the stomach contents were used to assess dietary preferences in the red mullet. Forty-one taxa co-occurred in the muddy habitat and in the stomachs, thirty-

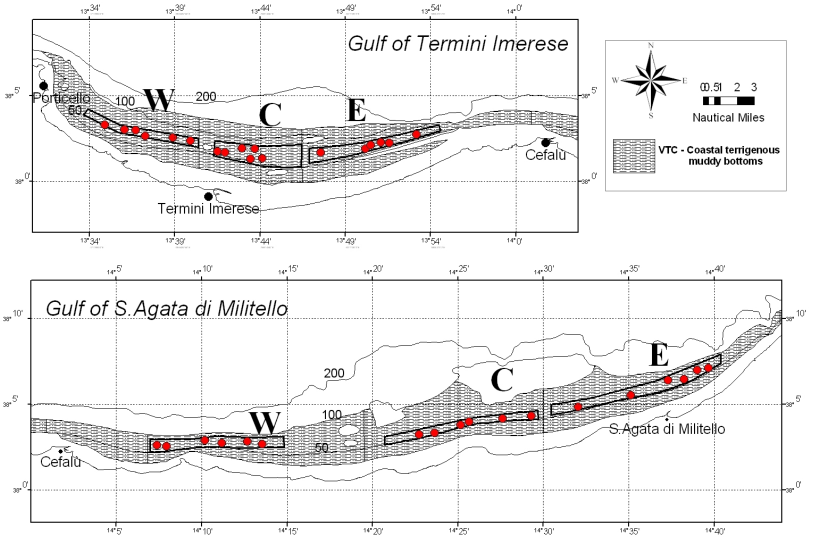

Figure 3: Gulfs of Termini Imerese and Sant'Agata. The red dots indicate the single benthic samples. The shaded area indicates the coastal terrigenous mud assemblage. W, C, E: west, centre and east sectors.

eight occurred in the habitat but not in the diet and eleven occurred in the stomachs but not in the habitat. Polychaetes were positively selected in the protected areas, especially in GCAST, whereas they were negatively selected (i.e., not eaten) in GSANT and only rarely eaten in GTERM. Crustaceans dominated the diet in the unprotected locations.

A general picture of the red mullet food web is given by Figure 3. δ^{15}N and δ^{13}C were correlated in each gulf, but more so in GCAST (Pearson's correlation coefficient: GCAST = 0.84, n=171, $P<0.001$; GPATT = 0.71, n=122, $P<0.001$; GSANT = 0.68, n=119, P=0.001; GTERM = 0.69, n=128, P=0.001). Eight potential predators of juvenile red mullet were identified: *Dicentrarchus labrax* (GTERM), *Epinephelus aeneus* (GSANT, GTERM), *Pagellus erythrinus* (GCAST, GSANT), *Raja radula* (GCAST), *Sparus aurata* (GCAST, GPATT, GSANT), *Torpedo marmorata* (GSANT), *Torpedo torpedo* (GCAST, GSANT) and *Uranoscopus scaber* (GPATT, GSANT). The potential predators of adults were: *Centrophorus granulosus* (GPATT, GSANT), *Dalatias licha* (GSANT), *D. labrax* (GSANT), *E. aeneus* (GCAST, GPATT, GSANT, GTERM), *Lophius budegassa* (GPATT), *P. erythrinus* (GCAST, GPATT, GSANT), *S. aurata* (GPATT, GSANT), *T. marmorata* (GSANT), *T. torpedo* (GCAST, GSANT, GTERM) and *U. scaber* (GPATT). Among the potential prey of adult red mullet, polychaetes had the higher δ^{15}N value. Sipunculids had the same position of deposit feeding polychaetes, whereas holothurians had a δ^{15}N value close to deposit feeding polychaetes but a much higher δ^{13}C, which excluded them from the group of potential prey. Crustaceans in all gulfs fell within two trophic guilds: omnivores (or large-sized deposit feeders) and deposit feeders; according to their isotopic values, they are potential prey of adult red mullet in all gulfs.

4 Discussion and conclusions

The abundance and biomass of the benthic assemblage differ among locations, with higher abundance and lower biomass in the protected gulfs (especially GCAST). The lower benthic biomass in the protected gulfs - which is compensated by higher P:B, especially in GCAST - could be due to the much higher groundfish biomass (red mullet biomass up to 47-fold higher [2, 3, 4] as a result of the trawl ban, and a consequent higher predation on the benthos.

In the unprotected gulfs the red mullet prefers suprabenthic prey, whereas in the protected gulfs (particularly in GCAST) they prefer infaunal prey, especially polychaetes. This difference, which is consistent across protected and unprotected locations, could be a result of adaptation to the level of trawling impact on the benthic community rather than due to natural variability.

This study clearly shows that the two methods used to calculate the trophic level of red mullet (i.e., stomach contents and stable isotope analysis) are not comparable. The isotope method seems to underestimate the trophic level of juveniles, whereas the stomach content method seems to underestimate that of adults. The result of the isotopic study of juveniles based on the deposit feeders is expected, because the reference material was not collected in the habitat of juvenile red mullet (which is coastal pelagic). What is unexpected is the result obtained with the mesozooplankton reference, which could be due to the different habitat (offshore pelagic) of the plankton samples. As a conclusion it is not advisable to collect the reference material in habitats different or far away from the habitat of the consumer, and this should be taken into account in future studies.

The mean isotope-based trophic level of red mullet is higher in the protected gulfs. At least two possible causes can be directly related to the trawl ban, that is (a) the absence of physical disturb on the bottom, and (b) the increased groundfish biomass. The absence of disturbance could have made new and different food sources available to the red mullet, as suggested by the higher number of positively selected prey items (e.g., polychaetes) in protected gulfs. A higher trophic level of polychaetes would explain the higher trophic level of red mullet in the protected locations (1/3 to 1/2 higher than unprotected), which in turn means that more energy is subtracted to the ecosystem by a unit biomass of red mullet. Most likely the ceased trawling impact has caused an increase in the trophic level of primary consumers (i.e., benthic invertebrates) and hence of their predators. Post and Takimoto [8] proposed a few possible ecological mechanisms that might apply to our case. The addition of a new intermediate species amid two species already linked by a prey-predator relationship might have increased the connectivity - and hence trophic level - in our protected gulfs [8].

The food web is similar in the four gulfs. The number of potential predators is small and basically only one species, the white grouper *E. aeneus*, is common to all gulfs. Less important are the pandora *P. erythrinus*, and the common torpedo *T. torpedo*. Remarkable is the absence of the hake *Merluccius merluccius* from the list of predators while the monkfish *Lophius* spp. appears only in GPATT. The stargazer *U. scaber* and the sea bass *D. labrax* could be occasional predators of juvenile red mullet. The observed differences between the four gulfs are consistent for all of the studied variables. A clear pattern is recognizable, with GCAST different from the unprotected gulfs (which are similar each other), and GPATT in an intermediate position between these two extremes. This pattern is similar to what has been observed for the red mullet CPUE in the four gulfs (authors' unpublished data), thus it seems correlated to the effect of protection. On one hand GSANT and GTERM are unprotected areas subjected to heavy trawling impact, while on the other hand GCAST and GPATT differ each other because they are subjected to a different degree of enforcement of the trawl ban. In GCAST, a major role in contrasting illegal fishing has been played by the presence of: (a) a huge system of artificial reefs within the -50 m isobath, (b) the CNR marine ecology laboratory who has contributed to develop the awareness among the different fishery stakeholders, (c) a great activism of the local Consortium for fish replenishment and (d) an effective enforcement of the trawl ban provided by the bodies in charge for patrolling the area.

The trawl ban in the Sicilian gulfs can be described as a successful management choice. It has contributed to reconstruct the benthic trophic food web and to increase the trawlable biomass, notwithstanding the higher average trophic level gained by secondary consumers. Due to the results obtained, the exclusion of trawl fishing from vast areas of the continental shelf has potential for application to other geographical areas in the context of an ecosystem approach to fishery. The data gathered in this study show the results of a true protection made possible by effective enforcement, especially in the earlier period. However, since fishermen are aware that trawlable resources are scarce and dramatically less abundant outside the protected areas, illegal trawling inside the protected area has increased recently [9]. For this reason strict enforcement is needed if gains are not to be lost, keeping in mind that the trawl ban in the Sicilian gulfs has allowed major enhancements from an ecosystem and fishery point of view, as well as new achievements in the field of marine ecology.

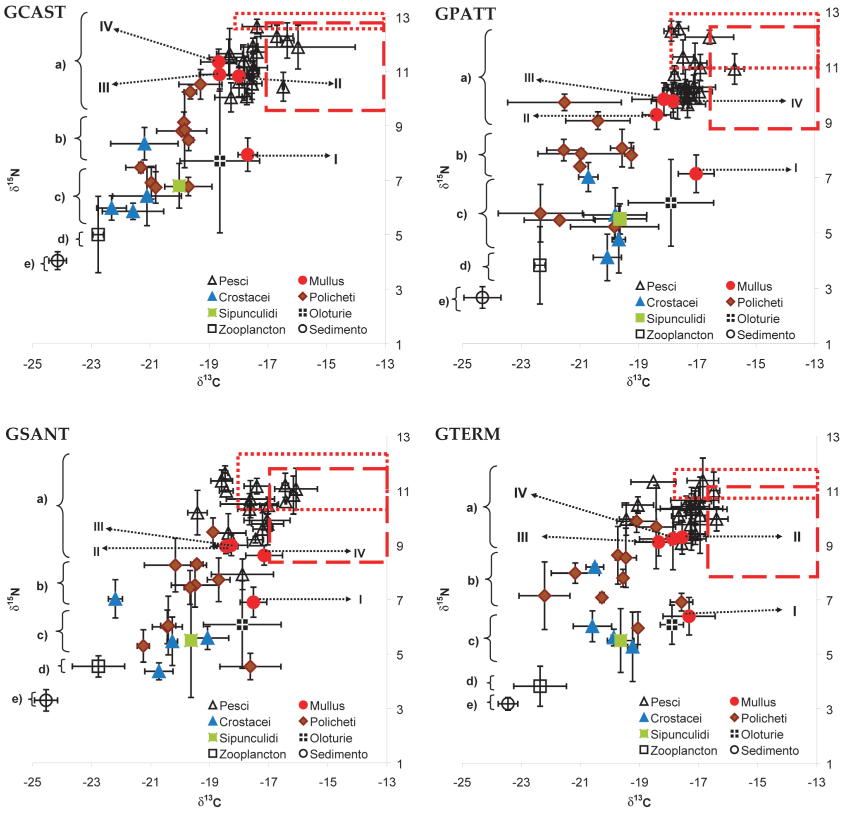

Figure 4: The red mullet, *Mullus barbatus*, food web reconstruction in the gulfs of Castellammare (GCAST), Patti (GPATT), Sant'Agata (GSANT) and Termini Imerese (GTERM) based on carbon and nitrogen stable isotopes. The mean δ^{15}N and δ^{13}C values of potential predators and prey are reported together with those of the main sources of carbon (zooplankton and sediment). I, II, III and IV show the red mullet size classes in cm of total length (I <7 cm; II 8-12 cm; III 13-17 cm; IV >17 cm). Dashed and dotted rectangles enclose respectively the potential predators of juvenile (size class I) and adult (size class II-IV) red mullet. Error bars are standard deviations. a) = carnivores; b) = omnivores or large-sized deposit feeders; c) = deposit feeders; d) = grazers (zooplankton); e) sediment.

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Inter-Annual Variability of the Trace Element Content in Seawater from the Western Ross Sea, Antarctica

C. Turetta[1], C. Barbante[1,2], G. Capodaglio[1,2], P. Cescon[1,2]
1, Institute for the Dynamics of Environmental Processes, CNR, Venezia, Italy
2, Department of Environmental Sciences, University “Ca’ Foscari”, Venezia, Italy
clara.turetta@idpa.cnr.it

Abstract

Total dissolved content of some trace elements (Cd, Cr, Cu, Mn, Pb, Zn), nutrients (nitrate, phosphate and silicate), fluorescence, salinity, dissolved oxygen and temperature were measured on several samples collected during 2 oceanographic surveys from 2001 to 2003 within the framework of the Italian expedition in Antarctica on board the R.V. Italica. The mean concentrations measured are consistent with literature data for seawater at high latitude. The concentration value of some elements, never detected in high latitude seawater before, were compared with the expected values for oceanic water: the results are in agreement with literature data. As for all the oceans, also in the Ross sea the relationship between Cd and phosphate is evident. Some trace elements like copper, cadmium and zinc may have the double role of micronutrient and toxicant. The geochemical cycle of some trace elements, like manganese, is principally controlled by the oxidation-reduction reaction. The obtained results were discussed taking in account the distribution of such trace elements as a function of the different water masses present during the austral summer in the western sector of the Ross Sea.

1 Introduction

The importance of trace elements as environmental contaminants and their diffusion at a planetary level has already been highlighted in various paper. Trace elements are released into the environment by a large number of natural processes, and, during the last few centuries, also by a number of uncontrolled human activities [1, 2, 3, 4, 5, 6]. With the aim to better understand the mechanisms that control the distribution of trace elements, we have focussed our attention on seawater because of the importance of this matrix in the global transport of chemicals. The Southern Ocean, plays a key role in the circulation of the world’s ocean, since the Antartic Circumpolar Current (ACC) extends around the globe, flowing eastwardly and driven by the world’s strongest westerly winds, being a unique global link which joins all major oceans [7], and the Ross Sea has an important role in the formation of Antarctic Bottom Water (AABW) which re-oxygenate all the oceans [8, 9]. Geochemical, physical and biological processes may control trace metals concentration in the various areas of the world ocean. Hence, the study of the distribution of trace metals is fundamental in better understanding processes, which happen in different geographic areas, in relation with their hydrological regime. The hydrological regimes, the atmospheric inputs, the ice covering, and nutrients availability may powerfully discriminate between the coastal and the pelagic areas. It has been observed that trace metals frequently covary with major nutrients and there is the evidence that they may also influence the plankton productivity and hence the community structure [10, 11, 12]. The knowledge of the trace elements distribution in the Southern Ocean is only partially known even if different areas have been investigated in this region. In the framework of the Italian Research Program in Antarctica (PNRA), the Ross Sea was investigated by two oceanographic expeditions, on board of the R. V. Italica during the austral summer of 2000-2001 and 2002-2003. The aim of this study was to recognize the different water masses, which can be observed in the western part of the Ross Sea, to study the vertical distribution of total dissolved cadmium, chromium, copper, lead, manganese and zinc in correlation with nutrients (nitrate, phosphate and silicate), fluorescence, salinity, dissolved oxygen and temperature in the Ross Sea during the austral summer and to understand the biological, physical, chemical processes, which may be involved.

2 Material and methods

2.1 Chemicals and laboratories

All materials used for sampling, treatment and storage of samples and solutions were carefully chosen, acid-cleaned and conditioned to minimise sample contamination due to the extremely low concentrations expected The cleaning procedures and controls are reported in details elsewhere [13, 14, 15, 16].

The cleaning steps can be summarised as follows: low density polyethylene bottles (LDPE) were cleaned first in a heated detergent solution for 2 weeks and then in a heated acid bath (10% HNO_3, suprapure grade - 65% Merck, Darmstadt, Germany); for 2 weeks all the materials were rinsed each time with ultrapure water (ELGA-Vivendi Water Systems, Bucks, UK). At the end of the preliminary cleaning steps the LDPE bottles were filled with a 0.1% solution of ultrapure concentrated HNO_3 (Romil Pure Chemistry - Romil Ltd., Cambridge, GB). After two weeks this step was repeated for additional two weeks and then, after further rinsing, the bottles were filled with a 0.01% ultrapure HNO_3 solution and sealed in double polyethylene bags until use. The Go-Flo bottles (General Oceanics, Teflon-coated, USA), used for the sampling, were repeatedly washed using ultrapure water and 1:100 (v:v) ultrapure HCl (Romil Pure Chemistry, Romil Ltd., Cambridge, GB) and were finally rinsed with ultrapure water to avoid contamination before sampling. Tests to check the cleanliness were performed on the rinse water.

All the pre-analytical procedures (preparation of materials, i.e. bottles for sample storage and dilution, standard solutions, and vials for analyses) were carried out in the clean laboratory equipped with a class 100 laminar flow bench, available at the CNR-Institute for the Dynamics of Environmental Processes, located in the Department of Environmental Sciences-University Ca’ Foscari, Venice.

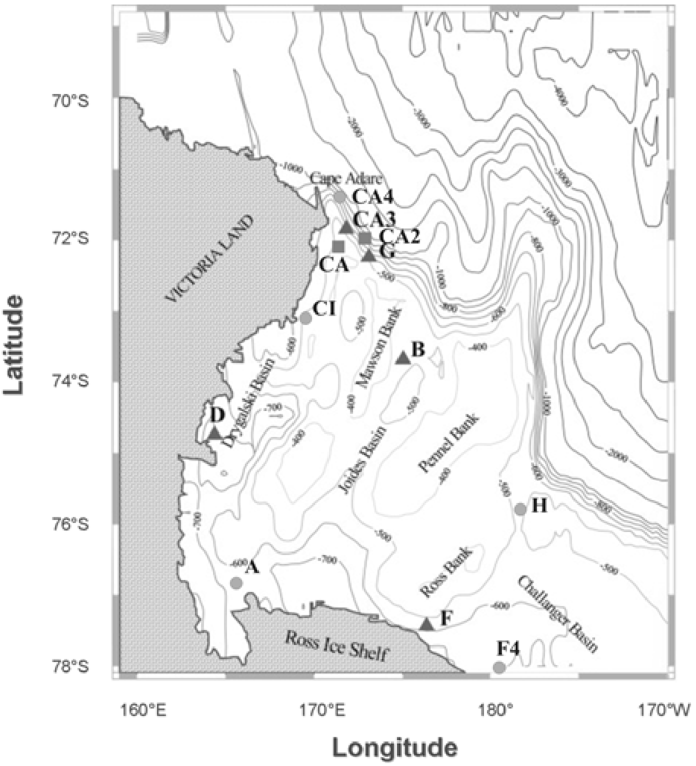


Figure 1: Sampling sites in the Ross Sea (square sampling survey 2000-2001, triangle 2002-2003, circle 2005-2006).

2.2 Sampling sites

Seawater samples from some depth profiles (see Figure 1 for location of sites) at six/ten different depths, depending on the site, were collected in the western sector of the Ross Sea (Southern Ocean, Antarctica) during the austral summer 2000-01 and 2002-03 within the framework of the Italian expeditions in Antarctica on board the R.V. Italica.

The sampling sites CA and G are located near Cape Adare, where the High Salinity Shelf Water (HSSW) sinks towards the deep sounding of the Ross Sea, to eventually form Antarctic Bottom Water (AABW), and where the Circumpolar Deep Water (CDW) intrudes around 200-600 m producing MCDW. The B site is located in the Joides Basin, a trough with a southwest-northeast direction, characterized by reduced horizontal transport and an efficient sedimentation process. Site F is near the ice shelf where the Ice Shelf Water (ISW) is formed and is overlain by a water mass fresher and warmer, a sort of winter water (WW) above which Antarctic Surface Water (AASW) is recognisable.

Site D is in the Drigalsky Basin, close to a glacier, which is located in the permanent polynia area where the HSSW is formed.

2.3 Sampling and sample handling

The sampling sites were chosen in order to evaluate some of relevant water masses present in this area; the sampling strategy was driven on the basis of the in situ measurements of salinity, temperature and dissolved oxygen along the water column. The samples were collected using five 20 L Go-flo bottles (high-capacity bottles are used in order to have the highest possible value of the volume/surface ratio minimising the possible releases from the walls into the sample); 2 or 3 Go-flo, mounted on a Kevlar line and triggered by means of Teflon messengers, were dipped at the same time; the constancy of water vertical structure during the sampling was checked by CTD profile at the beginning and the end of sampling. To verify the representativeness of results, occasionally, two samples were collected at the same depth. Sampling at each site was completed within 10-12 hours of the ship positioning.

After sampling, the Go-flo bottles were immediately transferred into a mobile clean-laboratory (Class 100) installed on board and the samples were filtered using a Teflon filtration system (Sartorius SM 16540, Gottingen, Germany) and cellulose nitrate membrane filters (pore size 0.45 μm). The membrane filters were previously washed using a weakly acid solution and can be considered clean for trace elements. The filtered samples were collected in 250 millilitre LDPE bottles previously washed as described above and conditioned with the same seawater. The samples were immediately stored in a freezer at -20 °C for transport to Italy without any additional treatment.

In Italy pre-treatment of the filtered samples before the analytical step was restricted to dilution, acidification and internal standard addition. Each sample was 10-fold diluted using ultrapure water obtained by coupling a Milli-RO system (Millipore, Bedford, MA, USA) with a Purelab-Ultra system (ELGA-Vivendi Water Systems, Bucks, UK), and acidified with UPA grade HNO_3 (1:10 v:v). An internal standard solution of Sc, In and Y (1 ng/ml) was added to correct the measurements for changes in sensitivity of the instrument ([17, 16]).

Treatments and analytical measurements were performed in clean laboratories, where the working area was in a class 100 laminar flow area.

2.4 Instrumentation and analytical parameters

Dissolved trace metal determinations were performed by ICP-SFMS (Element2, Thermo-Fischer, Bremen, Germany), as described in previously papers [16, 18]. Intensity optimization was carried out daily, using a 1 ng/ml tuning solution of indium in 10 fold diluted seawater.

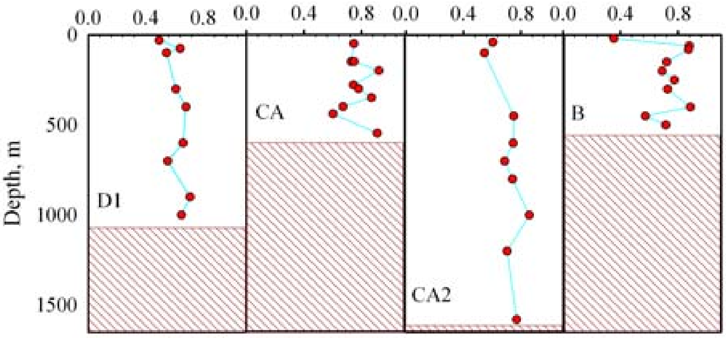
Before beginning the analyses, an accurate mass calibration was performed in low, medium and high resolution modes using a solution containing elements with m/z values covering the whole mass range of interests. Cd and Pb were determined in low-resolution mode ($m/\Delta m = 400$), while Cr, Cu, Mn and Zn were determined in medium resolution mode ($m/\Delta m = 4000$). The sample introduction system used was a micro-flow nebuliser (Elemental Scientific, 100 ml/min) coupled with a desolvation unit (Aridus, Cetac Technologies, Omaha, NE, USA) to reduce the interferences due to oxide and hydroxide formation. The introduction systems was coupled with an autosampler (Cetac Technologies, Omaha, NE, USA). Details of operating parameters for the ICP-SFMS and desolvation unit are reported in the original previous papers [19, 16, 18]. The sample introduction system, the desolvation unit and the autosampler were kept and handled under a laminar flow hood.

The accuracy of the measurements was determined using a certified reference material (NRCC-NASS 5); the measured values were in good agreement with certified: for the determined elements the mean difference between the measured and certified value was 5% of the certified value and always within the certified tolerance interval. The quantification of trace elements was carried out by a matched calibration method. Six aliquots of NASS-5 (Seawater Reference Materials for Trace Metals-NRC-CNRC), handled as just described for the samples, were spiked with a multi-element standard solution (0, 2, 5, 10, 50, 100 pg/ml, from a 1000 mg/l ICP-MS stock solution-Spex-CertiPrep, Metuchen, NJ-USA). All the materials, i.e. samples, internal standard and multi-element standard solutions, were prepared gravimetrically.

Tests on "blank solutions" (ultrapure water, HNO_3 1:10 v:v and internal standard at 1 ng/ml concentration) were performed in each analytical session before analysis of the seawater samples to carefully considered the effects of dilution, acidification and internal standard addition. For the considered trace elements no significant signal was observed. The results (mean and detection limit) of the analyses performed on six blank solutions (n=4) are reported in previous papers [16, 18]. Detection limits were calculated as three times the standard deviation of the blank solution signal.

3 Results and discussion

Different water masses, which affect the circulation of the world oceans, can be recognized in the Ross Sea: AASW (Antarctic Surface Water), MCDW (Modified Circumpolar Deep Water), HSSW (High Salinity Shelf Water), ISW (Ice Shelf Water) and LSSW (Low Salinity Shelf Water) [20, 21, 8]. AASW broadens with rather homogeneous properties from the Polar Front to the continental margins of Antarctica, where shelf waters are found at near freezing temperatures. Below AASW, we can recognise a warm and saline water mass: this is the CDW, the so-called "warm core" of the Ross Sea where it develops into several new water masses on and near the Antarctic continental shelves [8]. The MCDW, derives from the mixing of CDW with shelf waters [22], has a typical temperature between +1.0 and -1.5 °C [23]. HSSW is identified by a salinity $>34.6‰$ and a temperature at the sea surface freezing point (-1.89 to -1.8 °C). This neo-formation dense water mass flow northward along the Drygalsky and Joides Basins till downward to ocean floor re-oxygenating the bottom water. ISW is characterised by a temperature below the sea surface freezing point, and takes its origin from the part of HSSW that moves southward and flows under the Ross Ice Shelf (RIS) [22, 20, 21]. It is recognised in the west-central sector, where it comes out from the RIS [23]. While ISW is formed along the RIS, HSSW is formed in the large Ross Sea polynia and in the small,



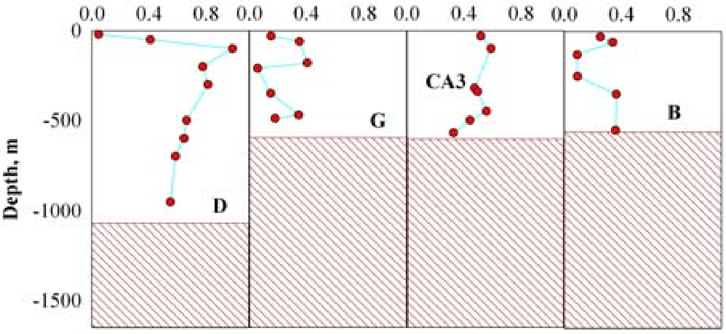


Figure 2: Total dissolved Cd distribution at the sampling sites.

persistent one close to theVictoria Land coast. LSSW is characterised by a slightly warmer sea surface freezing point temperature and low salinity (−1.89 to −1.7 °C and 34.4-34.7) [20] and overlies the ISW filling the intermediate and subsurface layers [24]. Its formation may be due to the interaction of AASW with colder waters in the subsurface layers [25].

The aim of the research activity was the geochemical characterization of the seawater masses which are recognizable in the Ross Sea and the study of the interaction between the neo-formation waters and the old one (in particular the CDW). Several seawater samples from some sites (see Figure 1 for location of sites) were collected in the western sector of the Ross Sea (Southern Ocean, Antarctica) at six/ten different depths, depending on the site, during the austral summer 2000-01 and 2002-03 within the framework of the Italian expeditions in Antarctica on board the R.V. Italica. The sampling sites were chosen in order to evaluate some of relevant water masses present in this area that are fundamental for the circulation both at local and global level. These water masses contribute to form the Antarctic Circumpolar Current (ACC), which flow continuously all around the Antarctic continent, connecting all the oceans [7]. The sampling strat-

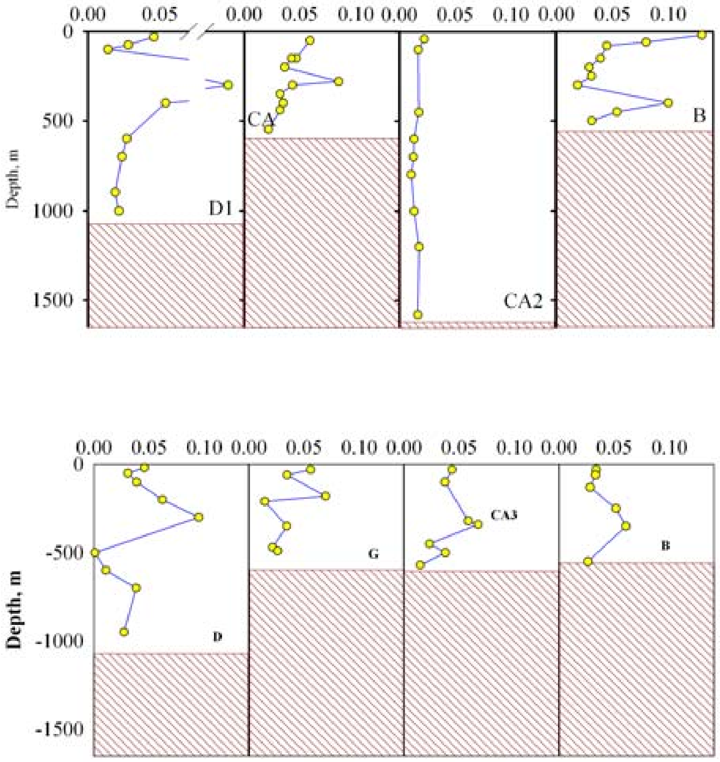


Figure 3: Total dissolved Pb distribution at the sampling sites.

egy was driven on the basis of the in situ measurements of salinity, temperature and dissolved oxygen along the water column. The sampled sites represent areas of new water formation (HSSW and ISW), or mixing area (shelf water and CDW), or sink area (where new waters flow downward to ocean floor re-oxygenating the bottom water) [26, 18].

The obtained results have highlighted the existing differences between the various seawater masses recognizable in the Ross Sea.

The dissolved cadmium concentration ranged between 0.1 and 0.9 nmol/L showing a general agreement with the concentrations reported in literature for the Weddel Sea and the Ross Sea [27, 28].

A variability on surface concentration of cadmium in the Ross Sea was observed; it seem to be related to similar and dishomogeneous distribution of phytoplankton, as highlighted in previous works [29], and with differences also in the major nutrient concentration and all the trace elements whose distribution is related to biological activity. Studies performed on the coastal areas of the Ross Sea have highlighted the strong relationship between cadmium and phytoplanktonic bloom along the vertical profile, expecially during the summer period, when the concentration vary from 0.8

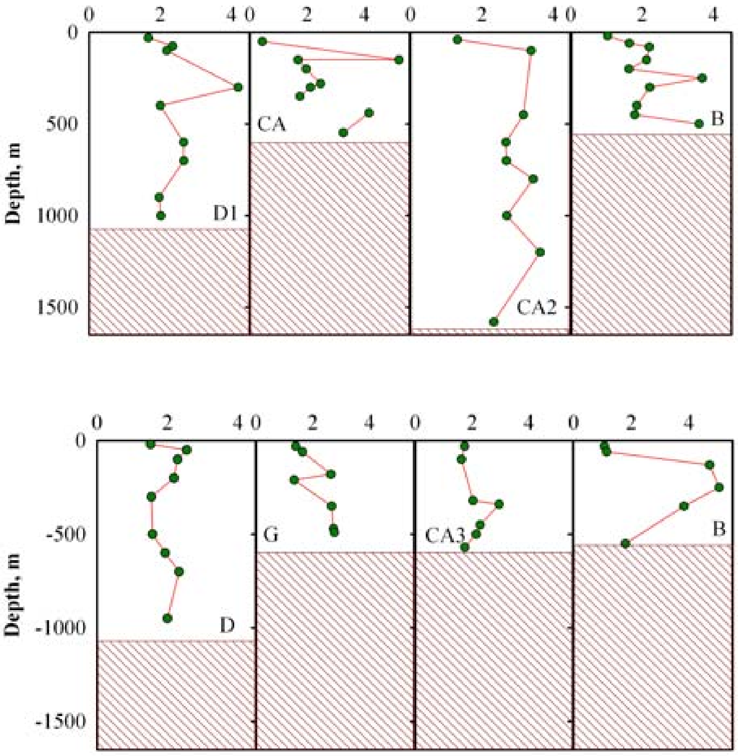


Figure 4: Total dissolved Cu distribution at the sampling sites.

nmol/L in austral spring time down to 0.1 nmol/L at the end of austral summer. This seasonal variability have repercussion also on interannual variability.

An increase of surface concentration of total dissolved lead with respect to underlying levels is in agreement with previous studies carried out in oceanic regions and reflect the atmospheric input and the short residence time of lead [30]. Recent studies performed on coastal area of the Ross Sea have highlighted that the total concentration of lead may vary with respect to geographycal position and seasonality. Total concentration of dissolved lead range between 0,01 nmol/L and 0,23 nmol/L in sampled sites (Figure 3).

Total concentration of dissolved copper in surface waters range between 0.4 nmol/L and 1.7 nmol/L in agreement with literature data on Southern Ocean. Total dissolved copper concentration in deep water range between 1.5 nmol/L and 4.0 nmol/L. In oceanic oligotrophic water the vertical distribution of copper presents an intermediate profile between nutrient-type and scavenged, showing a surface depletion in agreement with its behaviour as an essential micronutrient, and an enrichment in the intermediate and deep strata of the water column. The phytoplanktonic uptake affects the superficial distribution of copper

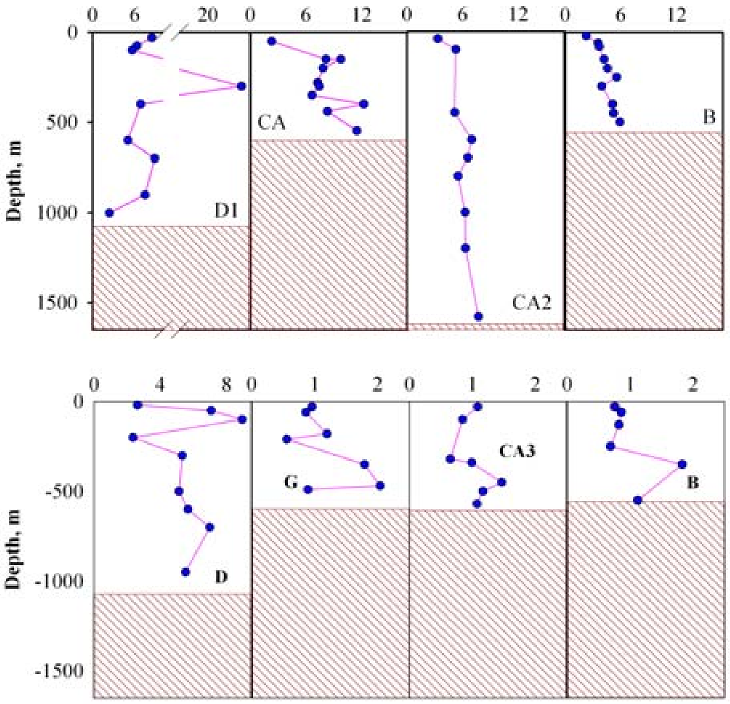


Figure 5: Total dissolved Zn distribution at the sampling sites.

(Figure 4) and the rapid diagenesis regenerate back copper at sediment/water interface justifying the relevant increment of concentration at intermediate and deep water; this sentence also justifies the different concentrations measured in the water of different ages.

In agreement with its nutrient-type characteristics zinc concentration shows a superficial depletion (Figure 5). The subsurface concentration was about half to one third of the value detected in deep water; only the samples collected in the permanent polynia site (Drygalsky Basin - site D in Figure 1) showed noticeably higher concentration than the mean value detected in the deeper layers, explained by the hydrodynamic characteristics of this area that shows a strong upwelling of deep water. We can hypothesize that the vertical distribution of zinc is ruled by the phytoplanktonic uptake in the superficial waters and by slow recycling processes deep along the water column.

The obtained results for chromium show a homogeneus surface distribution for all the studied sites, the surface concentrtion varying between 1,1 nmol/L and 2,8 nmol/L (Figure 6). At present no data for chromium concentration in Southern Ocean are available, but the results of this study are in agreement with concentration expected in oceanic areas, such as the Northeastern Pacific Ocean [31]. The

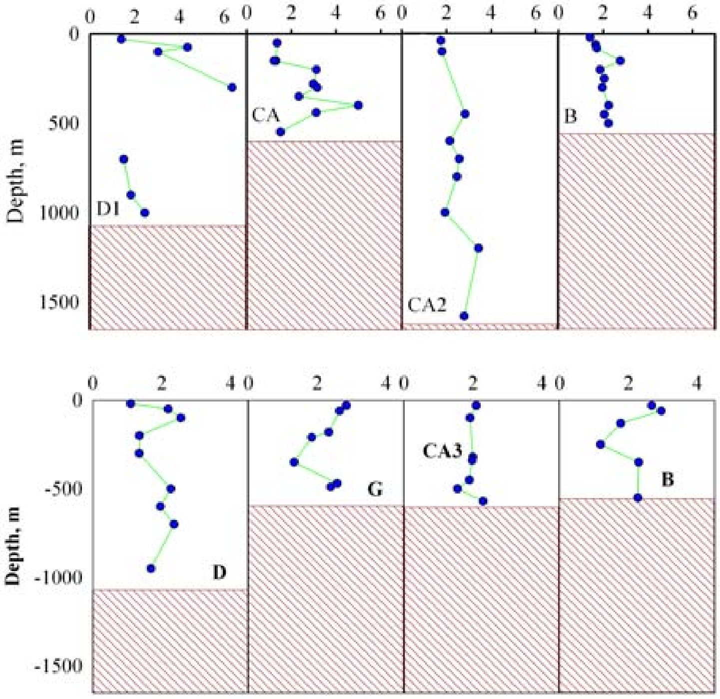


Figure 6: Total dissolved Cr distribution at the sampling sites.

expected oceanic distribution for this element is in correlation with the biological activity recognized in this area; it also seem to be complicated by the oxidation-reduction processes in anoxic waters. The measured performed on our samples show that the total concentration increase with depth. In oxigenated water Cr (IV) is the dominant species: due to the oxigenation of the studied area chromium should assume this state. Therefore the slow oxidation rate of Cr(III) could permit the coexistence of the two species in oxigenated waters, while we cannot exclude the presence of the reduced form close to the sediment.

The concentration distribution of total dissolved manganese is quite homogeneous in the studied sites (Figure 7), the total concentration varying in the mixlayer between 1.3 nmol/L and 2.0 nmol/L and between 1.4 nmol/L and 3.6 nmol/L in the intermediate water. A homogeneous distribution in the superficial and intermediate layers was underlined also in the Indian sector of the Southern Ocean; moreover, in the Ross Sea the concentration and the vertical distribution of manganese may vary with the seasonality, in particular between spring and austral summer. The obtained value of bottom water are in agreement with the value of polynia area in the spring time [32].

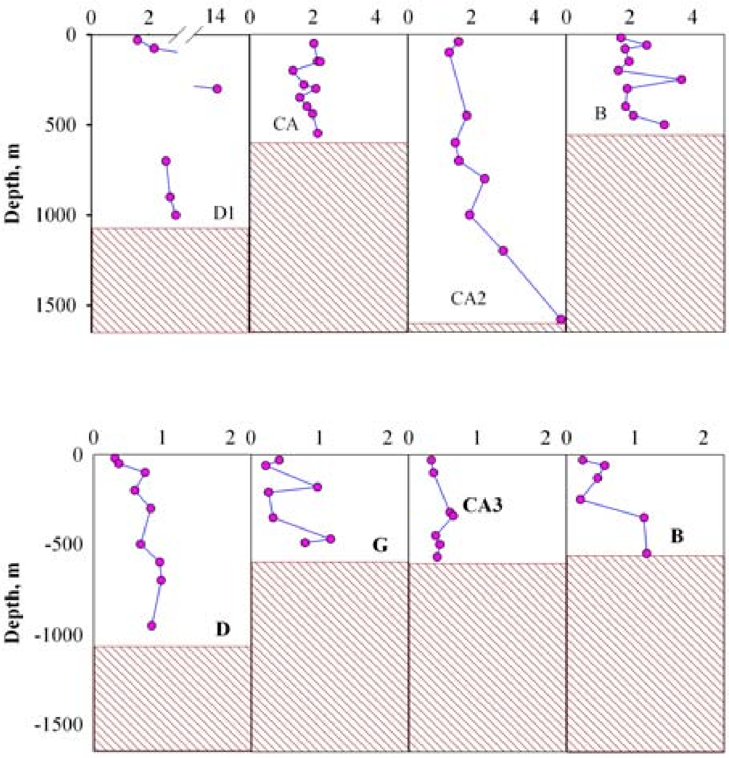


Figure 7: Total dissolved Mn distribution at the sampling sites.

4 Conclusion and future perspective

The vertical distribution of trace elements seems to be related not only to their geochemical property but also to other factors: seasonality, various local input, biological activity and to the different characteristics of the water masses recognizable in the western sector of the Ross Sea. On the basis of the acquired knowledge we may try to better understand the complex system of the Southern Ocean and to contribute to deeper known the role of the Ross Sea on the evolution of our environment.

In the framework of the Italian expeditions in Antarctica on board the R.V. Italica during the austral summer 2005-2006 we have collected numerous samples along five depth profile in different sites of the Ross Sea to have confirmation of our previous data and to evaluate the evolution of new-formation waters; we'll try to follow their movements till the mixing area of new and old waters and to quantify their interannual variability.

It will be necessary to deeply study the Antarctic waters, in particular in the areas of polynia, of formation of icewater and of sink from shelf to abyssal zone to better understand the mechanisms, the exchange entity between the water masses and the geochemical cycles of trace elements. The studies should be extended to different areas of all our planet to follow the exchanges between the different oceans till the Arctic Ocean.

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Effect of Sea Surface Temperature and Wind Forcing on the Potential Spawning Habitat of European Anchovy (*Engraulis encrasicolus*) in the Strait of Sicily

G. Basilone[1], A. Bonanno[1], B. Patti[1], R. Grammauta[2], A. Cuttitta[1], S. Aronica[1], G. Giacalone[1], I. Fontana[1], G. Buscaino[1], B. De Luca[1], C. Patti[1], E.M. Quinci[1], F. Falco[1]
1, Institute for Coastal Marine Environment, CNR, Capo Granitola (TP), Italy
2, Faculty of Mathematical, Physical and Natural Sciences, University of Palermo, Palermo, Italia
walter.basilone@irma.pa.cnr.it

Abstract

The European anchovy (*Engraulis encrasicolus*) represents one of the most important fishery resources of the Mediterranean. Because of its short living nature and its recruitment variability, associated to high environmental variability, this species undergo large interannual fluctuactions in the biomass levels. The aim of present study is to characterize the anchovy spawning habitat in terms of satellite-derived and in situ measurements of sea surface temperature (SST), and wind mixing and surface wind stress. This research work has been performed on the data collected during three oceanographic surveys carried in the Strait of Sicily out on board the R/V Urania in summer 2004, 2005 and 2006. The main spawning ground during 2004 survey was located in the coastal waters between Sciacca and Gela; during the 2005 survey the egg density spatial distribution pattern was complementary to the one observed in the previous year, with two main spawning areas located on the Adventure Bank and on the northern part of the Maltese shelf; the spatial pattern in 2006 was similar to 2005 but with lower densities. The results showed the existence of preferred values in the examined physical variables, associated to anchovy spawning areas. The analysis on SST indicates that the spawning habitat is characterized by a main peak at 23°C, while the optimal spawning conditions obtained by analysis on wind mixing and wind stress show a main peak in the range 300-500 $m^3 \cdot s^{-3}$and at 0.10 N/m^2.

1 Introduction

The European anchovy (*Engraulis encrasicolus*) constitutes one of the most important fishery resource in the central Mediterranean sea. Because of its short living nature and of its recruitment variability, associated to high environmental variability, this small pelagic species undergoes high interannual fluctuations in the biomass level [1]. The population abundance is determined especially by the high

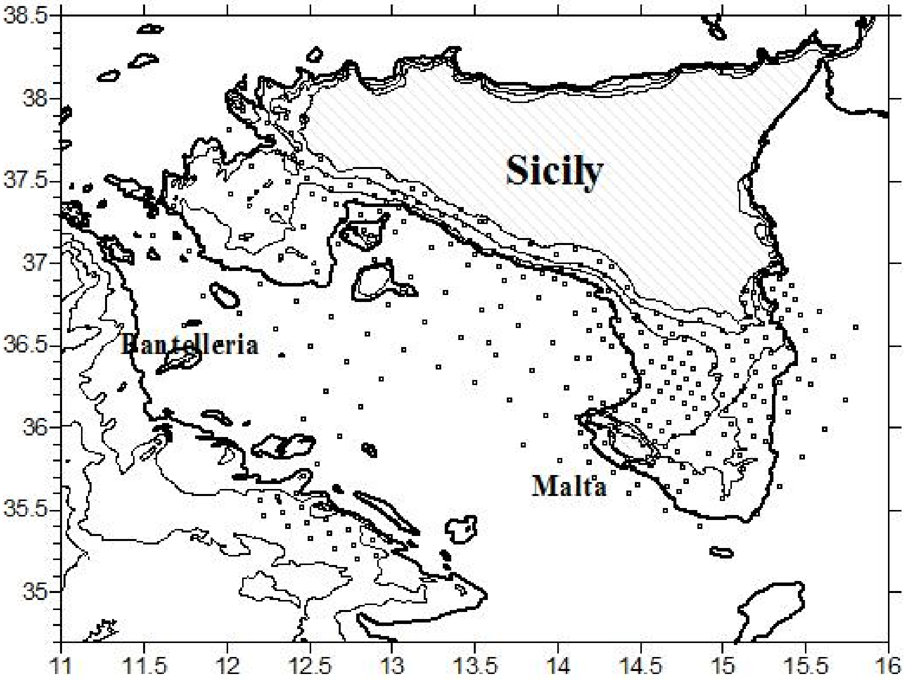


Figure 1: Study area with sampling stations in the period 2004 – 2006.

variability in recruitment success, which is generally considered to be linked to the survival of early life history stages, from eggs and larvae to juveniles [2]. Many studies highlighted the role of environmental conditions in determining the recruitment success of small pelagic fish species [3, 4, 5, 6, 7, 8] and several efforts were addressed to study and to model the key environmental features which drive European anchovy to the selection of a particular area as a spawning site [9, 10, 11, 12, 13, 14]. The main aim of the present study is to characterize the anchovy spawning habitat in terms of satellite derived sea surface temperature (SST), wind mixing and surface wind stress (τ, N$\cdot$m^{-2}). The magnitude and distribution of the shearing stress produced by the wind on the sea surface is fundamental to the wind-driven component of the ocean circulation and is essential to drive ocean models. The surface wind stress has a direct relationship with the remixing of the first layers of the water and the mixed layer depth is proportional to the magnitude of the forcing [15]. The marine ecosystem functioning is influenced by different physical processes, through changes in the temperature, light and mixing regimes [16]. Therefore, to better understand the ecosystem dynamics, it is important to know and to monitor both the abundance and distribution of marine biological populations and

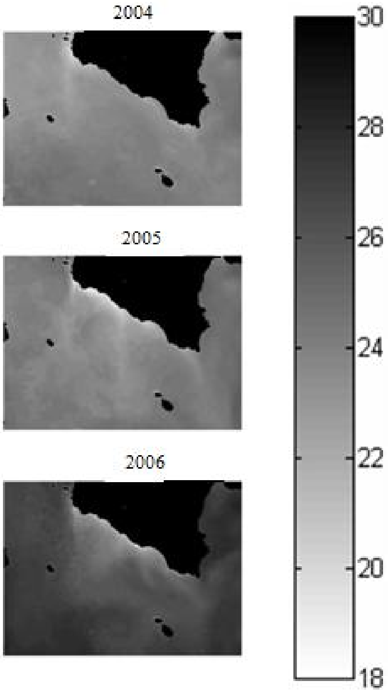


Figure 2: Maps of the Mean SST (°C) over the survey periods (years 2004, 2005 and 2006).

the physical environment, which may have important effects upon the biological resources. The actions of surface forcing, derived from the atmospheric energetic motions like wind stress, are the major causes of mixing of the upper layer sea. This part of water column is called mixed layer (ML) and is defined as the surface layer of the sea where there is nearly no variation in density with depth [17]. This homogeneity is caused by turbulence through the action of mechanical mixing by wind stress. The mixed layer of the ocean and the related processes influence the ocean's biological production. When the mixed layer deepens, nutrients from subthermocline depths are brought up into the mixed layer. As a result, phytoplankton production increases and the growth of fish is augmented [18]. Temperature, along with photoperiod, controls fish spawning, while wind-driven mixing effects determine the depth of the upper mixed layer [19]. In the Strait of Sicily (Central Mediterranean sea; Figure 1) the knowledge of the linking between physical environmental features and spawning grounds is still poor. The Strait of Sicily has a complex sea circulation scheme [20, 21], which is able to influence the spawning strategy and the recruitment success of many fish species, such as anchovy [22]. In the simplest circu-

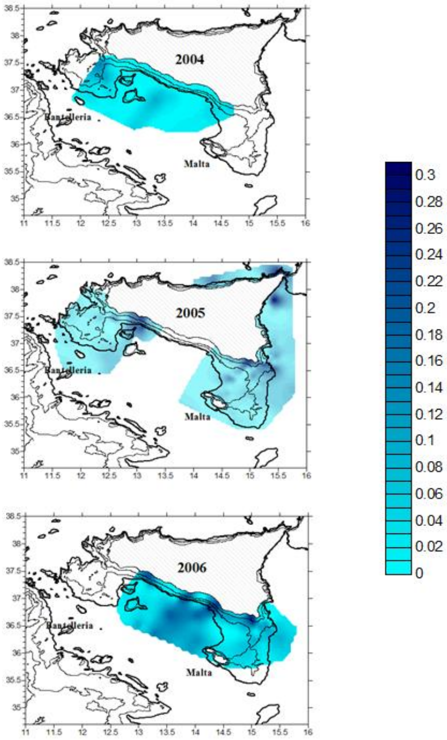


Figure 3: Maps of the absolute values of Wind Stress (N/m^2) based on the available stations from surveys 2004, 2005 and 2006.

lation model, the modified Atlantic water current (AIS) flows eastward through the Strait. Below the AIS a relatively dense and salty Levantine Intermediate Water (LIW) moves westward through the Strait. Local upwelling events arise when the AIS impinges with the southern coast of Sicily, as well as from wind-driven Ekman transport. Upwelling from such processes enrich the upper water layers and enhance primary production in the area [22], Patti et al., in press. The AIS's path varies seasonally but generally bounds two large cyclonic eddies, one over Adventure Bank in the north-western (NW) region of the coast and the other one over the Maltese Shelf in the south-eastern (SE) region of the coast.

2 Materials and methods

This research work is based on the analysis of data collected during three summer oceanographic surveys on board the R/V Urania in the Strait of Sicily (Figure 1) in the years 2004, 2005 and 2006. The first oceanographic survey "ANSIC 2004" was carried out in the period 17th June – 6th July 2004, the second survey "BANSIC 2005" in the period 3rd – 25th July 2005, and the oceanographic survey "BANSIC

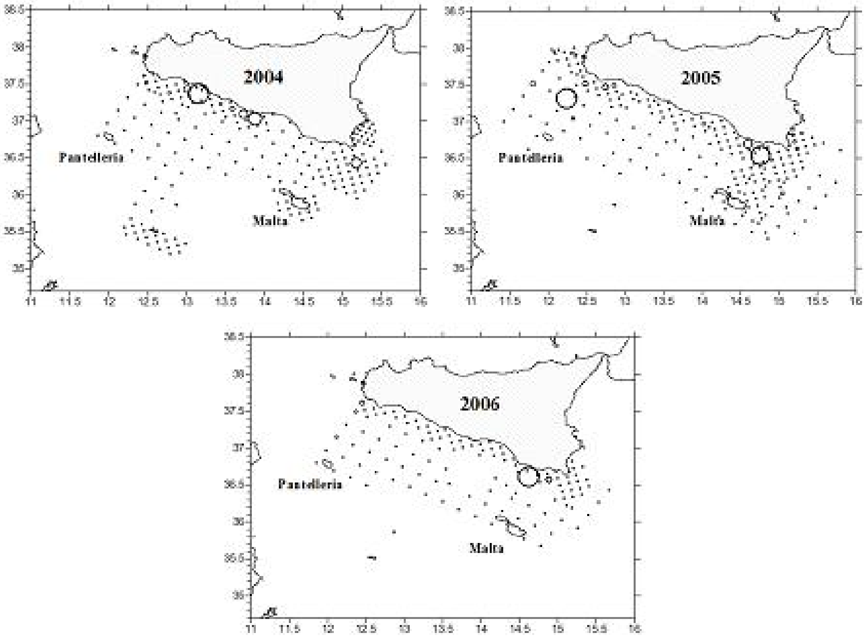


Figure 4: Eggs density distribution evaluated during the oceanographic surveys Ansic 2004, Bansic 2005 and Bansic 2006.

2006" in the period 29th July – 14th August 2006. A multidisciplinary set of data has been acquired in the study area (Figure 1) on a station grid of 4 x 4 nautical miles in sea zones closer to the coasts; a grid of 12 x 12 nautical miles was adopted for the off-shore areas. In each station ichthyoplankton samples have been collected by means of a Bongo40 net, which is composed by two coupled nets with the inlet mouth diameter of 40 cm and a mesh size of 200μm. The plankton oblique tows were carried out to a depth of 100 m, wherever possible, with a constant speed of 2 knots. The filtered water volume of each mouth was measured by a calibrated flowmeters (type G.O. 2030). A total of 206 samples were collected in the 2004 survey, 220 in the 2005 survey and 112 in the 2006 survey. Laboratory based sorting permitted to evaluate the numbers of anchovy eggs in each station. High eggs densities were considered as a proxy for the location of spawning grounds. Quotient analysis [23, 10, 14] was used to characterize spawning habitat preference. It was performed on anchovy eggs vs. sea surface temperature (SST, °C), wind mixing (U3, $\mathrm{m}^3 \cdot \mathrm{s}^{-3}$) and surface wind stress (τ, $\mathrm{N{\cdot}m}^{-2}$). For each variable divided into classes, the percentage frequency was divided by the percentage frequency of anchovy eggs giving the quotient values for each class. This semi-quantitative technique permits to establish whether the eggs distribution patterns are related to

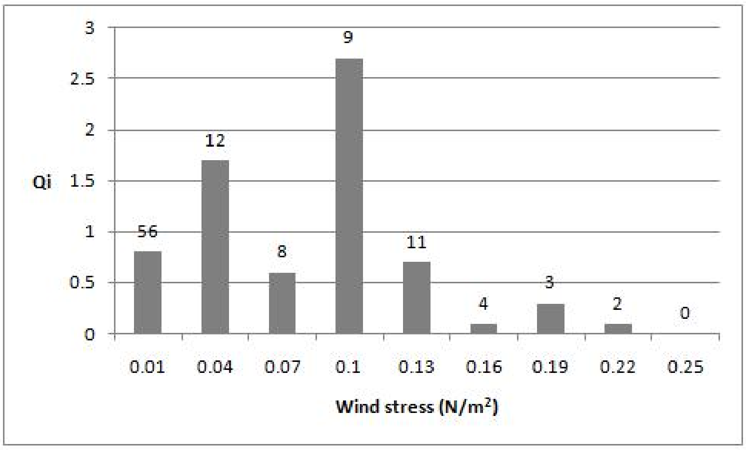


Figure 5: Anchovy quotients of eggs densities (eggs/m^2) vs. Wind Stress at stations sampled in the Strait of Sicily (the number of stations over any bar).

specific habitat conditions. Quotient values >1 indicate preference to a specific range of variable, while those <1 indicate avoidance of the specific class [10]. SST has been obtained from daily satellite images acquired by the AVHRR (Advance Very High Resolution Radiometer) sensor installed on board the NOAA satellites (http://eoweb.dlr.de:8080/servlets/template/welcome/entryPage.vm). Data collected by the hull mounted (2 m depth) temperature sensor were used both to check SST values obtained from satellites images and to estimate surface temperature in stations close to the coast. In such cases the estimation of SST was performed using the procedure suggested by Donlon et al. [24] and by Fairall et al. [25]. The data collected by the meteorological station installed on board the research vessel have been used for the calculation of wind stress, according to the Bulk formulae:

$$\tau = \rho_a C_D U^2.$$

In the above formula C$_D$ is the transfer coefficient for momentum, U is the mean wind speed and ρa is the density of the wet air at the surface. The energy transferred through the water column by the wind creates turbulence in the surface layers. Therefore, a wind-mixing index in the upper layer is usually calculated as the cube of wind speed (U^3) [26]. The estimation of wind-mixing index, based on measured mean parameters in each station, was not performed in all the stations of the study area (Figure 1) since the meteorological station did not work continuously for the whole period of each survey. 1./file/Tabx2x.jpgAverage values and confidence intervals of Wind Stress, Wind Mixing and SST in the whole study area.countdino.pattiiamc.cnr.it$_5 6tab$

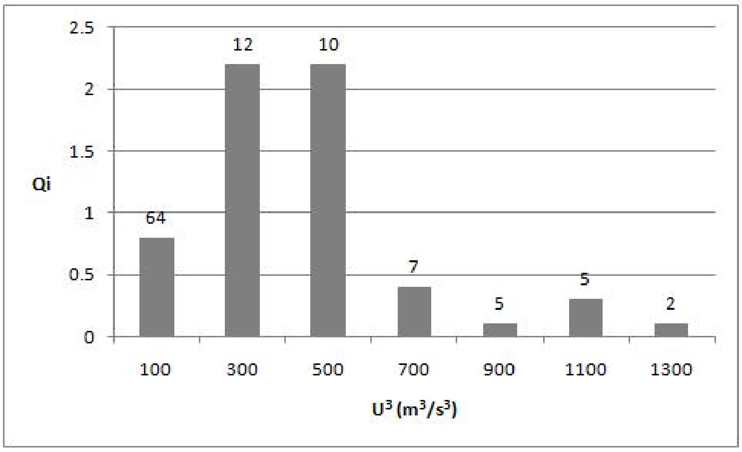


Figure 6: Anchovy quotients of eggs densities (eggs/m^2) vs. Wind-mixing turbulence at stations sampled in the Strait of Sicily (the number of stations over any bar).

3 Results

"Bansic 2006" survey was the only survey carried out in late summer, during the first half of August. This determined a higher average temperature regime over the Strait of Sicily compared with years 2005 and 2004. In both 2005 and 2006 surveys lower SST values were observed in more coastal areas due to the effects of coastal upwelling. During the 2004 survey a colder sea area was located in the north-western part of the study area (Figure 2). The average values and confidence intervals of Wind Stress, Wind Mixing and SST estimated for all the available stations are reported in Table 2. Maps of wind stress for surveys 2004, 2005 and 2006 are given in Figure 3. The main spawning ground during the 2004 survey was located in the coastal waters between Sciacca and Gela; smaller eggs densities were also present in more coastal waters close to Mazara and in the northern part of the Maltese shelf. During the 2005 survey (and, with lower densities, also in 2006 survey), two main spawning areas were observed, respectively located on the Adventure Bank and on the northern part of the Maltese shelf (Figure 4). Quotient analysis demonstrated that the preferred univariate conditions of spawning anchovy were generally well within the range of environmental conditions observed along the Sicilian coast. The main peaks of the quotient index analysis on wind stress data correspond to 0.04 and 0.1 $\mathrm{N{\cdot}m^{-2}}$; higher wind stress values correspond to very small number of eggs stations (Figure 5). The results of the QI analysis performed on wind mixing index showed the optimal spawning conditions in the range 300 – 500 $\mathrm{m^3 \cdot s^{-3}}$ (Figure 6). The quotient index analysis performed on SST (Figure 7) indicates that the

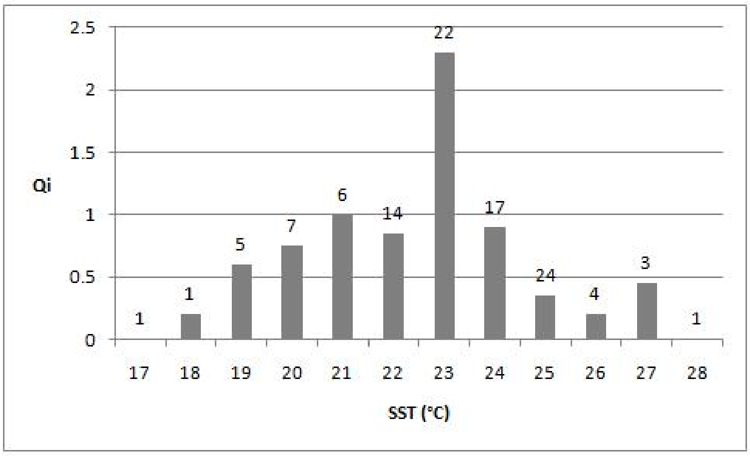
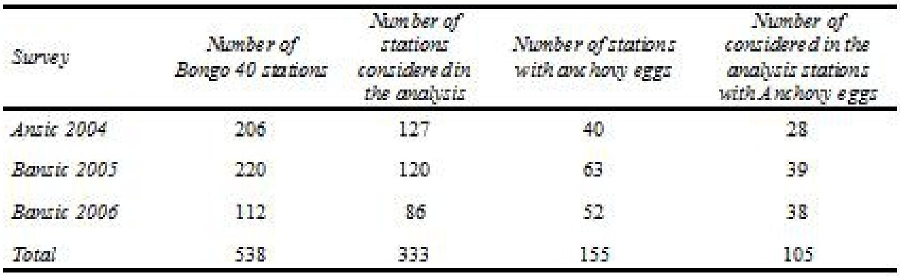


Figure 7: Anchovy quotients of eggs densities (eggs/m^2) vs. SST at stations sampled in the Strait of Sicily (the number of stations over any bar).

spawning habitat of anchovy is characterized by a main peak at 23 °C; such result is in agreement with values reported in literature in the study area, which indicate as optimal temperature for anchovy spawning the range 19 – 23 °C [22, 27].

4 Discussion and conclusions

In the Strait of Sicily from multidisciplinary surveys it was possible to recognize along the southern coast of Sicily several spawning and nursery areas for anchovy. Moreover, these surveys helped to schematize a transport model to explain the distribution patterns of anchovy larvae in relation to the surface circulation [22]. It has been hypothesized that fluctuations of small pelagic fish populations are affected by changes in ocean climate in terms of the extent and spatio-temporal location of suitable spawning habitats [28]. Several biological and environmental variables, like spawning stock biomass, mixed layer depth, turbulence and food availability, appear strictly linked to the selection of spawning locations [29, 14, 13]. From literature studies, anchovy eggs are mainly found in warm waters, with SST between 17 and 23 °C [29] and the spawning habitat of anchovy is characterized by a main temperature peak (17 – 19 °C). In the Strait of Sicily the main peak of the QI analysis is at 23 °C while the spawning habitat temperatures, as suggested in Figure 7, are in the range 21 – 24 °C. In the Central Mediterranean these temperatures are characteristic of the stratified season. In fact, anchovy spawning season begins when temperatures warms at the end of spring and extends throughout the summer [30]. Findings on SST are in agreement with other



Survey	*Number of Bongo 40 stations*	*Number of stations considered in the analysis*	*Number of stations with anchovy eggs*	*Number of considered in the analysis stations with Anchovy eggs*
Ansic 2004	206	127	40	28
Bansic 2005	220	120	63	39
Bansic 2006	112	86	52	38
Total	538	333	155	105

Table 1: Total number of stations in each survey and number of stations with anchovy eggs.

studies accomplished for the Bay of Biscay anchovy, where it was observed that when modelling the potential spawning habitat, the best hydrological predictors were mainly the bottom temperature and the mixed layer depth followed with less extent by the SST [13]. The turbulence, generated by wind or other forces, may enhance the survival of planktonic predators in food-limited environments through an increase in the prey encounter rate. Despite the existence of several literature studies focused on the relationship between water column turbulence and feeding success of fish larvae [31], it is not easy to derive clear relationships and it appears that the stratified nature of the water column, rather than turbulence or wind mixing, better explains the vertical distribution of fish larvae [32]. The seasonal (summer) stratification is not heavily affected by turbulence or wind mixing except in the shallower layers near surface, while the termocline stratification could be affected only by upwelling events driven in the study area by both AIS current and by NW winds. During these events the structure of the water column appear to be much more mixed with lower temperature and higher chlorophyll concentration [22]. One of the main reason for this discrepancy could be related to the assumption that derived indexes on water column turbulence accurately reflect the induced encounter probability. For instance, wind-based estimates of turbulence underestimate the dissipation of kinetic energy in the upper mixed layer and are of limited predictive value [31]. The instabilities produced by wind stress are one of the major causes of sea water mixing. In the present study the wind mixing index appears related to the spawning selection process, even though higher QI values were not strongly supported by the number of stations in the 300 – 500 turbulence classes, which were only 12 and 11 respectively. Indeed, further data may help in the future to better evaluate its importance on the spawning site selection behaviour of anchovy. The upwelling observed along the southern coast of Sicily is mainly driven by the AIS meanders and cyclonic vortices [21]. Moreover, it is well known and documented by bibliography [33, 20] that wind stress may modulate the upwelling strength in the study area. From the biological point of view the upwelling phenomenon tends to modify the temperature regime of surface waters, cooling them below the optimal temperatures for anchovy spawning [22, 27]. Therefore, in this paper the major effects of wind stress were taken into account by analysing both sea water mixing (wind mixing index) and temperature regime. The preliminary results obtained in this work suggest that SST and the wind induced turbulence may be considered proxies of a favourable anchovy spawning sites, where adult specimens may meet optimal environmental features for reducing the mortality of the spawning products. Further investigations and surveys for data collection are necessary in order to understand how the biological variability can be coupled with water column stability/turbulence.

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Identification of a Novel Helitron Transposon in the Genome of Antarctic Fish

E. Cocca[1], M.A. Morescalchi[2], S. De Iorio[3], T. Capriglione[3]
1, Institute of Protein Biochemistry, CNR, Napoli, Italy
2, Department of Life Sciences, Second University of Naples, Caserta, Italy
3, Department of Biological Sciences, University of Napoli “Federico II”, Napoli, Italy
e.cocca@ibp.cnr.it

Abstract

Rolling-circle eukaryotic transposons, known as Helitrons, are widely distributed, from protist to mammal species. Autonomous Helitrons show a peculiar open reading frame, encoding for a polypeptide that contains typical domains for the rolling circle replication: the Rep (rolling-circle replication initiatior) and the DNA helicase domains. These elements are believed to have an important role in the evolution of the host genomes, owing to their frequent capture of host genes, some of which can evolve into novel genes or become essential for Helitron transposition. In this work, we performed a molecular analysis on the suborder of the Notothenioidei, a Perciformes group of species that nowadays dominate the Antarctic waters, due to their remarkable ability in achieving cold-adaptation. We isolated and characterised a novel Helitron element, that we named HeliNoto (8.9 kb), from the genome of the icefish species Chionodraco hamatus, belonging to the Channichthyidae, the most derived family of the Notothenioids. The unique open reading frame obtained was analysed and compared to homologous sequences present in the genome of different species. The distribution of this element among Notothenioids was finally discussed.

1 Introduction

Rolling-circle eukaryotic transposons, known as Helitrons, are widely distributed, from protist to mammal species [1]. Autonomous Helitrons encode the so-called Rep/Hel protein, composed of the replication initiator (Rep) and helicase (Hel) domains. The Rep domain contains three motifs that are conserved in Rep proteins, which are encoded by plasmids and single stranded DNA viruses replicating by rolling circle mechanism (RCR). The Reps, such as transposases, perform both cleavage and ligation of DNA during rolling-circle replication. The Hel domain is a helicase that belongs to the SF1 superfamily of DNA helicases. In some species, Helitrons contain additional domains: apurinic endonuclease, cysteine protease, zinc finger motifs and replication protein A (RPA). Helitron is the only known class of transposons in eukaryotes that integrates into the genome without introducing target site duplications (TSDs). These elements are believed to have an important role in the evolution of the host genomes, owing to their frequent capture of host genes, some of which can evolve into novel genes or become essential for Helitron transposition. Therefore, Helitrons may function as a powerful tool of evolution, by mediat-

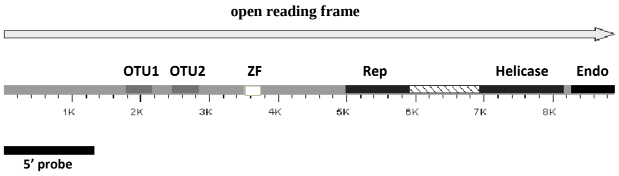

Figure 1: Schematic representation of the HeliNoto gene. The protein domains along the unique open reading frame are: OTU1 and OTU2 (OTU-like cysteine protease domain 1 and 2, respectively), ZF (DNA-binding zinc finger motif), Rep (replication initiator protein), Helicase, Endo (endonuclease domain). The Rep and Helicase domains, with the region in between, constitute the RepHel domain, that is the hallmark of all the Helitrons. The 5' probe segment correspond to the region used for the Southern analysis.

ing duplication, shuffling, and recruitment of host genes. Actually, Teleost fishes are receiving an increasing attention as models for studying the evolutionary impact of the mobile DNA in vertebrates, since their genomes contain many families of mobile elements perhaps actively involved in determining the high biological diversity of these species. In this work, we isolated and characterised a new Helitron element, that we named HeliNoto, from the genome of the icefish species Chionodraco hamatus, belonging to the Channichthyidae, the most derived family of the suborder of the Notothenioidei, a Perciformes group of species that nowadays dominate the Antarctic waters, due to their remarkable ability in achieving cold-adaptation. The unique open reading frame of the HeliNoto gene was analysed and compared to homologous sequences present in the genome of different organisms. Then we performed a molecular analysis on the presence of this transposon element in the genome of Notothenioids.

2 HeliNoto gene

We isolated and characterised a new Helitron element, that we named HeliNoto, from the genome of the icefish species *C. hamatus*. HeliNoto corresponds to a genomic fragment of 8.9 kb, isolated by PCR amplification from the DNA of *C. hamatus* using primers deduced from the alignment of fish Helitrons. It consists of a unique open reading frame that lacks of introns and contains both Rep and helicase domains, as well as the apurinic-apyrimidinic-like endonuclease domain (Figure 1). Moreover, two consecutive OTU-like cysteine protease domains and a potential DNA-binding zinc finger motif (ZF) are present in the 5' portion of the transposon. The 5' and 3' extremities of HeliNoto gene could not be determined, due to the lack of informative sequences to design amplification primers for these regions. The nucleotide sequence of the HeliNoto gene, as well as its overall structure, are very similar to those already described for one of the Helitrons of Danio rerio [2] and the only Helitron described from Xiphoporus maculatus [3].

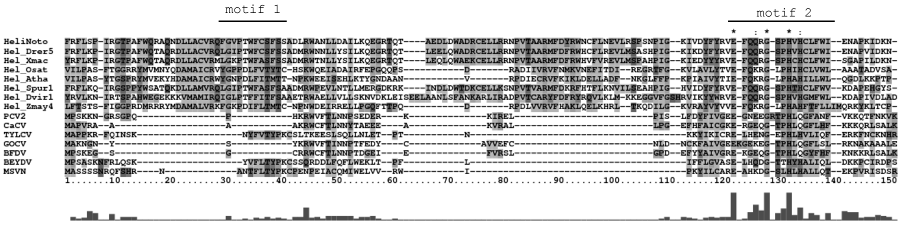

Figure 2: Alignment of Helitron Rep domains with Rep proteins. The sequences aligned with their Data Base accession number are: CaCV (Q912W1), BFDV (Q9YUD3), GOCV (Q91EK3), PCV2 (Q8BB16), BEYDV (O$_3$9522), MSVN (P14978), TYLCV (P27260), Hel_Drer5 (BK001161), Hel_Xmac (Q38JW4), Hel_Spur1 (XP790598), Hel_Dvir1 [Helitron-1_DVir, Repbase Reports 7(3), 130], Hel_Osat (BAC84865), Hel_Atha (AAD25621), Hel_Zmay4 [Helitron-4_ZM, Repbase Reports 8(7), 706]. The sequences correspond to the N-terminal portion of the Rep protein, containing two of the three conserved motifs, that are shown in the figure.

3 Rep domain

The HeliNoto gene from *C. hamatus* encodes a Rep domain consisting of 342 a.a., whose sequence has been compared to those of Rep domains from other Helitrons and of Rep proteins from other RCR replicons, such as circoviruses and geminiviruses. In a hypothetical model of the Helitron transposition [1], based on the replication of bacterial RC transposons, the Rep protein binds to both the 5'-terminus of the donor Helitron and the recipient target site DNA , where it catalyses two cleavage reactions, causing a double strand-transfer reaction. As a result, an ssDNA copy of the donor Helitron is integrated in the target site and can be replicated passively by the host. An alignment of Rep domains is shown in Figure 2, where sequences from Helitrons of fish (D. rerio - Hel_Drer5, X. maculatus - Hel_Xmac, *C. hamatus* - HeliNoto), sea urchin (Strongylocentrotus purpuratus – Hel_Spur1), fruit fly (Drosophila virilis – Hel_Dvir1), plants (Oryza sativa – Hel_Osat, Arabidopsis thaliana – Hel_Atha, Zea mays – Hel_Zmay4) are compared to the Rep proteins from circoviruses (PCV2, porcine c.v., GOCV, goose c.v., CaCV, canary c.v., BFDV, beak and feather disease c.v.), and geminiviruses (TYLCV, tomato g.v., MSVN, maize g.v., BEYDV, bean g.v.). The Rep domain of HeliNoto gene presents the three typical motifs of the Rep proteins [4, 5], but the sequence shown in Figure 2 covers only the motifs 1 and 2. The sequence similarity among the different protein groups suggests a viral evolutionary origin for the Helitron Rep domain.

4 Helicase domain

The sequence of the helicase domain encoded by the HeliNoto gene (410 a.a.) has been compared to those of helicase domains from other Helitrons and to those of helicase proteins from several organisms, belonging to the SF1 superfamily [6]. In Figure 3 the HeliNoto

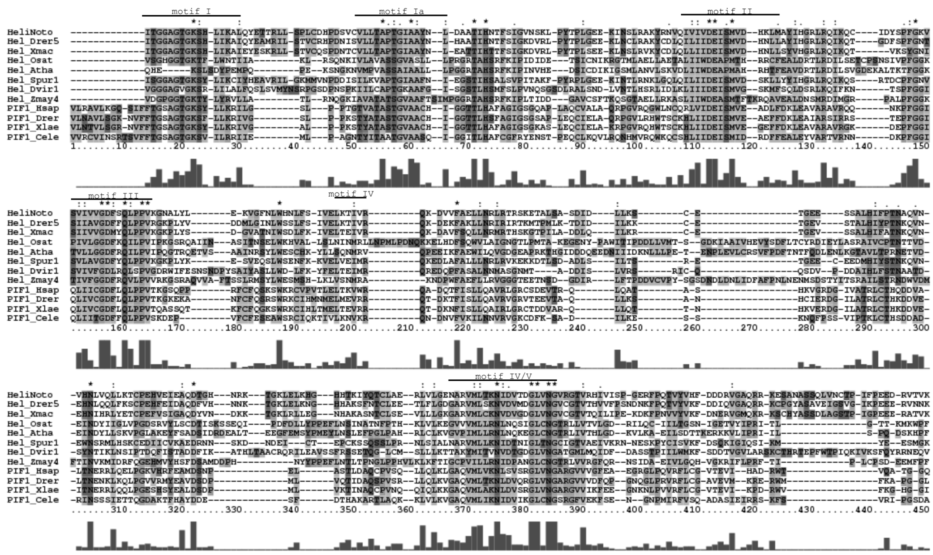

Figure 3: Alignment of Helitron helicase domains with cellular PIF1 helicases from several organisms. The sequences and their accession number are: Hel_Drer5 (BK001161), Hel_Xmac (Q38JW4), Hel_Osat (BAC84865), Hel_Atha (AAD25621), Hel_Spur1 (XP790598), Hel_Dvir1 [Helitron-1_DVir, Repbase Reports 7(3), 130], Hel_Zmay4 [Helitron-4_ZM, Repbase Reports 8(7), 706], PIF1_Hsap (Q9H611), PIF1_Drer (Q7ZV90), PIF1_Xlae (Q0R4F1), PIF1_Cele (Q9BL90). Six of eight of the conserved motifs of helicases are indicated.

helicase domain is aligned to homologous sequences from other Helitrons of fish (D. rerio - Hel_Drer5, X. maculatus - Hel_Xmac), sea urchin (Strongylocentrotus purpuratus – Hel_Spur1), fruit fly (Drosophila virilis – Hel_Dvir1), plants (Oryza sativa – Hel_Osat, Arabidopsis thaliana – Hel_Atha, Zea mays – Hel_Zmay4), and to PIF1 helicases from several organisms (Caenorhabditis elegans - PIF1_Cele, D. rerio – PIF1_Dre, Xenopus laevis – PIF1_Xlae, Homo sapiens – PIF1_Hsap). As the PIF1 helicases from which they originated, the helicase domain from Helitrons present eight conserved motifs, six of which are indicated in Figure 3. PIF1-like helicases are found in diverse organisms [7]. These enzymes seem to play a very important role for the cell life promoting genetic stability: they mediate the inhibition of telomerase by suppressing telomerase-mediated healing of double-strand breaks [8]. We submitted the HeliNoto helicase sequence to the Phyre (Protein Homology/analogY Recognition Engine) webserver to get a structure prediction for this domain. The 3D model shown in Figure 4 is the best result of this analysis, obtained comparing the query sequence to those of homologue proteins whith known 3D structure. The model of HeliNoto helicase domain has been ob-

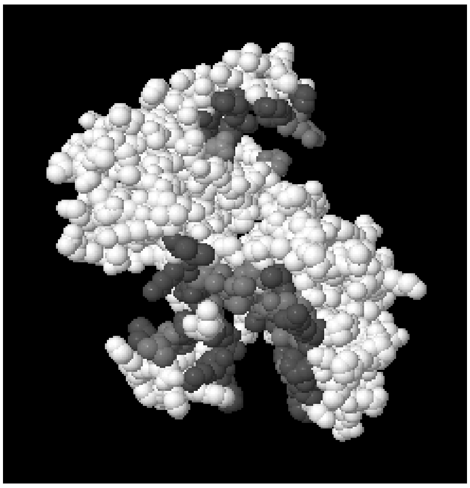

Figure 4: A 3D model of HeliNoto helicase domain obtained using as template the 3D structure of the helicase RecBCD from E. coli. This model is given with a prevision of functional sites, shown as grey-colored residues.

tained using as template the 3D structure of the helicase RecBCD from E. coli. This model is given with a prevision of functional sites (grey-colored residues), indicating a good likelihood for a functional 3D structure of the HeliNoto helicase. In the model of the Helitron transposition, the DNA helicase facilitates the unwinding of the DNA duplex formed by the action of the Rep domain, enabling leading-strand DNA synthesis by the host DNA polymerase [1].

5 Endonuclease domain

The Helitrons of teleost fishes, cephalochordates, urochordates and echinoderms encode apurinic-apyrimidinic (AP)-like endonuclease [2]. The acquisition of the Endo domain by the primordial Helitron is an example of the primary characteristic of this family of transposons: the capture of host genes, some of which can become essential for the Helitron transposition mechanism. The endonuclease domain of HeliNoto (213 a.a., uncomplete at the C-ter) has been compared to homologues sequences from other Helitrons and from several endonuclease proteins. BLAST searches indicated that the Helitron Endo domain may have been derived from the Endo domain of non-LTR (LINE-like) retrotransposons [9, 10], that belongs to the AP family of endonucleases. In the alignment of Figure 5 are indicated the seven conserved AP endonuclease domains [11], as well as the three putative catalytic sites, for the sequences analysed (Endo domain from Helitrons: HeliNoto, D. rerio - Hel_Drer5, X. maculatus - Hel_Xmac, S. purpuratus – Hel_Spur1, Endo domain from non-LTR elements of the CR1, Jockey and L1 clades: Homo sapiens – ApexHs, Gallus gallus - CR1Gg,

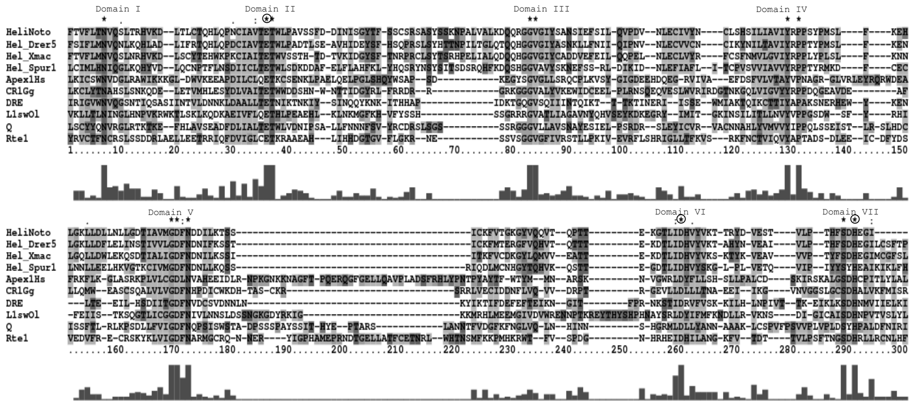

Figure 5: The endonuclease domain of Helitrons have been compared to homologues sequences from non-LTR mobile elements. The sequences aligned and their accession numbers are the following: Hel_Drer5 (BK001161), Hel_Xmac (Q38JW4), Hel_Spur1 (XP790598), ApexHs (P27695), CR1Gg (U88211), DRE (X57034), L1swOl (AF055640), Q (U03849), Rte1(AF054983). The seven conserved AP endonuclease domains, as well as the three putative catalytic sites (circled asterisks), are also shown.

Dictyostelium discoideum - DRE, Orizia latipes - L1swOl, Anopheles gambiae - Q, Caenorhabditis elegans - Rte1). The high level of sequence conservation suggests that the Endo domain of HeliNoto transposon may still retain its functionality.

6 OTU-like cysteine protease domain

In the 5' half of the HeliNoto gene, two OTU-like cysteine protease domains were identified. These domains have been found so far only in one family of Helitrons from fish (where they appear as duplicates), lancelet and sea urchin [3]. Their sequences are related to the ovarian tumor protein of Drosophila (OTU) [12]. Their activity during the transposition might be the cleavage of the large Helitron precursor polyprotein into functional domains, similarly to the proteases encoded by retrovirus and LTR retrotransposons. Alternatively, they might participate in the degradation of the RepHel protein through the ubiquitination pathway [13]. The sequences of the two OTU-like domains have been compared to those of their homologues from other Helitrons and to those of proteins belonging to this family of cystein proteases, identified in a variety of eukaryotic cellular genes and viruses. The phylogenetic tree (1000 bootstrap replicates) in Figure 6, obtained using two sequences from iridescent viruses (Tiri and IRV6) as the outgroups, shows a well-defined cluster for the Helitrons OTU domains, containing two distinctive sub-groups corresponding to the OTU domains of type "1" and to those of type "2". These domains seem to be strictly related to non-viral sequences from nematodes (Bmal, Cele1, Cele2, and Cbri). This result suggests that these He-

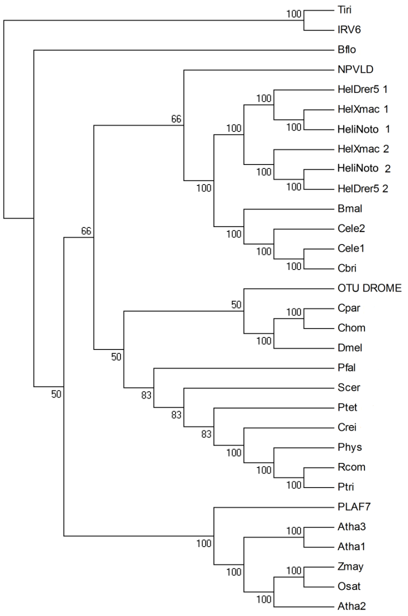

Figure 6: Phylogenetic analysis of OTU-like proteases domains. The tree was calculated by the maximum likelihood method using the LG+I+G+F amino acid substitution model [parameter: alpha+p-inv (+I+G) = 0.95]. The sequences from iridescent viruses (Tiri and IRV6) are considered as the outgroups. The numbers at the branches represent the confidence limits computed by the bootstrap procedure (1000 replicates). Internal nodes with bootstrap values under 50% are not shown.

litron domains might be derived from a cellular gene. Other sequences fall in different clusters, phylogenetically more divergent from the Helitron OTU domains, such as the cluster containing sequences of fruit fly (OTU DROME and Dmel) and of Cryptosporidium parasites (Cpar and Chom), the cluster containing sequences from protozoan cells (Pfal, Ptet, Crei), yeast (Scer) and plants (Phys, Rcom, Ptri), the cluster containing another sequence from P. falciparum (FPL7) and several OTU domains from plants (Atha1, Atha2, Atha3, Zmay, Osat).

7 Distribution of HeliNoto gene among Notothenioids

The distribution of the HeliNoto element in the *C. hamatus* genome, as well as its presence in the DNA of other Notothenioidei, have been analysed. A DIG-labelled probe corresponding to a 5’-portion of HeliNoto (1307 bp, shown in the figure of the gene as “5’ probe”), was hybridised against genomic DNA from *C. hamatus* and from other 12 notothenioid fishes (listed in Table 1), belonging to five of the eight families

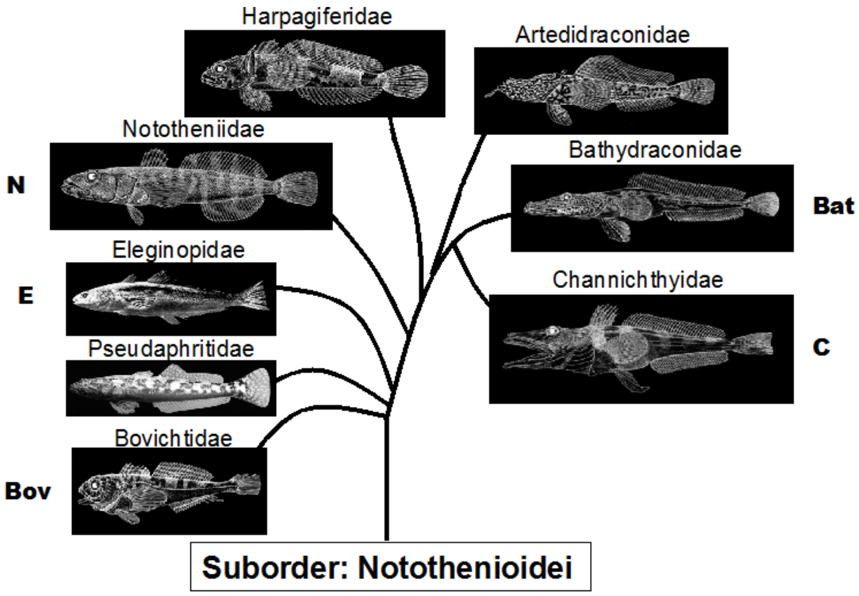

Figure 7: Cladogram of the eight families of the suborder of Notothenioidei. The abbreviations for the five families analysed with Southern hybridisation are indicated with bold characters.

of this suborder (see the phylogram of Figure 7 and Figure 8). The probe was chosen because it covers a region that is distinctive of this family of Helitrons. As it was expected, the genomes of *C. hamatus* and of the other two icefish analysed (Chaenocephalus aceratus and C. rastrospinosus) present a similar and very strong hybridisation pattern, with a major band of about 8.6 kb that appears in most of the DNAs. Good hybridisation signals are visible for all of the six nototheniid species analysed (indicated by an “N”), as well as for the two bathydraconids (“Bat”), that suggest the presence of HeliNoto-related sequences in the genome of the fish belonging to these two families. Interestingly, there are not evident signals for the two sub-Antarctic species: Bovichtus diacanthus (“Bov”) and Eleginops maclovinus (the unique member of Eleginopidae, “E”). Assuming for the Helitrons a mode of vertical transmission, and considering the evolutionary history of notothenioids, this result indicates that the HeliNoto element might have been lost by the genome of these two species. On the other hand, HeliNoto insertion might have occurred later on, during Antarctic species radiation, and might have played a role in their diversification. *C. hamatus* HeliNoto, with its apparently uncorrupted open reading frame, seems to be an autonomous Helitron that might be still active in the icefish genome. The structural analysis of its proteic domains, that appear to be very well conserved in comparison to their functional homologues, offers further elements that seem to support this hypothesis.

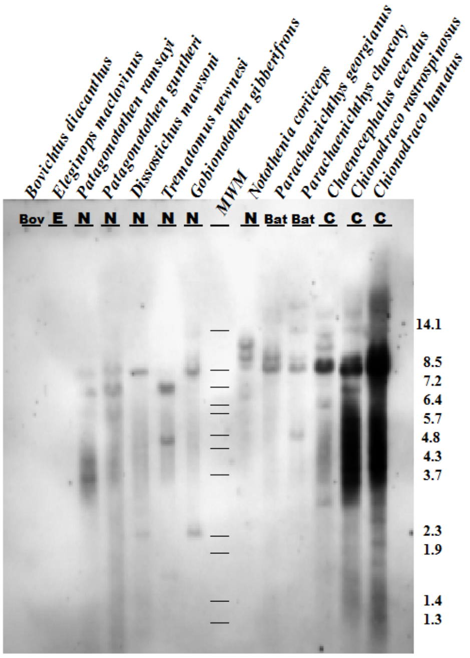

Figure 8: Genomic DNAs (10 μg for each lane, digested with Bam HI) from fish belonging to five families of Notothenioids were analysed for the presence of HeliNoto gene using as probe the fragment shown in Figure 1. The family and the species of the Notothenioids analysed are listed in Table 1. The sizes (kb) of DNA standards are indicated.

Family	**Species**
Bovichthidae (Bov)	*Bovichtus diacanthus* (Bd)
Eleginopidae (Ele)	*Eleginops maclovinus* (Em)
Nototheniidae (Not)	*Patagonotothen ramsayi* (Pr) *Patagonotothen guntheri* (Pg) *Dissostichus mawsoni* (Dm) *Trematomus newnesi* (Tn) *Gobionotothen gibberifrons* (Gg) *Notothenia coriiceps* (Nc)
Bathydraconidae (Bat)	*Parachaenichthys georgianus* (Pg) *Parachaenichthys charcoty* (Pc)
Channichthyidae (Cha)	*Chaenocephalus aceratus* (Ca) *Chionodraco rastrospinosus* (Cr) *Chionodraco hamatus* (Ch)

Table 1: Notothenioid species analysed in the Southern blot.

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Mucilage Monitoring Program in the Northern Adriatic Sea (Oceanographic Parameters)

F. Grilli, E. Paschini, M. Marini
Institute of Marine Sciences, CNR, Ancona, Italy
f.grilli@ismar.cnr.it

Abstract

A research program MAT, supported by the Italian Ministry of the Environment and Protection of the Territory, has been devoted to monitor for four years the environmental conditions of the northern Adriatic Sea, with the main aim to study the mucilage phenomenon. These phenomena increased since the late eighties. Meteorological conditions, circulation patterns and water column stratification can affect the development and spreading of mucilage. Starting from June 1999, a monthly monitoring along three transects located in the northern Adriatic was carried out for three years. CTD casts were performed from the Italian to the Croatian coast, with water column sampling for a subset of stations. Some unusual oceanographic situations, heating of the sea surface in May–June and domination of eastwards transport of freshened waters formed in the Po Delta area, were observed during the investigated period in the years with more intense mucilage events (2000 and 2002).

1 Introduction

The northern Adriatic (NA) is a very shallow continental sub-basin extending approximately to the 100 m depth and characterized by a mean depth of about 35 m and by strong river runoff [2]. This sub-basin identifies distinct physical [3] and biological [4] oceanographic characteristics. The western coastal area has a different dynamical regime compared to the offshore zone. The coastal area north of the Po delta is dominated by the fresh water plumes of many rivers, while the circulation south of the Po delta is dominated by a boundary intensified current, the Western Adriatic Coastal Current (WACC) which is a wind and fresh water input driven current. The southward spreading of this surface water mass along the Italian shore is compensate by a northward current along the eastern coast, the Eastern Adriatic Current [5, 6, 3, 7]. The NA is an important shelf area where the occurrence of critical environmental issues such as extensive algal blooms, sea water anoxia and in particular mucilage events may affect the economic activities of the area. The mucilage phenomenon is represented by the appearance of suspended gelatinous material in marine waters horizontally transported by currents and subjected to vertical movements due to water temperature and salinity changes. There is a general consensus that the mucilage phenomenon is generated by synergic combination of several factors [8]. Among these, significant changes of nutrient concentration can increase the phytoplankton excretion rate of polysaccharide mucus that comprises the matrix of the mucilaginous material [9]. Moreover various perturbation

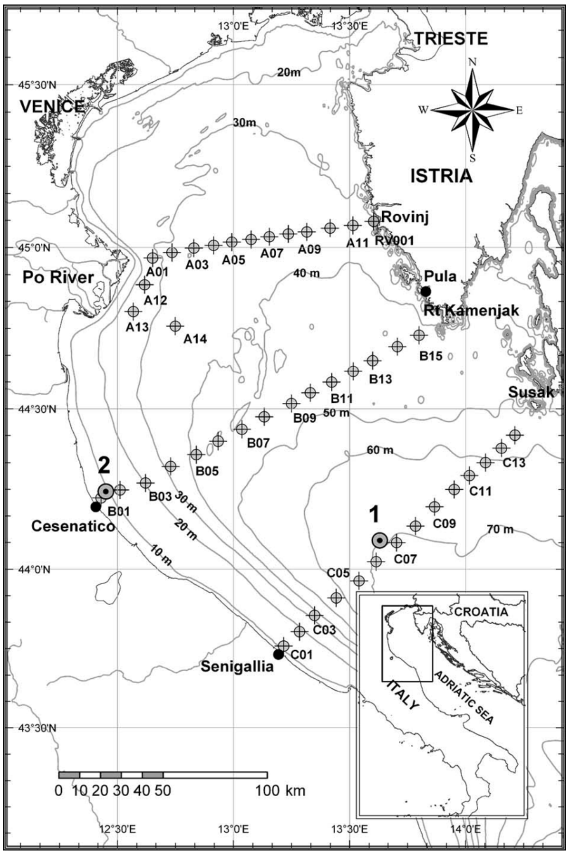
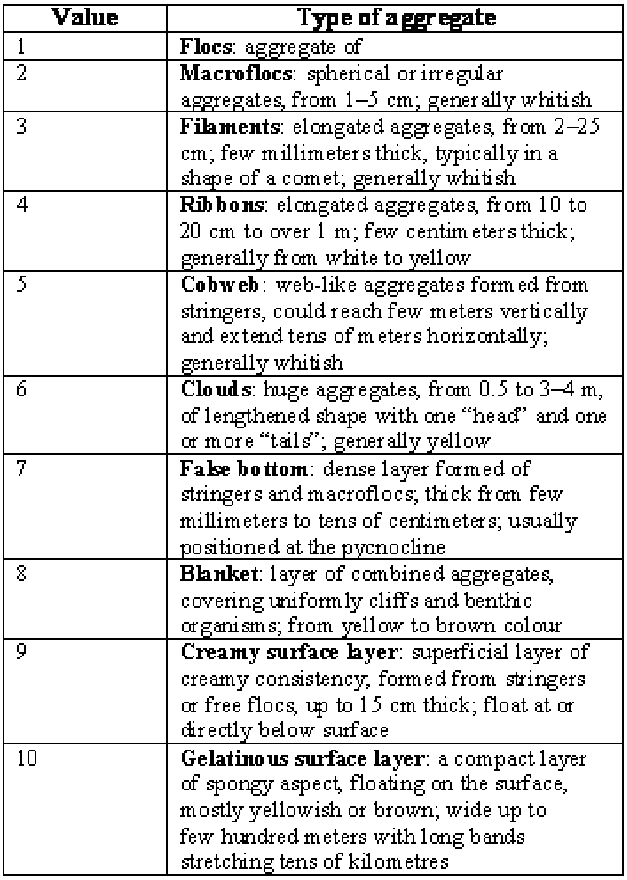


Figure 1: The investigated area with sampling station positions along the three transects with locations of the 2 moorings denoted by 1 and 2 (from [1]).

in the organic carbon flow may favour the production and accumulation of mucilage [10] while particular climatic and oceanographic conditions seem to play an important role in triggering the phenomenon [11]. Since 1988 in the NA the production of mucilaginous material has occurred with increased frequency and intensity causing negative consequences for the ecosystem, the tourism industry and the fishery by hampering work with nets. For these reasons a research program (“Monitoring and study of processes of mucilage formation in the Adriatic and Tyrrhenian Seas”-MAT) has been devoted to monitor for four years (June 1999 - May 2003) the environmental conditions of the NA and Tyrrhenian Sea with the aim of evaluating the importance of meteorological conditions, circulation patterns and water column stratification on the aggregation and dispersion process and estimating the possible consequences of the mucilage impact on marine organisms and communities. The scien-



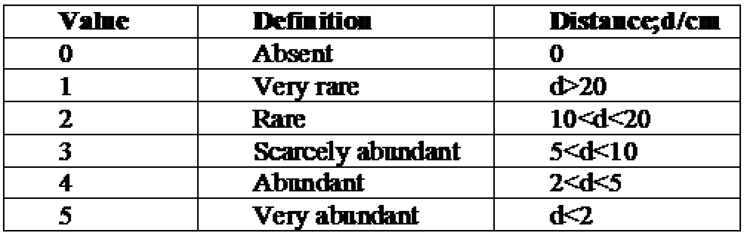
Value	Type of aggregate
1	**Flocs**: aggregate of
2	**Macroflocs**: spherical or irregular aggregates, from 1–5 cm; generally whitish
3	**Filaments**: elongated aggregates, from 2–25 cm; few millimeters thick, typically in a shape of a comet; generally whitish
4	**Ribbons**: elongated aggregates, from 10 to 20 cm to over 1 m; few centimeters thick; generally from white to yellow
5	**Cobweb**: web-like aggregates formed from stringers, could reach few meters vertically and extend tens of meters horizontally; generally whitish
6	**Clouds**: huge aggregates, from 0.5 to 3–4 m, of lengthened shape with one "head" and one or more "tails"; generally yellow
7	**False bottom**: dense layer formed of stringers and macroflocs; thick from few millimeters to tens of centimeters; usually positioned at the pycnocline
8	**Blanket**: layer of combined aggregates, covering uniformly cliffs and benthic organisms; from yellow to brown colour
9	**Creamy surface layer**: superficial layer of creamy consistency, formed from stringers or free flocs, up to 15 cm thick; float at or directly below surface
10	**Gelatinous surface layer**: a compact layer of spongy aspect, floating on the surface, mostly yellowish or brown; wide up to few hundred meters with long bands stretching tens of kilometres

Table 1: Description of the aggregate types observed in the northern Adriatic Sea.

tific activities were performed by 150 scientists and technicians belonging to 24 Italian and two Croatian institutions. During the first three years (June 1999 - July 2002) oceanographic cruises were monthly performed in the NA. In addition, several laboratory and in situ experiments were conducted to explain mechanisms and evaluate processes observed in the field. The meteorological, physical, chemical, biogeochemical, planktonic, benthonic parameters and visible mucilaginous aggregates have been monitored. In this paper some of the results obtained in the framework of the project concerning physical and chemical oceanographic conditions during the mucilage events and the spatial distribution of the mucilaginous aggregates are analysed.

2 Methods

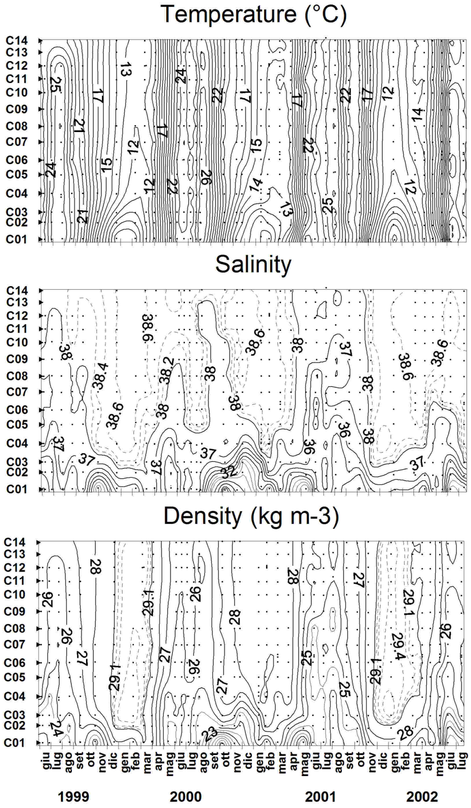
Enviromental monitoring was taken place over a network of 43 stations, spaced by



Value	Definition	Distance;d/cm
0	Absent	0
1	Very rare	$d>20$
2	Rare	$10<d<20$
3	Scarcely abundant	$5<d<10$
4	Abundant	$2<d<5$
5	Very abundant	$d<2$

Table 2: Scale for the semi-quantitative estimation of flocs abundance.

about 10 km, situated along three transects in the NA, (A) Po Delta-Rovinj (Rovigno), (B) Cesenatico-Cape Kamenjak (Promontore), and (C) Senigallia-Susak Island (Sansego; Figure 1) where monthly, in the period June 1999 - July 2002 during 39 cruises, oceanographic data were collected. The Conductivity-Temperature-Depth (CTD) data were collected with a SeaBird Electronic and processed according to UNESCO [12] standards, obtaining pressure-averaged data (0.5 db interval). During the period of study, two currentmeter moorings were deployed (Figure 1 in the NA). The first one (station 1) was located mid-way along transect C (bottom depth 68 m), and the second (station 2) 4.2 km offshore from Cesenatico (bottom depth 9 m). The sampling interval was 10 min. Recovered data were qualitatively checked, and hourly and daily averages were calculated after removing tidal components. Nutrient concentrations (ammonium—NH_4, nitrite—NO_2, nitrate— NO_3, orthophosphate—PO_4 and orthosilicate—SiO_4,) were determined at 20 stations (bottom depths 9–71 m) along all three transects. Water samples were collected with 5 L Niskin bottles at 2–5 oceanographic depths (surface, 5, 10, 20, 30, 40 and 2 m above the bottom), depending on station sonic depths. The samples were filtered (HA Millipore, 0.45 μm) and stored at -22 °C in polyethylene vials (transect B and C), or analysed immediately aboard after collection (transect A). Dissolved inorganic nitrogen (DIN) was calculated as the sum of the NH4, NO_2 and NO_3 concentrations. The nutrient concentrations and geostrophic velocities (calculated using a standard dynamical method [13]) estimated across transects A, B and C were interpolated on a regular grid (distances between grid points: 2 km in horizontal and 2 m in vertical) using the Kriging method [14]. Nutrient transports were computed by multiplying nutrient concentration by water transport (calculated by integrating geostrophic velocities over the cross-sectional area). Net transports represent the difference between northward (positive values) and southward transports (negative values). The distribution of visible aggregations in the water column was monitored using underwater video cameras. The identification of the typology of the aggregates (Table 1) was based on the morphologic differences as defined by Stachowitsch et al. [15] while the abundance (Table 2) was determined on a semi-quantitative scale from 0 to 5 on the basis of the average distance between aggregates. The observations along transect A are presented for stations A05, A07 and



Temperature (°C)

Salinity

Density (kg m-3)

Figure 2: Surface temperature (°C), salinity and density (kg$\cdot\mathrm{m}^{-3}$) along the transect C from June 1999 to July 2002.

A09 only because of the difficulties in processing video images due to low water transparency at stations closer to the Po River delta. The data on transects B and C are not presented as for transect A due to differences in the semiquantitative evaluation and only data on aggregate type could be compared to observations from transect A.

3 Results

3.1 Oceanographic conditions: geostrophic circulation and nutrients transport

The shallow NA is characterized by fluctuations of oceanographic and chemical conditions, mainly due to atmospheric forcing, very variable and complex circulation and freshwater nutrient contributions [18, 6, 19, 20]. Relevant oceanographic process such as stratification dynamics of

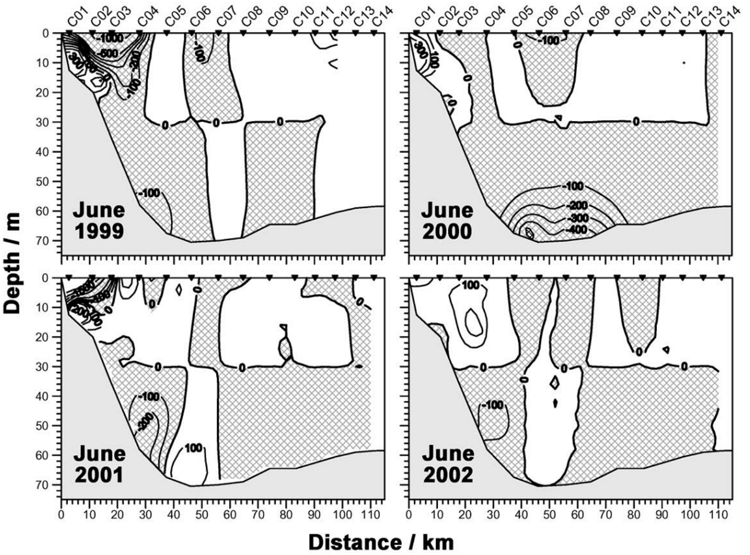


Figure 3: Vertical distribution of DIN net transport (mol·s^{-1}) at transect C in June 1999, 2000, 2001 and 2002. Negative values (shaded area) indicate southward transport, and positive values (white area) northward transport (from [16]).

the water column were studied in detail to verify the importance of their role in the formation, persistence and dispersion of the mucilaginous aggregates. Some unusual oceanographic situations were observed during the investigated period in the years with more intense mucilage events (2000, 2002 and for a short period in July 2001) in the NA. In particular the thermoaline properties (temperature, salinity and dansity) along three transects (only transect C shown, Figure 2) were analysed to identify recurrent environmental conditions that might have favoured the mucilage events. During spring-summer 2000 and 2002, surface temperature were higher than during corresponding period of 1999 and 2001. Surface salinity distribution showed the influence of freshwater discharge mainly from the Po River on all transects, although seasonally very variable and with decreasing intensity southwards. Surface salinity minima along the transect C (Figure 2) were recorded in autumn 2000 and winter 2001 close to western coast when the Po River flow rate was exceptionally high. During summer 2001 values 37-38 were measured in the eastern part and low values of density (23-24 kg·m^{-3}) were measured in the western part of the transect C. Surface density maxima were recorded in winter 2002. The nutrients role in the development of the mucilage phenomenon, suggested by laboratory and controlled in situ experiments, could become critical in particular circulation patterns that can be established in the investigated area. Nutrient concentrations were generally much more variable in lower salinity and oxygenated surface waters, in which the mucilage phenomenon primarily developed, than in other water types of the northern Adriatic [21]. During the mucilage events (2000, 2001 and 2002) the nutrients transport (in particular DIN, Figure 3) on the westernmost transect C was principally northward indicating a semi-enclosed circulation of a major part of the nutrient-rich water that forms in the area off the Po Delta and along the western coastal belt within the investigated area [22]. This circulation pattern mainly coincided with a very weak or even reversed WACC, the principal mechanism of nutrient export from the northern Adriatic.

3.2 Typology and distribution of mucilaginous aggregates

Mucilaginous aggregates formed during spring/summer of 2000, 2002 and for a briefer period in July 2001, but were absent during the first year of investigation (1999). The distribution of visible aggregates on transect A according to Table 2 is presented in Figure 4. Smaller aggregates (flocs; $<$ 1 cm) were present through the entire investigated period. The maximal particle concentration was roughly correlated with increases of the Po River flow, stimulating phytoplankton growth [1]. In general the flocs appearance was more accentuated when the circulation was weak and transversal currents were favoured [16]. In these conditions the residence time of waters originated from the Po River in the NA may be longer (e.g. in 2000 and 2002). The flocs appearance was also more accentuated in the western part of the transect, where they accumulated in a marked pycnocline layer. Macroflocs (1–5 cm) and filaments (2–25 cm) mainly appeared after a maximum abundance of flocs. The first sign of the upcoming mucilage aggregation were noted at the end of May 2000 (29-31) and in the last 10 days of May 2002 when a rise in the abundance of filaments (up to 50 cm long) was observed in the entire water column together with the maximal abundances of flocs [17]. This indicates that during the mucilage events all types of aggregates were formed. The appearance of larger aggregates (ribbons and clouds) as well as complex structures (cobwebs and false bottoms) were again mainly located in the upper water column, below the pycnocline. Their accumulation on the surface was dependent on oceanographic conditions such as freshwater discharge, temperature and turbulence.

4 Conclusion

The results of Project MAT represented a significant contribution to the knowledge of the mucilage phenomenon. The monitoring activities aimed at recognising the inception of the phenomenon in order to activate system of prevention and control at an early stage of its development. Extremely important have been investigations on the oceanographic process, principally water dynamics and nutrient fluxes, to explain mechanisms and evaluate processes observed in the field as well as to estimate possible consequences of the formation, persistence and dispersion of the mucilaginous aggregates.

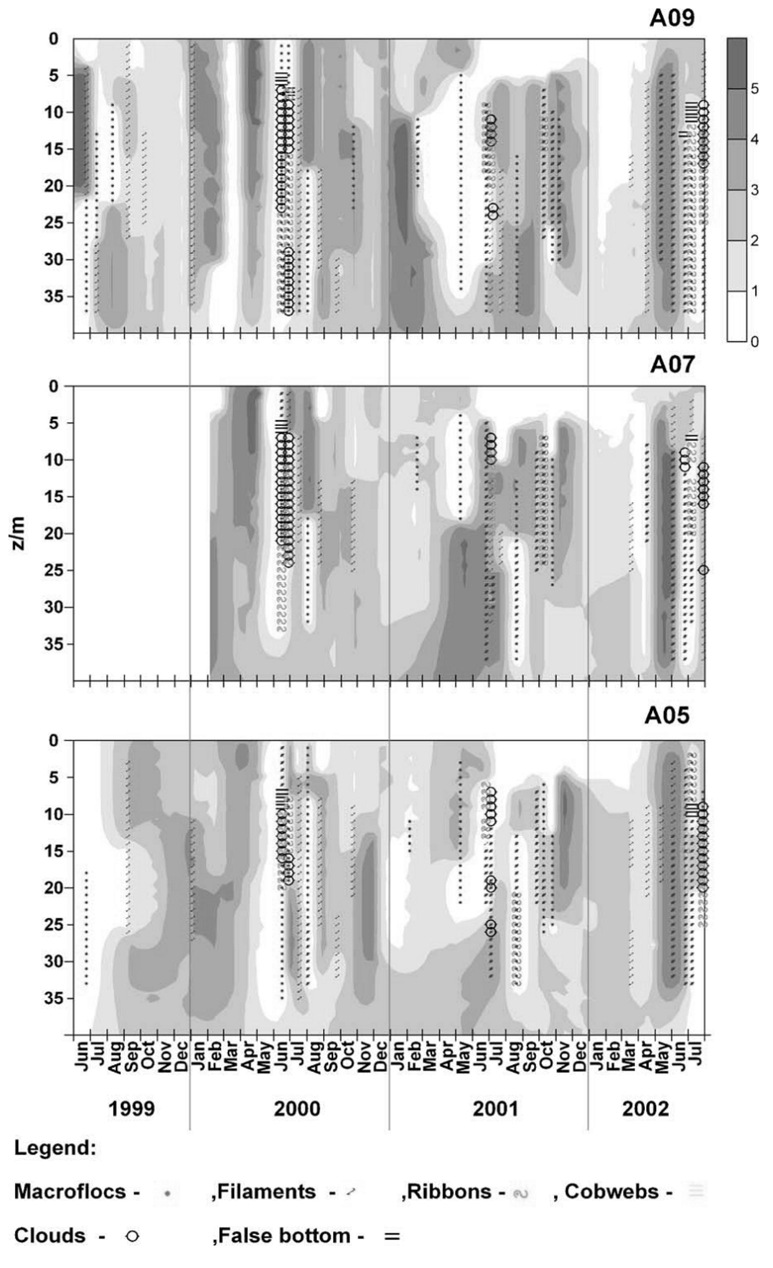


Figure 4: Distribution of aggregates in the water column at stations A05, A07 and A09 from June to July 2002. The scale (0-5) represents the abundance of flocs as described in Table 1 (from [17]).

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The Exploration of Deep Hypersaline Anoxic Basins of the Easterm Mediterranean Sea

G. Bortoluzzi[1], M. Borghini[2], V. La Cono[3], L. Genovese[3], F. Foraci[4], A. Polonia[1], F. Riminucci[1], G. Marozzi[1], M. Yakimov[3]
1, Institute of Marine Sciences, CNR, Bologna, Italy
2, Institute of Marine Sciences, CNR, Pozzuolo di Lerici (SP), Italy
3, Institute for Coastal Marine Environment, CNR, Messina, Italy
4, Institute for Coastal Marine Environment, CNR, Mazara del Vallo (TP), Italy

g.bortoluzzi@ismar.cnr.it

Abstract

The world's deepest and most hypersaline anoxic basins (DHAB) are located on peculiar depressions of the eastern Mediterranean Sea, SW Greece, and their formation appears to be controlled by regional tectonics and the dissolution of halite/magnesite of Messinian evaporites. The interface between normal and dense water (5/10 times sea water) is generally deeper than 3000m, brine thickness can be hundred meters, and some of the basins show intense microbial activity at the interfaces. The presence of life in these very hostile environments challenges biologists and genetists, and gives the chance for the study of similar potentially life-containing sites in outer planets. Five of these DHABs, discovered since '80 (Tyro, Bannock, Discovery, L'Atalante, Urania), have been extensively studied from geological, biogeochemical, oceanographical points of view. Nontheless, the presence of other DAHBs was postulated in the area, and the Medee basin, SW Crete, was discovered by a French/Japanese EU HERMES 2006 cruise. During two R/V Urania cruises (September 2008,2009), directed by IAMC Messina (EU-ESF Projects MIDDLE-MAMBA), we investigated possible DHAB candidates revealed by morphobathymetric analysis. CHIRP data presented the same acoustical sharp reflection at the sea water/brine interface as in other known basins, and the existence of anoxic brine pools was proved by CTD, water and bottom sampling. Some data and results are discussed, and the importance of studying extreme environments is stressed.

1 Introduction and setting

The Deep Hypersaline Anoxic Lakes (DHAB) of the Eastern Mediterranean Sea (Figure 1), Tyro, Bannock, Urania, L'Atalante, Discovery, Medee [1, 2, 3, 4], are produced by the exhumation and dissolution of Messinian salt strata [5], fluid migration and precipitate confinement in basins and depressions at water depth >3000 m [6, 7, 8, 9]. These features are widespread in the inner portion of the Mediterranean ridge accretionary complex, at the transition between the prism and the continental backstop of the Eurasian and Aegean plates to the NE; active shear is localized at this contact and a major dextral flower structure has been described as the first order structural control on massive mud expulsion [10]. The Africa plate subducts along the Hellenic arc, and shallow-focus earthquakes occur on faults in the region (Figure 1); recent instrumental data recorded magnitudes up to ∼7.2; historical events are thought to have had M8+, and produced catastrophic tsunamis. The back-arc volcanism on the Aegean Sea is also a source of tsunamis, as documented for the ∼1600 BC explosion of Santorini. The extreme oceanographic and chemical properties of DHABs' are characterized by sharp interfaces at the halocline (density, salinity, temperature, oxygen), varying percentages of Na, K, Cl, Mg, SO_4 ions, and salinities up to 5-10 times higher than that of seawater. Temperature is normally some degrees higher, except for the Urania Basin, where values of 45 °C were reported from the pit on the seafloor [11]. The DHAB interface is detected by extended-range CTD sensors, and, being an equipotential surfaces, is shown as a perfectly straight line on reflection seismic profiles.

Cores collected from DHABs' revealed that recent sedimentation is mainly related to homogeneous mud interbedded by sapropels and coarser materials. Sediment recovered near mud volcanoes (Urania and Medee) can be made up entirely by mud flows. Hemipelagic, oxic conditions are found outside the basins, where past anoxic episodes can be intercalated to the normal sedimentation. Gypsum precipitates with different morphologies and dimensions are present in sediments of all the DHABs', and high concentration of sulphates and methane can be found in the brines, e.g. on Urania.

At the sea water/brine interface, that can be as thick as 1m, microbial diversity is incredibly rich; microbes largely live by sulphide oxidation, surviving independently of sunlight and oxygen, and can be an important stock of organic C. These communities include unique types of uncultivable extremophiles, specifically adapted to high pressure, cold, low water activity and chaotropic agents, and present a large amount of unexploited genomic information [12, 13, 11, 14, 15].

The development of cultivation-independent methods, based on the extraction and analysis of DNA from entire environmental microbial communities and the current advances in metagenomics have revolutionised the research in microbial ecology and biotechnology. New insights may be espected into the biogeochemical cycles and lifestyles of the organisms, toghether with the discovery of new enzymes. Metagenomic expression libraries from a DHAB isolate was already constructed and successfully screened for proteins of biotechnological interest [16]. The structural studies of proteins from DHAB metaproteomes will highlight the processes responsible for the specific molecular adaptation to the environment and may lead to the discovery of novel biocatalysts.

In the framework of EU MAMBA and ESF EuroDEEP-MIDDLE projects Yakimov's team of IAMC-Messina has already identified more than ten new lineages of bacteria and *archaea*. During two recent cruises on R/V Urania (MIDDLE, 2008-09 and MIDDLE&MAMBA, 2009-09), the Discovery, Urania, L'Atalante and Medee basins were investigated. During every transits and stand-by of principal operations we used the SBP system to explore the likely occurence of other anoxic basins. We were aided in this by morphobathymetric analysis to find confined depressions deeper than or similar to known basins's interface

depth. On every studied basin we were able to recognize a sharp, crisp interface with the SBP, except on Medee, where it appeared to be more diffuse.
Some results from the two cruises are presented hereafter.

2 Materials and Methods

We used CNR's R/V Urania, a 61 m vessel equipped with integrated navigation system, single and multi-beam, CHIRP SBP, ADCP, and water and sediment sampling down to 6000m.
CTD (SBE-911, conductivity, temperature, oxygen, pressure) and rosette sampling casts were performed; on brine pools extended range, high accuracy and resolution SBE and Paroscientific sensors were used.
A large amount of water and sediment samples, their immediate filtering, manipulation and storing in the field according to standard procedures, and time-consuming laboratory work are necessary for exploring and investigating the biodiversity and biogeochemical pathways that sustain microbial metabolic network systems and genomic richness of DHABs'.
Three types of CTD-casting were performed on water column (WC), at the interface (IF) and on the brine pools (BP). Samples from WC were used for investigating microbial dark production, diversity analysis at maximum depth and metabolic-arrays. On samples collected at the IF the salinity gradient for each bottle was measured by Refractometer and the concentration of oxygen was checked by Winkler titration. IF and BP sub-sampling were performed as described in [13, 12] and filtered by tangential flow system with Pellicon cassettes and direct filtration on Sterivex; the filtrates were stored at -20°Ctill transfer to the laboratory.
Sediment was sampled by a 60 cm cylindric Box-Corer. After measurement of pH, T and Eh, and sampling of the interface, 3-5 sub-cores were collected. Some of them were subsampled. The bulk was washed and sieved.
Microbial diversity has been analysed by DNA and RNA-based techniques and metagenomic and clone libraries of taxonomic and functional genes were built. Molecular fingerprinting techniques (SSCP, DGGE) were applied and real-time PCR technique performed to analyse and quantify functional genes.
Sediment cores had magnetic susceptibility measured and X-ray was performed, and once opened, were described, photographed and subsampled.
The DTM data of [17] was used for morphobathymetric analysis and SBP windowing for brine interface detection.

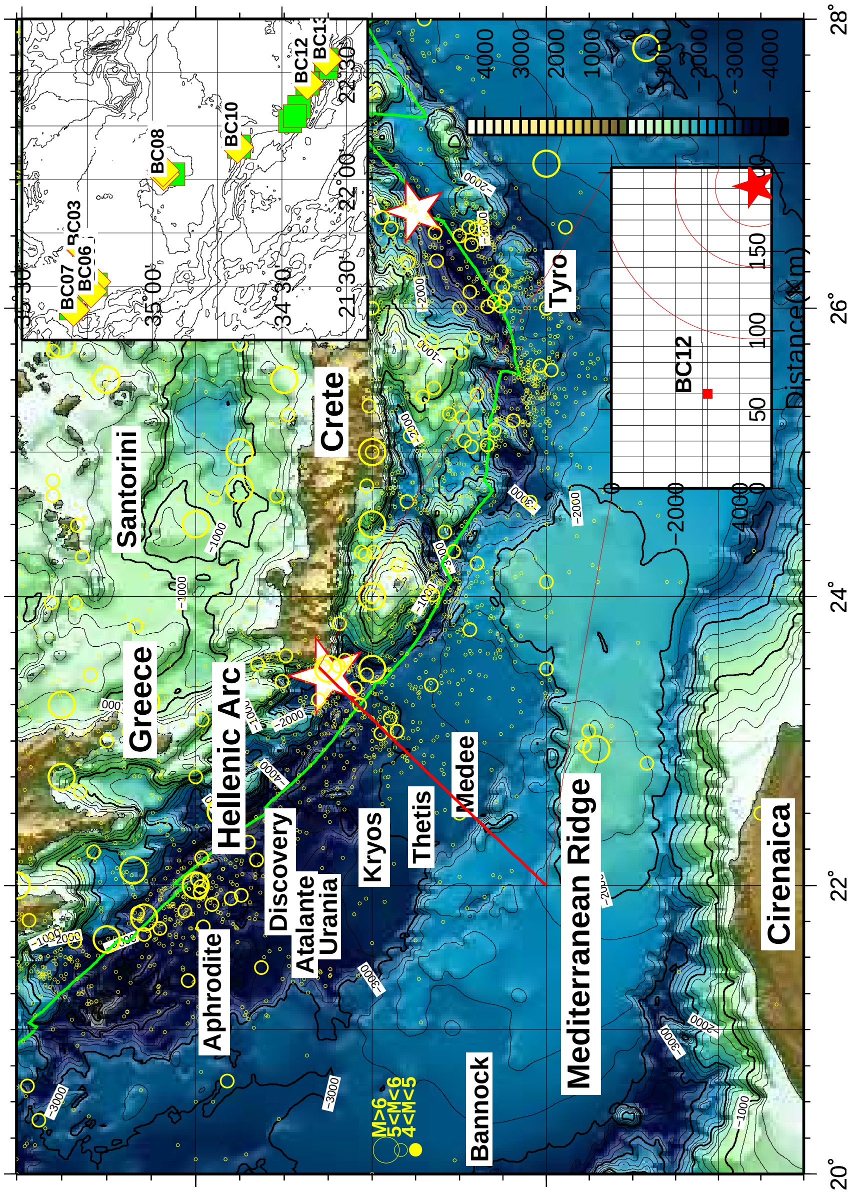
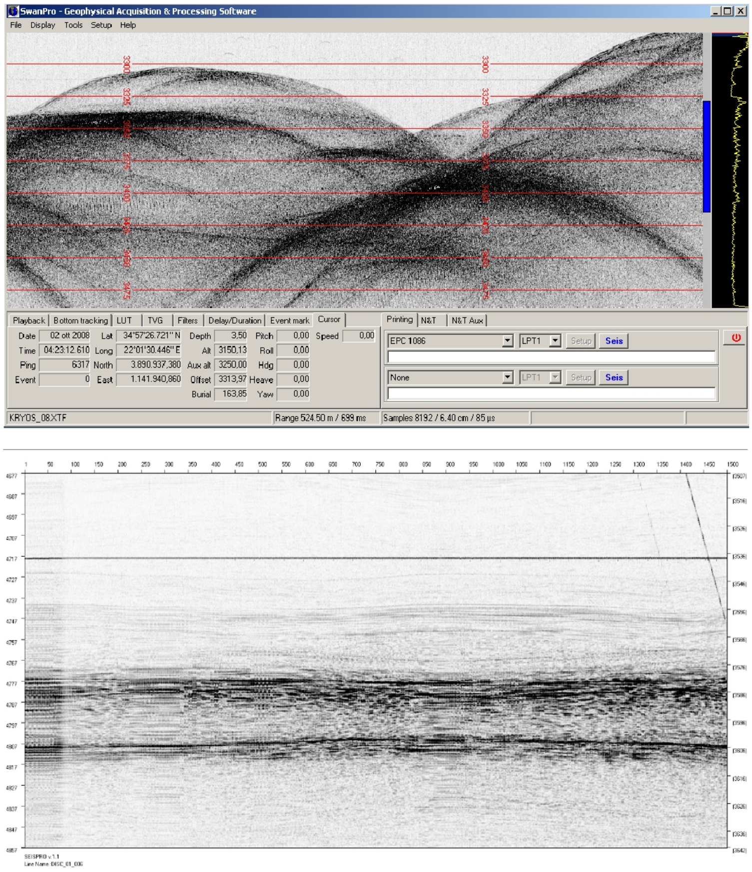


Figure 1: The Anoxic Basin Area. The yellow circles are the ISC epicenters of earthquakes from 1998 to 2009 (www.isc.ac.uk). The two white stars are the M8.3 AD 365 and M8.0 AD 1303 tsunamigenic earthquakes. Insets: (top) sampling stations (2008,2009); (bottom) bathymetric profile from Mediterranean Ridge to Western Crete, crossing BC12 station on Medee. Bathymetry by GEBCO.



Date 02 ott 2008
Time 04:23:12.610
Ping 6317
Event 0
Lat 34°57'26.721'' N
Long 22°01'30.446'' E
North 3.890.937,380
East 1.141.940,860
Depth 3,50
Alt 3150,13
Aux alt 3250,00
Offset 3313,97
Burial 163,85
Pitch 0,00
Roll 0,00
Hdg 0,00
Heave 0,00
Yaw 0,00
Speed 0,00
EPC 1086 LPT1
None LPT1
KRYOS_08.XTF
Range 524.50 m / 699 ms
Samples 8192 / 6.40 cm / 85 µs

Figure 2: The sharp, straight line of seawater/brines interface by CHIRP profiles: (above) during acquisition, (bottom) after filtering.

3 Results

On cruise MIDDLE08, after having succesfully sampled the Discovery, Urania and L'Atalante basins and on the way to the Medee Basin, we investigated by SBP profiling two depressions at about 20 and 40 NM SE of the Urania Basin. The signature of a possible brine interface shown by SBP, was confirmed by CTD casts and bottom coring. Both of them were found to be anoxic. The northern basin was already cited on naval maps as ”Kryos”, probably derived from the name of a depression located in the proximity, whereas the second appeared to be unknown, and we named it Thetis. On cruise MIDDLE&MAMBA we started by investigating the Tyro Basin, then we studied the Medee, the new basins, and the Urania and the Atalante. On the way back home we explored the Aphrodite Basin.

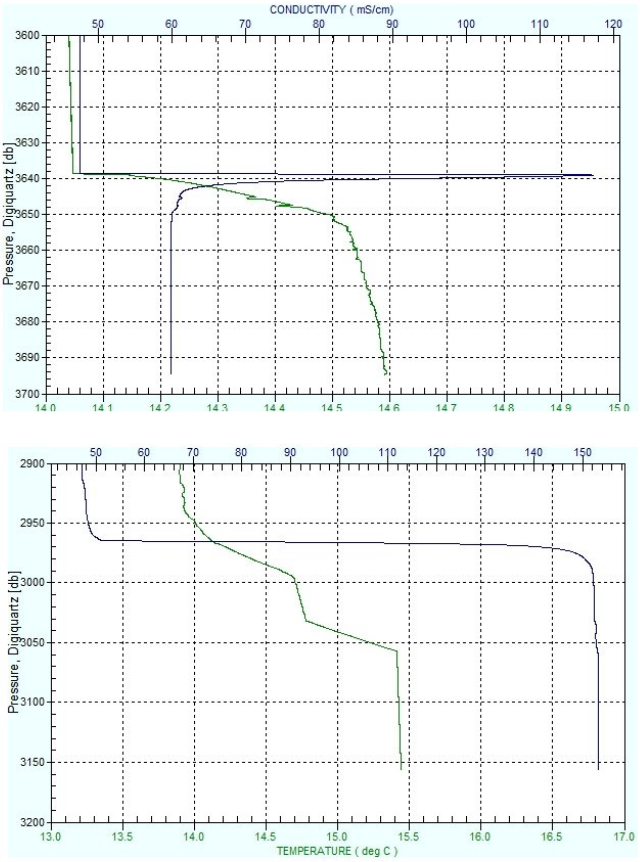


Figure 3: Oceanographic data on brine pools: (top) Discovery, (bottom) Medee.

Figures 2 and 3 show data from SBP profiles and CTD casts in the brine pools.
Table 1 shows the DHABs' interface data. The sediment cores of MIDDLE are shown in figures 4 and 5, that report also images of gypsum precipitates and of subsamples.

Discovery, l'Atalante, Urania

The three basins were visited during MIDDLE08, while only Urania and l'Atalante on MIDDLE&MAMBA. Seawater and brines were sampled and filtered onboard, especially on the Urania. Among the interesting findings of sediment coring we point the attention to core BC01 (Discovery, bischofite saturated, anoxic, 'oily' on top, see [8]), BC03 (Urania, oxic, with 'exotic' layers below 20 cm, studied by [18]), and BC07 (L'Atalante, anoxic, showing gypsum precipitates confined on a thin layer at a depth of 15 cm). The core near the hot pit on Urania's Western arm recovered gray, fluid mud with gassy vesicules.

LON	LAT	DATE	STATION	BRINE	INTERF.	INTERF.	BOT.	BOT.
ddmm	ddmm				dB	m	dB	m
02141.41	3516.60	2008-09-26	Discovery	YES	3638.5	3581.4	3694.5	3636.0
02131.24	3513.28	2008-09-28	Urania	YES	3523.0	3466.6	3609.5	3553.1
02123.98	3519.00	2008-09-29	L'Atalante	YES	3638.5	3581.4	3558.0	3502.8
02209.12	3440.25	2008-10-01	Thetis	YES	3307.5	3258.0	3414.5	3467.5
02226.43	3423.14	2008-10-01	Medee	YES	2967.6	2925.0	3063.5	3019.6
02602.32	3352.70	2009-09-12	Tyro	YES	3378.0	3327.5	3576.9	3521.6
02051.08	3551.43	2009-09-19	Aphrodite	NO	-	-	3261.5	3212.9

Table 1: CTD Stations interface data.

New basins

The Kryos Basin, is a narrow, N-S elongated fracture. The brine composition, bischofite saturated, and the recovered black, 'oily' at top, sediment, were found to be similar to that of the Discovery Basin. This transparent and water soluble 'oily' fraction is likely a polymer of biogenic origin (C.Ferreri, personal communication). The bottom core revealed an undisturbed, laminated, gray to black muddy sediment [19].

The Thetis Basin as depicted by bathymetry and halocline depth [20] shows an elliptical shape, oriented WNW-ESE, with a bulge to the S. Its morphology shows a linear trough or valley, bending to NE and E as a semicircular ring around a relief, and pointing to the S toward the Medee area The brine is halite saturated, with values close to that of the L'Atalante's composition. The bottom core revealed an undisturbed, laminated, gray-greenish muddy sediment [19]. During the 2009 cruise the NE basin was sampled and was found to be oxic.

Medee Basin

The Medee brines fill a trench at the base of a 1800 m relief, SW of the western side of Crete. Box corer BC12 (MIDDLE, Figure 1), recovered an ∼8 cm layer, characterized by a coarse, fining upwards, sandy basal unit located at ∼23 cm from top (Figure 5). This base stands over compact gray mud and is composed mainly by foraminifera, rounded white and gray grains, with some black aggregates, green crystals, and less abundant orange to red crystals; the top shows two levels of slightly coarser, silty sediment. Sediment above and below is gray mud.

In the absence of additional samples and data, we interpret it as a turbidite from the NE flanks of the ridge driven by earthquake-triggered slope instabilities.

Local seismicity in the area is low, although some M5+ events were recorded to the South and East. The 365 AD earthquake [21], epicenter 150 km NE (Figure 1), and related tsunami may be good candidates (according to [22] the average sedimentation rates on DHABs' is ∼14 cm/ka). The mechanism for the turbidite triggering can be the passage of the tsunami wave, the shaking of the earthquake itself, or both. This reconstruction is higly questionable, especially without accurate datings and knowledge of local sedimentation rate, and needs further analysis and dating of carbonatic materials sampled from key-layers. Weiss [23] argues that the tsunami amplitude in deep water should not be able

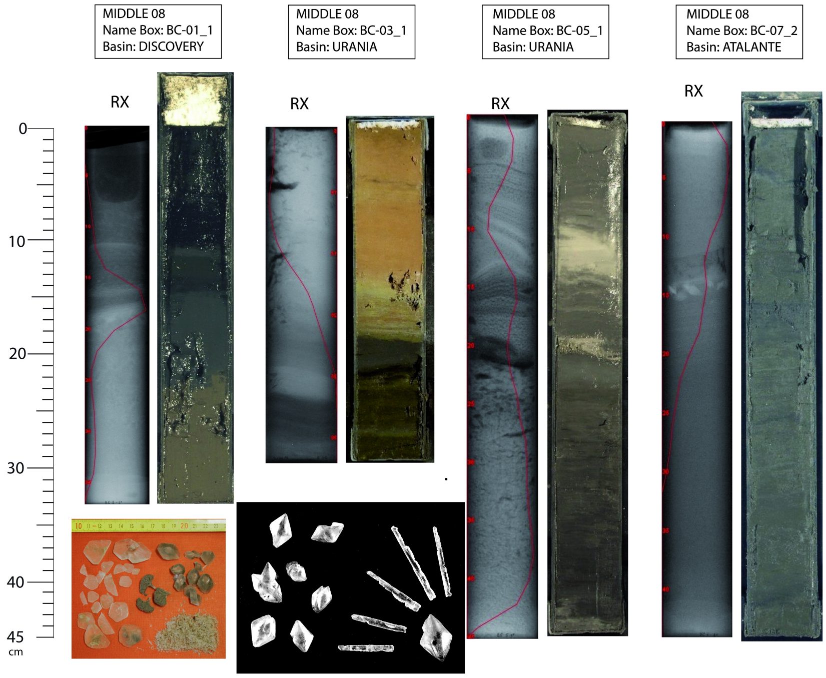


Figure 4: Cores collected during cruise MIDDLE08: (left) X-Ray and magnetic susceptibility; (right) photograph. Bottom panels show gypsum precipitates recovered from box-corers after washing/seiving (left, 2009; right, 2008, not in scale).

to remove sandy sediments from the ridge (top depth at 1800-2000m), and we can only guess amplitude and period of wave, and bottom stress to be large [24, 25], even without possible shoaling effects. Among others, the scenario involving the destabilization of the sedimentary cover of the ridge by the earthquake shock, that arrived after tenths of seconds, and its further mobilization and transport by the tsunami, that arrived after minutes from the event, seems reasonable.

The cores collected elsewhere in the 2008 and 2009 cruises did not contain layers of comparable thickness and grain sizes, therefore we may conclude that its deposition must considered local to BC12 site. Additional coring transects and analysis will be necessary, also in the light of the models of [26]. The cores of MIDDLE&MAMBA cruise picked up the layer again near the BC12 site, whereas muddy sediments were recovered to the S and the N; these last sample showed some possible injection of flows from the mud volcano to the NW of the lake.

Tyro and Aphrodite

The CTD data in the Tyro Basin (Tab.1) revealed a brine thickness of $\sim$145 m. In contrast, [27] reported the interface at 3383

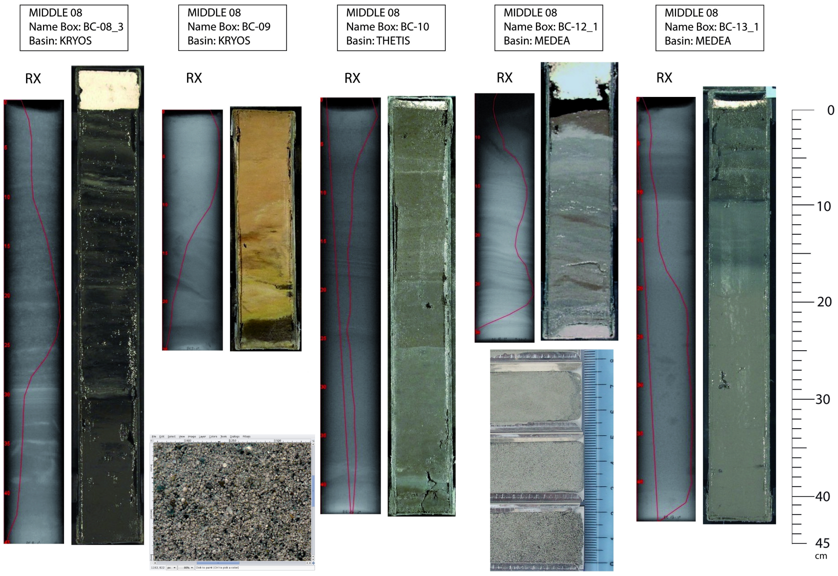


Figure 5: Cores collected during cruise MIDDLE08: (left) X-Ray and magnetic susceptibility; (right) photograph. Bottom panels show BC12 subsamples of coarse grained layer (left, base, right, first 6 cm from bottom).

db and the bottom measurement at 3441 db, with thickness of ∼60m. The Aphrodite Basin (Figure 1) was described by [6] as a possible DHAB candidate. We visited it and the CTD cast revealed that it is still oxic, at least 1m above the seafloor.

3.1 Microbiology at IAMC

Phylogenetic analysis of the MedSea DHABs' revealed a pronounced stratification of prokaryotes within the brines (Figure 6) and an astonishing diversity of autochthonous prokaryotes. We found many novel groups of microorganisms, suggesting the existence of a very specific microbiota, highly adapted to thrive under anoxic conditions at elevated pressure and salinity, that we termed as Mediterranean Sea Brine Lake (MSBL) candidate divisions, which have more than ten different groups among *Eubacteria and Archaea* (Figure 7). Another characteristic feature of DHAB ecosystems is the dominance in brine and lower interface of members of KB1 division whereas the class of *Deltaproteobacteria* appeared to be the most prominent group in the less saline, upper and middle layers. The candidate division KB1 includes species deeply branched with *Thermotogales-Aquificales* orders and have been found exclusively in anaerobic hypersaline systems [28]. Candidate division MSBL2 is phylogenetically related to the candidate division SB1, previously identified in the Shaban Deep (Red Sea). MSBL2-related sequences were also detected in other hypersaline basins, but not in seawater (Figure 6), indicating that these bacteria are anaerobs and specifically

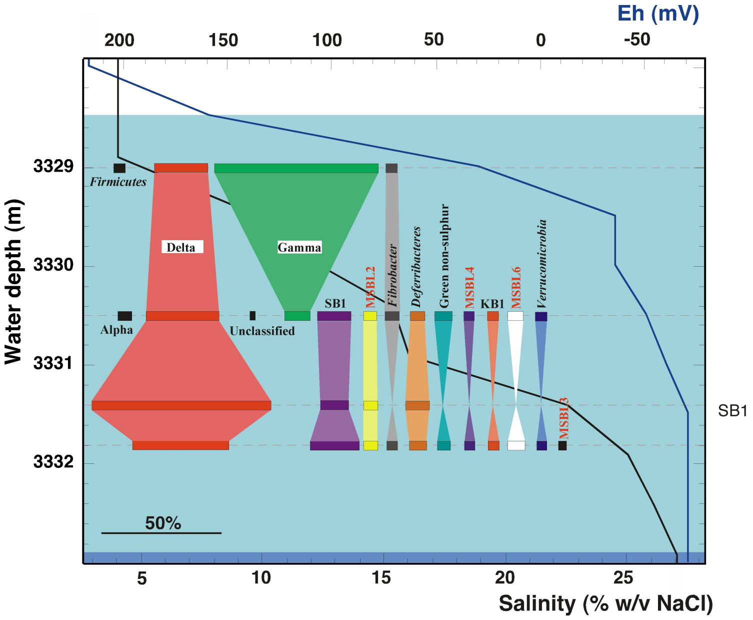


Figure 6: Stratification of the bacterial phylogenetic clusters typically observed along the DHAB lake interfaces.

adapted to the DHABs'. Metabolically-active members of the domain *Archaea* in the upper level of halocline were represented by Marine Group I *Crenarchaeota* [29], typical meso-pelagic CO_2 scavenger, while the downward compartments were inhabited by different groups of halophilic *Euryarchaeota*. Organisms affiliated with the MSBL-1 Candidate Division initially discovered only in Mediterranean DHABs' exhibited a distribution similar to that of ANME-1 (Figure 7), but likely possess a broader halotolerance. Members of this division have only thus far been found in anoxic, hypersaline environments, like Mediterranean DHABs', deep-sea mud volcanoes and endoevaporitic microbial mats [30], indicating adaptation to extreme anaerobic lifestyle.

4 Conclusions

MedSea DHABs' contain much more diverse uncultured prokaryotic assemblages than any other shallow anoxic marine hypersaline basin, and up to now they resisted to any cultivation attempts; their role in biogeochemical functioning of these ecosystems remains yet unknown and unravelling of this circumstance is the aim of our future studies.

The results from two R/V Urania successful cruises point to the need of further exploring the Eastern Mediterranean Sea for discovering new DHAB candidates, and increasing our knowledge on life processes and biogeochemistry of these extreme environments.

Brine lakes are small and closed basins whose formation is generally driven by tec-

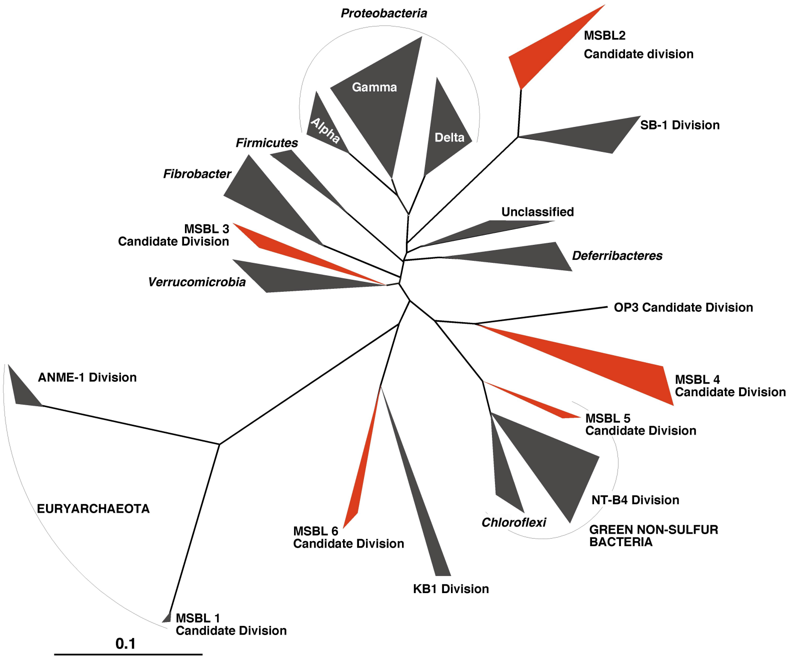


Figure 7: Phylogenetic positioning of 16S rRNA sequences of the most abundant prokaryotic phylotypes recovered in interface of DHABs'.

tonic processes and being very conservative environments that may record high energy sedimentary events triggered by seismic activity and/or slope instabilities in the surrounding regions.

The already good coordination between IAMC and ISMAR could be improved and extended to produce new insights in fields like seismotectonics, paleontology, palaeoceanography, biogeochemistry and nutrient/carbon fluxes at the interfaces.

5 Acknowledgments

We would like to give the name Thetis to the new discovered basin, to remember CNR's research vessel who sunk dramatically in Mazara del Vallo 2007-08-03. The Russian researcher Petr Mikheychik died during this accident. This work was done thanks to financial support of CNR in the frame of ESF Project 06-EuroDEEP-FP-004 MIDDLE and funds obtained in the frame of EC Project FP7-226977 MAMBA. We thank Captain E.Gentile and all crew of R/V Urania for their valuable professionalism and support during the cruises. The discoveries were possible only because good topographic data were made available by other Institutions, and we thank Dr. Loubrieu of IFREMER for providing the 500m resolution DTM data of [17].

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High Efficiency Feeding Strategy of *Tursiops truncatus* During Fishing Gear Depredation

G. Buffa, G. Buscaino, F. Filiciotto, A. Bellante, V. Maccarrone, G. Basilone, C. Angela, B. Patti, M. Sprovieri
Institute for Coastal Marine Environment, CNR, Capo Granitola (TP), Italy
gaspare.buffa@iamc.cnr.it

Abstract

It is well known that the Bottlenose dolphin (*Tursiops truncatus*) has a diet made up by a great variety of preys, including many fish that are also target species of the small scale fisheries activities. Previous studies has showed that this dolphin species often interacts with fishing activities feeding on the entangled fishes in order to catch preys at a low energetic cost.

The Bottlenose dolphin's depredation on artisanal fisheries is a serious problem because it represents a real economic threat to fishermen damaging the nets and reducing the fish catch.

In order to better characterize the foraging strategies of *Tursiops truncatus*, a comparative study on two different artisanal typologies of nets was carried out during the depredation activities in the South-Eastern coast of Sicily. Two multifilament gillnets named "Palamitara" and one Trammel net were used. For each haul (29), data on damage, weight and species composition of the catch were collected. CPUE (Capture Per Unit Effort) and Damage Factor, as indicator of depredation level, were calculated for the three nets in order to asses the feeding strategies in relation to the net efficiencies. The analysis of results (ANOVA) shows that the Palamitara net with the highest CPUE has also the highest value of Damage factor. These results suggest that the *Tursiops truncatus* is able to discriminate among different opportunities, choosing those providing greater benefits.

1 Introduction

Diet and feeding behavior of marine predators, such as Bottlenose dolphins (*Tursiops truncatus*), are the result of the combination of the availability of resources and foraging strategies [1]. It is well known that this species has a diet made up by a great variety of preys, mainly teleosts and cephalopods [2, 3]. Furthermore, the distribution of Bottlenose Dolphin populations can be often linked to the distribution of their preferred preys [4, 5, 6]. Preys' patch sizes and their spatial and temporal distributions are important factors that influence the predator's foraging decisions and strategies [7, 8, 9]. Some foraging behaviours are associated with habitats or specific conditions [10, 11] while other ones may depend on human activities and can be related to fishing performance [12, 13, 14]. The fishing exploitation by dolphins represents a new source of food that plus allows to foresee the availability of resource.

The Bottlenose dolphin's behaviour can

vary according to the type and distribution of preys [15, 16]. Moreover, the behavior, habitat and distribution of mammals may also change in response to the fishing effort [17, 13]. Previous studies showed that the Bottlenose dolphin interacts with fishing activities feeding on the entangled fishes [14].

The analysis of dolphin behaviour, during the interaction with the net, suggests that there is a complex feeding strategy. This species was observed directly engaged in the depredation of gillnet, with a "common strategy" in which specimens removed fishes from the net [18, 19]. Another strategy could consist in the use of the net as a "supermarket" [20] in which it is possible to select the preferred prey and the most nutritious portion [21]. Particularly, this behavioural was evident in the trammel net for red mullet (Mullus surmuletus) that was considered the most affected trammel net by depredation events [22, 23]. In her studies, Shane [15] listed 27 different feeding behaviours exhibited by Bottlenose dolphins, including herding fish into a net. The net may act as a wall by which prey cannot escape [24], making it beneficial for dolphins that use it as fish safety. Understanding the breadth of *Tursiops truncatus* foraging behavior during interaction with fishing activities, it is important to answer questions on behavioural flexibility in this social species and their survival.

The competitive interaction between cetaceans and fisheries is probably as old as the first human attempts to catch fish with a net [13]. Today, the Bottlenose dolphin is a species involved in many cases of interactions with coastal fisheries, but not always it benefits from them. In fact, it is well-known that the biggest problem for the dolphins is the risk of by-catch in the nets, considered the main threat to the conservation of different species of cetaceans [25, 26]. Although different studies report frequent interactions, other ones show few or none entanglements of dolphin [27, 19, 28], concluding that dolphins are entrapped when they are unaware of the net or are distracted by other stimuli [25]. It was also assessed that this species is able to identify the fishing net (Kastelein, 2002). However, we must also consider that the depredation strategies of artisanal fisheries is a serious problem since it represents a real economic threat to fishermen damaging the nets and reducing the fishing catch [17, 22, 28]. Based on this assumption, data were analysed in the past in order to quantify the impacts of dolphin depredation on artisanal fisheries [13, 20, 23, 28].

To better characterize the foraging strategies of *Tursiops truncatus*, a comparative study on two different artisanal typologies of net, through the analysis of damages and fished during dolphin depredation, was carried out.

2 Materials and methods

The study was carried out in autumn 2005 inside the Ognina bay (South-East of Sicily, 36°59.826' N; 15°17.231' E) (Figure 1), where a family of fishermen claimed frequent *Tursiops truncatus* depredation raids in the nets.

2.1 Fishing gears

The Palamitara is a multifilament gillnet of small-scale fisheries of Sicily. This fishing technique is inspired to the strategies used in Tuna traps [29] and its fishing efficiency depend on the fisherman's ability to placing the gear in the area where there is a

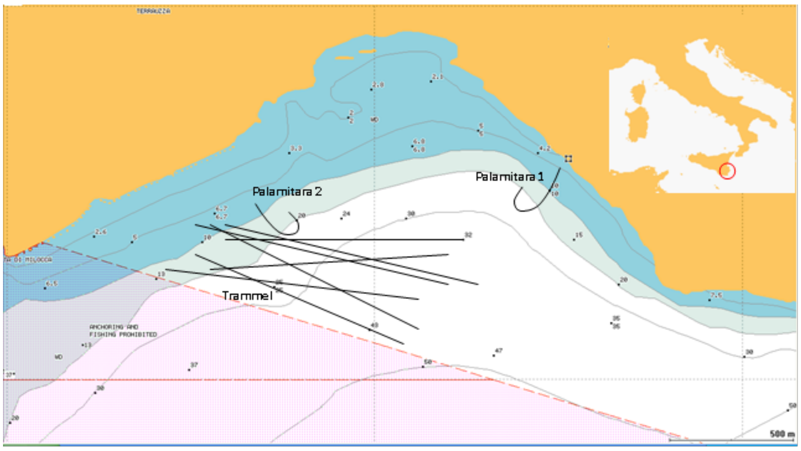


Figure 1: Map showing the study area in the Bay of Ognina as bounded by a red circle (36°59.826' N, 15°17.231' E) and the geographical positions of Trammel and Palamitara nets.

$$CPUE_{gr} = \frac{Catch}{Length \times Time}$$

$$DF = \frac{Damages}{Length \times Time}$$

Figure 2: CPUE and Damage Factor index. Catch is the total weight per haul per net in grams, Damage is the number of holes per haul, Length is the net's length in meter, Time is the fishing time in hour.

greater likelihood of block the path to the fish. This gillnet is usually positioned perpendicular to the coast and forms a barrier from the surface to the bottom because the pieces of which it consists are of different heights to each other, as they have a growing number of mesh in the vertical with increasing depth.

The net consisting of three sections called Coda, Petto and Campile having different features and functionality. The Palamitara is placed in the sea so as to form a "hook". Coda and Petto have the function of directing (or occasionally capturing) pelagic fish into the Campile that positioned to form an arc (>180°) is designed to capture fish. Two multifilament gill nets named Pal 1 and Pal 2 with a length of 254 meter (Petto 72 m, Coda 72 m and Campile 110m) and 85 mm stretched mesh were used (Figure 1).

Moreover one 1080 m long Trammel net (with 35 mm stretched mesh), was used. During the experiment, the two Palamitara nets were fixed in the same geographical positions while the trammel net was positioned daily in different sites of the bay (Figure 1).

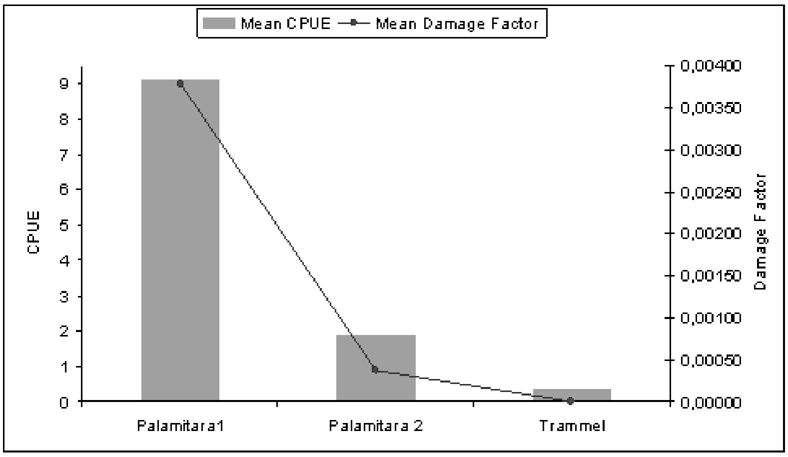


Figure 3: Average of the CPUE and of the Damage Factor values for the three nets.

2.2 Data collected

For each haul, data on fishing effort, species composition, weight of fish and nets damages (in terms of number of holes) were collected. After the data collections the holes were repaired in order to ensure maximum efficiency of the fishing gears and enable the detection of actual damage in each sampling session. Moreover, the visual monitoring of Palamitara 1 was performed in order to report dolphins' presences and to assess, using an ad libitum sampling method [30, 31] if the behaviour of dolphins near the net was a depredation strategy. Then, the photo-ID technique of specimens was used to investigate if the depredation phenomenon observed was a customary behavior of dolphin populations.

2.3 Data analysis

CPUE (Catch Per Unit Effort) was calculated as described in Figure 2. The indicator of damage, the Damage Factor (DF), was created in order to assess the depredation level for each fishing net and for the sections. The presence of holes in the net (Rocklin et al 2008) was assessed as evidence of dolphins depredation actions. In fact, considering the robustness of multifilament of Palamitara, which has a breaking strength per single multifilament of a 9 kg-force (where 1 kg-force = 9.81 Newton, [32]), the damages can be imputable only to dolphins and not to the others smallest predators species (Murena Helena, Octopus vulgaris, Loligo vulgaris) or to accidental contact to the rocks, as it is the case for others typologies of net [20, 28]. Then ANOVA analysis was used in order to asses the differences of CPUE and Damage Factor among the depredated nets. Finally, a linear regression was applied to the CPUE and the Damage Factor values for each depredated haul.

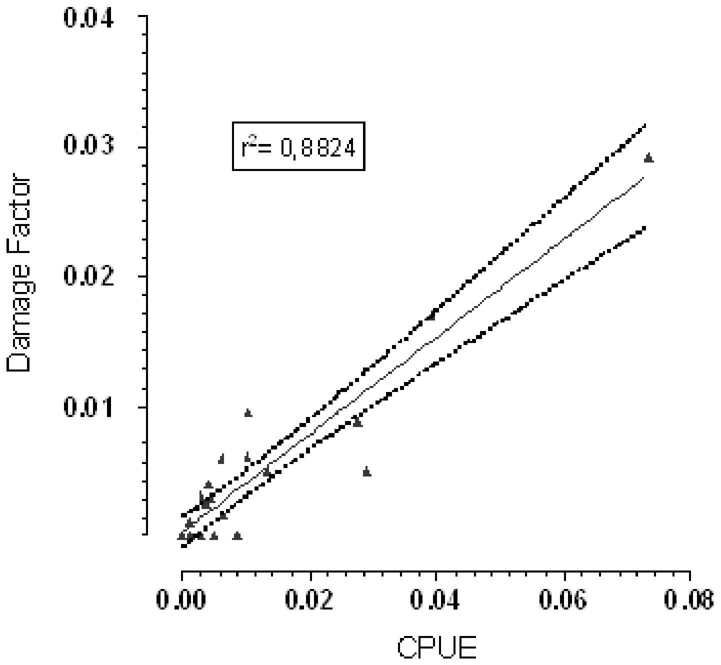
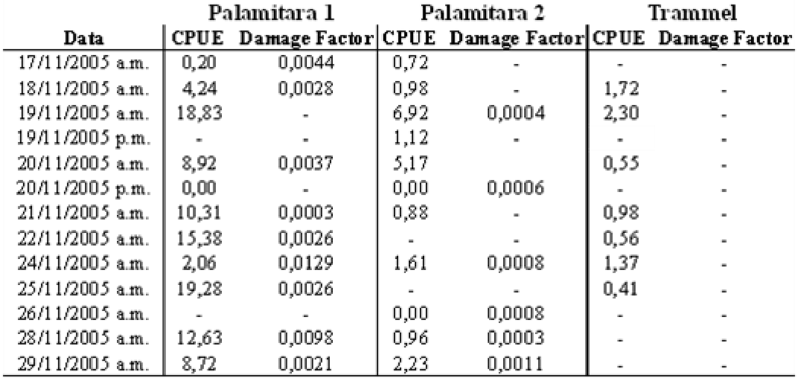


Figure 4: Linear regression between the Damage Factor and CPUE values of Palamitara1.

3 Results

29 sampling sessions were carried out (11 on Palamitara 1, 11 on Palamitara 2 and 7 in the trammel net) with a fishing effort of 248 hours (Table 1). The analysis of data show that the composition of catch is different between the two types of nets (Table 2). The Palamitara 1 net has a mean CPUE of 9.1 ± 2.2 $\mathrm{g{\cdot}m^{-1} \cdot h^{-1}}$ with a total catch of 236.1 kg and Euthynnus alletteratus (53.6% of the total catch) and Sarda sarda (44.8% of the total catch) are the most abundant species caught. The Palamitara 2 has a mean CPUE of 1.9 ± 0.7 $\mathrm{g{\cdot}m^{-1} \cdot h^{-1}}$ with a total catch of 50.1 kg and the main species are Euthynnus alletteratus (74% of total catch) and Sarda sarda (21.37% of total catch). Finally, the Trammel net has a mean CPUE of 0,38 ± 0.06 $\mathrm{g{\cdot}m^{-1} \cdot h^{-1}}$ (25.238 kg), and catches 13 different species among which the most abundant is Mullus surmuletus (40% of total catch).

Considering the number of hauls with damages to gear and consequently with dolphin's depredation events, it can be assumed that dolphins interacted more with the Palamitara 1 (82% of hauls; Mean Damage Factor 0,0038 ± 1,3 10-3 $\mathrm{n{\cdot}m^{-1} \cdot h^{-1}}$, than with the Palamitara 2 (63% of hauls Mean Damage Factor 0,0004 ± 1 10-4n$\mathrm{{\cdot}m^{-1} \cdot h^{-1}}$ and never with the trammel net (Table 1).



Data	Palamitara 1		Palamitara 2		Trammel	
	CPUE	Damage Factor	CPUE	Damage Factor	CPUE	Damage Factor
17/11/2005 a.m.	0,20	0,0044	0,72	-	-	-
18/11/2005 a.m.	4,24	0,0028	0,98	-	1,72	-
19/11/2005 a.m.	18,83	-	6,92	0,0004	2,30	-
19/11/2005 p.m.	-	-	1,12	-	-	-
20/11/2005 a.m.	8,92	0,0037	5,17		0,55	-
20/11/2005 p.m.	0,00	-	0,00	0,0006	-	-
21/11/2005 a.m.	10,31	0,0003	0,88	-	0,98	-
22/11/2005 a.m.	15,38	0,0026	-	-	0,56	-
24/11/2005 a.m.	2,06	0,0129	1,61	0,0008	1,37	-
25/11/2005 a.m.	19,28	0,0026	-	-	0,41	-
26/11/2005 a.m.	-	-	0,00	0,0008	-	-
28/11/2005 a.m.	12,63	0,0098	0,96	0,0003	-	-
29/11/2005 a.m.	8,72	0,0021	2,23	0,0011	-	-

Table 1: Values of CPUE and Damage factor for each haul of the three fishing gears.

The most damaged net was the Palamitara 1 (94 holes). Significant differences in the values of Damage Factor were recorded (ANOVA $p<0,001$) (Table 1).

In Figure 3 values of mean CPUE and Damage Factor for the three nets are shown. Finally, in Figure 4 the linear regression (positive correlation, $p<0.01$) between the CPUE and the Damage Factor values for each haul of Palamitara 1 was given. No by-catch events were registered during the experiments. Two adults and one juvenile were identified in two different hauls. In the observation of dolphins' behaviour, only immersion events and sudden bursts of speed at the surface in perpendicular direction respect to the floating line of the Palamitara 1 were recorded.

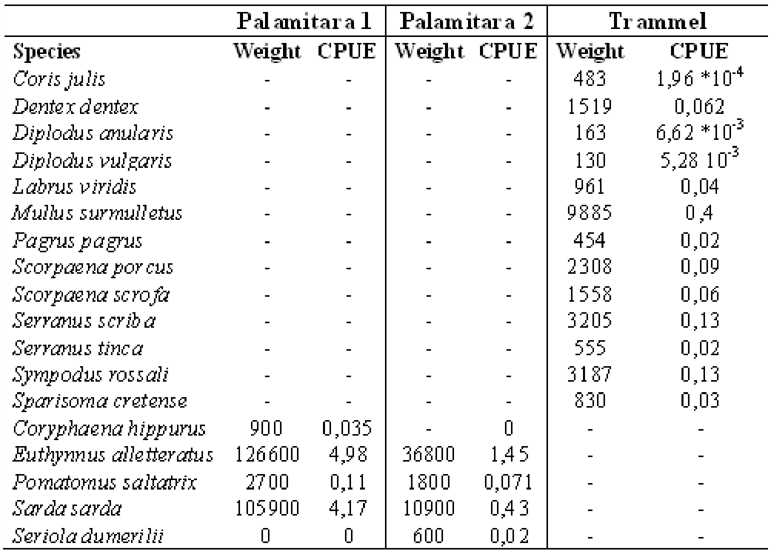
4 Discussion

Considering the Damage Factor of the nets, it can be assumed that dolphins depredated more the Palamitara 1 than the Palamitara 2 and never the trammel net (Table 1).

The data analysis shows that the Palamitara net with the highest values of CPUE has also the highest values of Damage Factor. These results verify the efficiency of Bottlenose Dolphins' strategies and confirm their ability to discriminate among different opportunities; in fact, dolphins depredated differently the nets in relation to their fishing efficiency and to species composition (as related by other authors: [17, 23, 28].

The correlation between the CPUE and the values of the Damage Factor of Palamitara 1, during hauls with depredation, indicates that dolphins depredated more the Palamitara 1 net when there was a greater presence of fish in its meshes.

To better understand the strategy of the Bottlenose dolphin's depredation we should consider that the trammel net, positioned at different sites with greater depths, with a smaller catch distributed on a 1080 meters of net (lowest CPUE), was not damaged by the dolphins. On the contrary, the Palamitara nets positioned always in the same sites at shallow depths, with a bigger catch distributed only on 254 meters of net (greater CPUE), suffered enormous damages. Consequently, we can suppose that the Bottlenose dolphins have adapted



	Palamitara 1		Palamitara 2		Trammel	
Species	Weight	CPUE	Weight	CPUE	Weight	CPUE
Coris julis	-	-	-	-	483	1,96 $*10^{-4}$
Dentex dentex	-	-	-	-	1519	0,062
Diplodus anularis	-	-	-	-	163	6,62 $*10^{-3}$
Diplodus vulgaris	-	-	-	-	130	5,28 10^{-3}
Labrus viridis	-	-	-	-	961	0,04
Mullus surmulletus	-	-	-	-	9885	0,4
Pagrus pagrus	-	-	-	-	454	0,02
Scorpaena porcus	-	-	-	-	2308	0,09
Scorpaena scrofa	-	-	-	-	1558	0,06
Serranus scriba	-	-	-	-	3205	0,13
Serranus tinca	-	-	-	-	555	0,02
Sympodus rossali	-	-	-	-	3187	0,13
Sparisoma cretense	-	-	-	-	830	0,03
Coryphaena hippurus	900	0,035	-	0	-	-
Euthynnus alletteratus	126600	4,98	36800	1,45	-	-
Pomatomus saltatrix	2700	0,11	1800	0,071	-	-
Sarda sarda	105900	4,17	10900	0,43	-	-
Seriola dumerilii	0	0	600	0,02	-	-

Table 2: Catch composition of the three fishing gears: Weight and CPUE values for the species caught into each fishing net.

to prey on the Palamitara, because they consume less energy to search the net, dive and detect the prey.

It can not be excluded that the Bottlenose dolphins' preferences of prey may have influenced the strategy of depredation (as observed in precedent studies, such as [20, 23]. Indeed, the species composition of the two types of net were different and the dolphins preferred the species caught by Palamitara nets. However, other studies show that the trammel net for red mullet (Mullus surmuletus) is the most affected by depredation events [22].

Another result of this study is the lack of by-catch events. This confirms the high ability of Bottlenose dolphins to locate the net even in low visibility conditions to avoid being entangled [33, 33]. Moreover, the presence of a juvenile in the group indicates that dolphins consider the entrapment in the net a low risk [34] and there might be a passage of information from one generation to another, through forms of parent education [35]. These processes can lead to population segments becoming apparently dependent on fishery interactions [36].

The behavioural observations of specimens show very complex foraging strategies adopted by the Bottlenose dolphins. Their trajectories allowed to make the hypothesis that dolphins use the net as a barrier against which they push fishes and then dive in order to capture preys or strip them from the meshes. These feeding behaviours have been observed also in other species of dolphins that, taking advantage of the presence of rocks or artificial barriers, prevent the escape of preys and increase the number of catches [18, 37]. Furthermore, according to other studies [16], the presence of sudden bursts of speed at the surface by dolphins was used as indicator of prey chasing.

The results highlight the opportunistic behaviour of Tursiops truncatus and his ability to discriminate among different feeding opportunities, exploiting the ones from which obtain the greatest benefits and generating strong competition with fishermen.

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Findings of Phototrophic Picoplankton in the Aphotic Zone of the Ionian Sea (Mediterranean)

R. La Ferla, G. Maimone, M. Azzaro, F. Azzaro, M. Leonardi, F. Raffa
Institute for Coastal Marine Environment, CNR, Messina, Italy
rosabruna.laferla@iamc.cnr.it

Abstract

The paper refers on the findings of picophytoplankton cells in the dark water column of the Ionian Sea (Mediterranean Sea). In the framework of NEMO Project (NEutrinos Monitoring Observatory), coccoid cyanobacteria, genus *Synechococcus* were detected by image analysis. The *Synechococcus* cells ranged between 0.1 and 4.2 x10^3 cells ml^{-1} and were observed along the whole water column (max depth 3331 m) in both study periods (Autumn and Summer). Moreover, an unidentified group of very small red fluorescing cells were detected. The occurrence of both microorganisms at the meso- and bathy-pelagic layers - without light or nutrient limitation - was comparable to those at epi-pelagic zones. Differences were evidenced between the study periods with high abundances in Summer than Autumn, but no clear correlations with hydrological or trophic parameters were computed. The recovery of picophytoplankton at great depths puts stimulating questions to their ecological function as well as to the trophic dynamics in the ocean interior. Several comparative hypotheses have been performed for understanding the pathway and significance of these cells at great depths. Anyhow, a till now unconsidered resource of C exists in the deep biota, mainly in terms of C sink. This topic should be investigated by future studies to clarify the true meaning of such organisms in the dark water column.

1 Introduction

Picophytoplankton are prokaryotic autotrophic organisms and the major contributors to primary production and photosynthetic biomass in both marine and freshwater habitats [1, 2, 3]. The photoautotrophic community is dominated by the cyanobacteria *Synechococcus*, *Prochlorococcus* and a complex assemblage of small picoeukaryotes. Specific studies on the distribution and ecology of picophytoplankton in the surface waters of the oceans and Mediterranean Sea have been carried out [4, 5, 6, 7, 8, 9] but there are few knowledge on the presence of such organisms in the deep realm. [10] have studied the sedimentation of *Synechococcus* embedded in sinking organic aggregates. The resilience of cells and persistence of their distinctive phycoerythrin fluorescence at confocal microscopy have been observed in the sediment traps located at 120 and 550 m depths in sub-antarctic and sub-tropical waters, respectively. As concern the Mediterranean sea, [11] reported the occurrence of autofluorescing picoplankton until great depths in the Eastern basin. In 1999 during the EMTEC cruise (in the framework of SINAPSI project) *Synechococcus* as well as very small red cells at surface and deep layers of the Ionian Sea were observed (unpublished data). As concerns our findings, another paper was previously published [12]. Vilibić, et al. [13] have described the conspicuous findings of *Synechococcus* in the deep Adriatic Sea, by cytofluorimetric technique, assessing the major role of both the deep convection in the Southern Adriatic Sea and the downslope density current in the Bari canyon for bringing these cells down to the bottom. This work is aiming to acquaint the presence of the coccoid cyanobacteria, genus *Synechococcus* and an unidentified group of very small red fluorescing cells in the aphotic zones of the North-Western Ionian Sea. The recent experimental observations on autotrophic organisms in the marine depth could mean that an unconsidered resource of C exists in the deep biota, mainly in terms of C sink. This paper might stimulate future contributions to the knowledge of new ecological implications of such organisms in the deep biota.

2 Materials and methods

In the frame of the NEMO Project (NEutrinos Monitoring Observatory), aimed at the realization of a deep submarine astrophysical observatory, two multidisciplinary surveys were carried out in the North-Western Ionian Sea (TALASTRO 2: Autumn – November 2000; TALASTRO 3: Summer – July 2001). Three sampling stations (L1 stn.: 36°43'N, 15°27'E; KM3 stn.: 36°30'N, 15°50'E; KM4 stn.: 36°20'N 16°00'E) along an inshore – offshore transept in front off Cape Passero were investigated. Water samples were collected from surface to a maximum depth of 3331 m, using a rosette sampler equipped by 10-liters Niskin bottles. A CTD probe 911plus SeaBird was used to record conductivity, temperature, pressure and oxygen content at all the stations. Salinity and potential density were calculated by means of the algorithms of Fofonoff and Millard [14]. The values of salinity were calibrated through comparison with measurements made with an induction salinometer AutoSal Guildline Model 8004B. Samples for nutrients ($\mathrm{NO_2}$ + $\mathrm{NO_3}$, N-$\mathrm{NH_4}$, P-$\mathrm{PO_4}$) were performed according to [15], except for N-$\mathrm{NH_4}$ ([16]. For particle material sampling, 2000ml of seawater, previously screened through 200 μm net to take out the larger zooplankton, were filtered on pre-combusted (480 °C for 4h) pre-weighted glass fibre filters (Whatman GF/F), and subsequently oven-dried at 60°C for 24h. Total suspended matter (TSM) was evaluated by gravimetric method according to [17]. Particulate Organic Carbon and Nitrogen (POC and PON) incidences were estimated, after removal of carbonates [18], by combustion at 980°C with a "Perkin-Elmer CHN Elemental Analyser", utilizing Acetanilide as standard. Water samples for picophytoplankton enumeration were analysed according to [19] and observed by an Axioplan 2 Imaging microscope (Zeiss) equipped with Zeiss Axiocam digital camera, and Zeiss Axiovision 3.1 software. At least 20 microscope random eyefields were observed for each sample. *Synechococcus* sp. (SYN) and the smaller reddish spherical cells called in the following text Other Picophytoplankton (OP), were distinguished according their fluorescence and dimension (SYN: cells emitting an orange fluorescence, range size: 1.15 - 2.2 μm OP cells emitting a red fluorescence, range size: 0.4 – 0.5 μm).

		Autumn			Summer		
		L1	KM3	KM4	L1	KM3	KM4
T°C	min	13,45	13,30	13,31	13,46	13,40	13,35
	max	23,19	24,86	24,82	24,11	19,71	24,61
Salinity	min	38,47	38,59	38,68	38,54	38,55	38,37
	max	38,79	38,87	38,86	38,83	38,83	38,86
NO_2+NO_3 µM l^{-1}	min	0,01	0,01	0,95	0,37	0,01	0,18
	max	4,21	4,33	4,50	5,00	4,28	5,26
NH_4 µM l^{-1}	min	0,02	0,01	0,01	0,05	0,01	0,02
	max	0,97	1,01	1,14	0,78	0,27	0,55
PO_4 µM l^{-1}	min	0,01	0,14	0,01	0,01	0,01	0,01
	max	0,26	0,30	0,40	0,45	0,31	0,25
TSM mg C L-1	min	0,83	0,96	0,69	1,73	2,20	2,19
	max	3,26	3,68	4,23	6,47	6,42	12,06
POC µg C L-1	min	14,84	18,35	18,07	0,31	7,60	5,20
	max	63,9	67,63	61,43	60,25	42,55	54,77

Table 1: Range of T °C, Salinity, NO_2+NO_3, NH_4, PO_4, TSM and POC values in autumn and summer at L1, KM3 and KM4 stns

3 Results

3.1 Environmental data

Table 1 summarizes minima, maxima, mean values and standard deviations of physico-chemical data in Autumn and Summer. Generally, the examined stations exhibited no great difference each other in the hydrological parameters. T°C values ranged from 13.30 to 24.86 and 13.35 to 24.61 °C in Autumn and Summer, respectively; S values ranged 38.47 to 38.87 and 38.37 to 38.86 in Autumn and Summer, respectively. The concentrations of nutrients in all the study periods, sampling sites and depths greatly ranged between 0.01 and 5.26 μM for NO_3+NO_2; between 0.01 and 1.14 μM for NH_4; between 0.01 and 0.45 μM for PO_4.

3.2 Total Suspended Matter and Particulate Organic Carbon

The amount of TSM in the analysed waters was very low in Autumn, while a little higher values were recorded in Summer (Table 1). Marked decreasing from the surface to the deeper layers were found in both the analysed periods. The POC incidences were low in both study periods, with slightly higher values in Autumn (range: 14.84 - 67.63 μg$\cdot$C $\cdot$ l^{-1}) than in Summer (range: 5.20 – 60.25 μ g$\cdot$C $\cdot$ l^{-1}). Vertical POC distribution was generally decreasing from the epi-pelagic to the meso- and baty-pelagic layers, even if some notable increases were locally recorded in Autumn in the deeper layer (1000m – 3000m). It is interesting to remark that they ever occurred in concomitance of OP and/or SYN abundance peaks.

3.3 Picophytoplankton

The abundances of both SYN and OP were in the order of 10^2 -10^3 cells ml^{-1} and were higher in Summer than Autumn. Minima, maxima, mean values and standard deviations of the abundances are summarized in Table 2. Generally, the maximum densities were achieved in all the study periods for OP than for SYN, with the exception at L1 stn where SYN showed

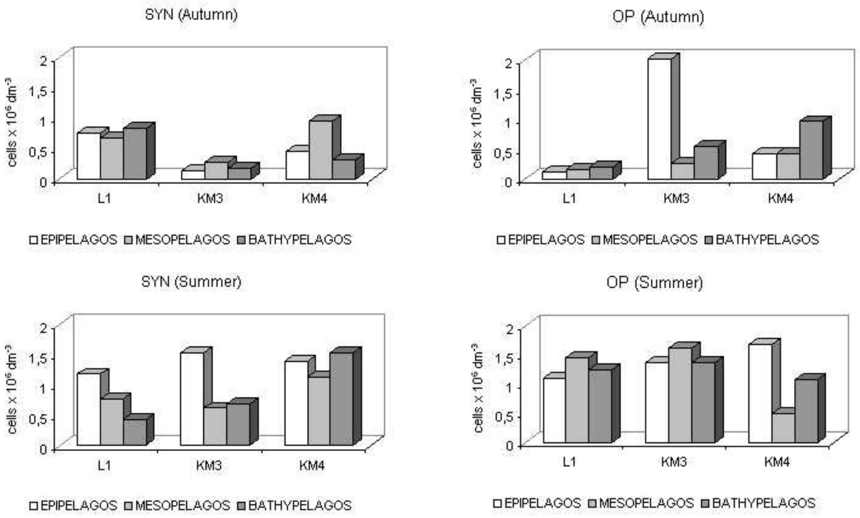
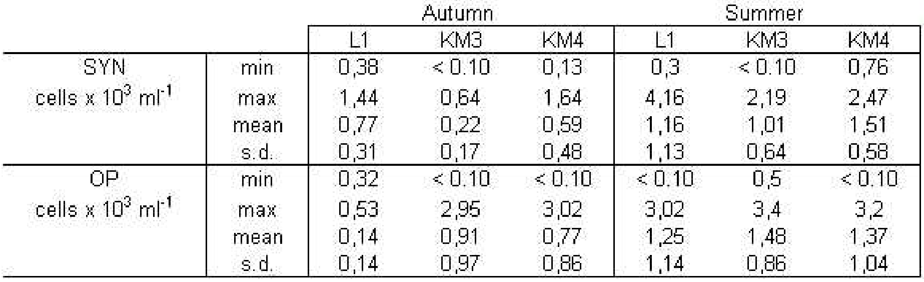


Figure 1: Depth-integrated and normalized abundance of SYN and OP in the epi- meso- and bathy-pelagic zones in the two study periods.

the highest abundances in Summer (value max 4.16 cell x 10^3 ml^{-1}). The depth-integrated and normalized abundances of SYN and OP in the epi– meso– and bathy-pelagic zones showed for each station different patterns (Figure 1). In Autumn, high SYN occurrences were in the meso-pelagic zone at KM4 (0.96 cell x 10^6 dm^{-3}) and in the bathy-pelagic zone at L1 stn. (0.83 cell x 10^6 dm^{-3}). The highest OP abundance was achieved in the epi-pelagic layer at KM3 stn. (2.2 cell x 10^6 dm^{-3}); moreover, two significant secondary increases were observed in the bathy-pelagic zones at KM3 and KM4 stn. (0.54 and 0.96 cell x 10^6 dm^{-3}, respectively). In Summer, in all the examined stations, SYN showed high concentrations in the epi-pelagic layer (mean value 1.37 ± 0.18 cell x 10^6 dm^{-3}), and high values were also detected in the meso- and bathy-pelagic zones at KM4 stn (1.13 and 1.54 cell x 10^6 dm^{-3}, respectively). OP contribution was important in all examined layers, with the maximum value at KM4 stn. at epipelagic layer (1.68 cell x 10^6 dm^{-3}). Pearson correlation coefficients were computed among the different examined parameters. Few significant correlations were found between the entire data set: SYN abundance positively correlated with salinity, TSM and NH_4 in autumn. OP positively correlated with temperature and POC, and negatively with salinity and NO_3+ NO_2, both in Autumn and Summer (Table 3).

4 Discussions and conclusions

The main purpose of this paper is to signal a new scientific problem: the occurrence of picophytoplankton in the aphotic oceanic layer and, in particular in the meso- and



		Autumn			Summer		
		L1	KM3	KM4	L1	KM3	KM4
SYN cells x 10^3 ml^{-1}	min	0,38	< 0.10	0,13	0,3	< 0.10	0,76
	max	1,44	0,64	1,64	4,16	2,19	2,47
	mean	0,77	0,22	0,59	1,16	1,01	1,51
	s.d.	0,31	0,17	0,48	1,13	0,64	0,58
OP cells x 10^3 ml^{-1}	min	0,32	< 0.10	< 0.10	< 0.10	0,5	< 0.10
	max	0,53	2,95	3,02	3,02	3,4	3,2
	mean	0,14	0,91	0,77	1,25	1,48	1,37
	s.d.	0,14	0,97	0,86	1,14	0,86	1,04

Table 2: Range, mean and standard deviation of picophytoplankton cell concentrations in Autumn and Summer (SYN = *Synechococcus* sp., OP = Other picophytoplankton).

	Autumn				Summer		
SYN *vs*:				**SYN *vs*:**			
S	n = 14	r = 0,684	P < 0,01				
TSM	n = 14	r = 0,54	P< 0,05				
NH_4	n = 12	r = -0,556	P < 0,05				
OP *vs*:				**OP *vs*:**			
T °C	n = 13	r = 0,538	P < 0,05	T °C	n = 12	r = 0,72	P < 0,01
S	n = 13	r = -0,688	P < 0,01	S	n = 12	r = -0,814	P < 0,01
POC	n = 13	r = 0,57	P< 0,05				
NO_2+NO_3	n = 13	r = -0,735	P < 0,01	NO_2+NO_3	n = 12	r = -0,556	P < 0,05

Table 3: Coefficients of significant correlations between SYN and OP versus other parameters

bathy-pelagic layers of the oligotrophic Ionian Sea. Picophytoplankton distribution, and particularly SYN, generally reflects adaptations to different light and nutrient regimes [2, 20]. Our results showed surface values similar to those referred in nutrient depleted areas of Mediterranean [6, 21, 22, 23] and Pacific Ocean [24]. As expected, our data were lower than those detected in nutrient-enriched areas (i.e. upwelling, coral reef, lagoon or transition areas) [25]. In general, our abundances were higher in Summer than Autumn. [26] showed the high dependence of SYN growth rate on temperature. In our study case, only OP correlated with temperature. Significant positive correlations were often observed with salinity. This findings partially agreed with those reported by [27] and [28], that observed a strong response of SYN even to small-scale fluctuations in salinity. Respect to nutrients, SYN negatively correlated with NH_4 in Autumn but not in Summer, OP indeed showed significant negative correlation with NO_2+NO_3 in both study periods. Phylogenetic differences between surface and deep picophytoplankton populations as well as different ability to use various nitrogen sources were determined by [29], [30], and [31]. According with [32] the vertical distributions of nitrogen pools could select for different SYN ecotypes and so could be responsible for the vertical distribution in the water column. In this study case, picophytoplankton abundances resulted quite low and might have been underestimated. In fact, the use of fluorescent microscopy instead of flow citometry had probably neglected the counts of *Prochlorococcus* and picoeukaryotic cells [8]. However, in our opinion, the repeated microscopic observations of the small red cells (OP) constitute an important topic, since such picosized cells showed characteristics similar to the marine prochlorophytes. As regards the novelty of this paper, namely the occurrence of SYN and OP in the meso- and bathy-pelagic layers of the Ionian Sea, the majority of researchers consider unexpected their presence in the "dark water column". As a consequence, most of the studies on picophytoplankton are restricted to the upper ocean. Formerly, [33] attributed the measurements of deep maxima in-situ fluorescent profiles in the deep Pacific Ocean (500 and 1000m) to biological causes, like as the occurrence of cyanobacteria or nitryfing bacteria or purple non sulfur bacteria. They associated these findings with the transport of faecal pellets, of which cyanobacteria constitute one of the main vital components [34]. This hypotesis has been confirmed by [10] that observed the accumulation of *Synechococcus* cells in sediment traps located at 150 and 550 m in sub-antarctic and sub-tropical waters, respectively. Recently, [35] emphasized the possibility to find picophytoplankton in the deep biota as a consequence of different pathways including indirect export through the consumption of picoplankton aggregates by organisms at higher trophic levels, and direct routes such as aggregation and incorporation into settling detritus. As a consequence the contribution of picophytoplankton to export could be proportional to their production rates. Our findings confirm the possibility that the aggregation of picophytoplankton cells into larger detrital particles determines their vertical settling and export fluxes from the upper layers. In fact, significant correlations between SYN and TSM as well as OP with were detected. Moreover, aggregate cells or cells adhering to detritus of OP were often observed, above all in the deep samples. Unfortunately, they were discarded from the enumerations because of the difficulties in counting. A different intriguing hypothesis by [13] showed that *Synechococcus* could be a tracer for deep water ventilation. A significant vertical transport of *Synechococcus* to oceanic depths was imputed in winter 2006 to the convective processes of water masses, like the deep water formation in the Southern Adriatic Sea (AdDW, Adriatic Deep Water) as well as to the downslope density current of NAdDW (North Adriatic Dense Water) through the Bari canyon for bringing these cells down to the bottom. The authors found the maximum abundance at subsurface layer and at 800 m depth (3.5 x 10^5 cells ml^{-1}). Nevertheless, in our study case the picophytoplanktonic cells occurred along the whole water column in an almost constant way and the circulatory patterns of the study area were not as strong as in the Southern Adriatic Sea. However, the importance of SYN in carbon cycling and fixation is still a novel topic also assessed by using hierarchical model, genomic and proteomic studies [36]. We don't exclude that the more and more frequent experimental observations of picophytoplankton in the marine depth could mean that an unconsidered additional resource of C exists in the deep biota. The weight of picophytoplankton biomass in the aphotic water layers, as suggested by depth-integrated data, open a new scenario about the C sink in oceans. These populations need to be better known because their true role in deep marine habitats is not yet understood. Further experimental efforts must be performed for understanding the pathway of sinking and the ecological significance of these cells at great depths.

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Study of the Decomposition of Organic Polymers in Mediterranean Basins Through the Measurements of Microbial Enzymatic Activities

G. Caruso, R. Zaccone
Institute for Coastal Marine Environment, CNR, Messina, Italy
gabriella.caruso@iamc.cnr.it

Abstract

Knowledge of the processes involved in the turnover of organic matter is of utmost importance to understand ecosystem functioning. In most of the aquatic ecosystems, microorganisms are major players in the cycling of biogeochemical nutrients (Nitrogen, Phosphorus) and Carbon through their enzymatic activities (leucine aminopeptidase, alkaline phosphatase and beta-glucosidase) on organic polymers (proteins, organic phosphates and polysaccharides, respectively). The small monomers released by decomposition may be used by microbes themselves for their growth. Estimates of the decomposition rates of organic polymers are performed using fluorogenic compounds, analogues to natural substrates, whose hydrolysis rate allow us to obtain information on the potential metabolic activity of the prokaryotic community.

This note refers some of the results obtained during recent oceanographic cruises performed in the Tyrrhenian and Ionian Seas, with the aim of evaluating the contribution of the prokaryotic community to organic matter decomposition and the changes in microbial activity patterns during different seasonal conditions. Enzyme measurements revealed significant variations in the rates of hydrolytic activities in relation to space and time, with the highest levels generally found during summer and in upper epipelagic layer; sporadic peaks (hot spots) of enzyme activity are also detected in meso- and bathypelagic layers, due to the presence of different water masses.

1 Introduction

Prokaryotes are an important component of the planktonic community of aquatic ecosystems. Other than producers, some of them, such as heterotrophic bacteria, are also the main consumers of dissolved organic matter (DOM) and therefore they are major players in organic matter turnover within the “microbial loop” [1]. Microorganisms contribute significantly to the regulation of the flux of organic matter and energy from the biotic to abiotic compartment, acting in both decomposition (by DOM hydrolysis and nutrient release) and production processes (new living biomass production). Enzymatic hydrolysis of organic matter, which is mostly composed of both dissolved and particulate polymers, is recognised as a critical process for organic matter utilization. In fact, heterotrophic microorganisms may uptake directly only low molecular weight substrates, such as amino-acids, simple sugars, fatty acids [2]. Many microorganisms, such as heterotrophic bacteria, microalgae, cyanobacteria, fungi and yeasts, are able to synthesize enzymes. Therefore, the determination of enzymatic hydrolysis rates allows to quantify the contribution of the microbial community to organic matter turnover [3, 4, 5, 6, 7, 8]. Since the last decade, the flux of Carbon and Phosphorus through microbial decomposition of organic polymers (proteins, polysaccharides and organic phosphates) in the Mediterranean Sea has been studied at the Istituto per l'Ambiente Marino Costiero (IAMC) CNR of Messina. Particularly, enzyme measurements have concerned three enzymes: leucine aminopeptidase (LAP) and beta-glucosidase (B-GLU), which are both involved in the decomposition of proteins and polysaccharides, respectively, and therefore in the Carbon cycle and alkaline phosphatase (AP), which plays a key role in the regeneration of inorganic Phosphorus from phosphoric esters. Although enzyme measurements provide only a "potential" estimate of microbial activity, they can give insights on the metabolic functionality of the microbial community and therefore improve the current understanding of the whole functioning of aquatic ecosystems.

The Mediterranean Sea is considered to be an ecosystem particularly suitable for biogeochemical studies due to its particular characteristics of a semi-closed basin, with short residence times of water masses and the occurrence of deep water formation phenomena. Moreover, particular attention towards the biogeochemistry of this basin has been addressed in recent years, because of its vulnerability to climate changes [9]. Particularly, the study of the role of climate changes on Carbon cycle in the Mediterranean Sea has been the main objective of VECTOR (VulnErability of the Italian Coasts and marine ecosystems to climaTe changes and their rOle in the Mediterranean CaRbon cycles) Project, funded by Italian Ministry of University and Research (2006-2010).

2 Materials and methods

2.1 Study projects and areas

The samples examined in the present study come from several oceanographic cruises carried out both in the Tyrrhenian and Ionian Seas, which had similar scientific approaches, sampling strategies and analytical methods. The Tyrrhenian Sea is a Mediterranean basin about 475 miles long and from 60 to 300 miles wide, between the Ligurian Sea, the Italian peninsula, Sicily, Sardinia, and Corsica. It is characterised by mixing and diffusion of water masses coming from the Eastern and Western Mediterranean. The Tyrrhenian Sea was sampled during the following projects:

- FIRB-MIUR Project: Biodiversity and organization of the communities in different environmental contexts, FIRB cruise, performed by R/V Universitàtis of CoNISMa in July 2005 [5].
- VECTOR Project- Task 8 Cycle of Carbon in the pelagic areas of the Mediterranean Sea, where the VECTOR station (Lat. 39°30' N 13°30' E), was chosen as the reference station for the study of Carbon transfer through the food web along the epi-, meso- and bathypelagic layers. Sampling were performed in November 2006, February and June 2007, by the Italian R/V Urania of CNR and in April 2007 by the R/V Universitàtis. The Ionian Sea represents a crossing area between the Western and the Levantine Mediterranean Sea; it is a site of wa-

ter mass transformation and it is directly influenced by Adriatic outflow. Seawater samples were collected from the Ionian Sea during the following research projects:

- NEMO project (NEutrin Monitoring Observatory) performed from 1999 to 2002 onboard of the R/V Urania and R/V Thetis of Italian CNR, with 4 cruises (December 1999, October 2000, July 2001, March 2002), to study the hydrobiological characteristics of marine area in front off Capo Passero [4].
- FIRB-MIUR Project: in the framework of the MED-BIO cruise carried out in November 2004 [10].
- VECTOR Project Task 8: TransMed cruise performed during June 2007.

Seawater samples were collected at different depths from surface to bottom depth (varying as a whole from 5 to 4900 m), using a rosette sampler equipped with 10-L acid rinsed Niskin bottles. They were either immediately processed onboard the R/V or stored for subsequent analyses within 24 hours of sampling.

2.2 Enzyme assays

Microbial enzyme activities were measured using specific fluorogenic substrates, which are methylcoumarine (MCA) or methylumbelliferone (MUF)-derivates and are analogues to natural compounds. Particularly, L-leucine-4-methylcoumarinylamide hydrochloride, 4-methylumbelliferyl B-D-glucopyranoside and 4- methylumbelliferyl phosphate (Sigma) were used for LAP, B-GLU and AP measurements. Increasing amounts of each substrate were added to 10 ml subvolumes of each sample [11]. The fluorescence released by substrate hydrolysis was measured with a Turner TD-700 model fluorimeter, at 0 time (initial time) and 3 hours after incubation at the "in situ" temperature. Calibration curves with the standards 7-amino-4-methylcoumarin (MCA) or 4-methylumbelliferone (MUF) were performed for LAP or for B-GLU and AP, respectively. Data were expressed in terms of maximum reaction velocity (Vmax) of hydrolysis of the substrates, namely as nanomoles of Leucine, Glucoside and PO4 potentially released per litre and per hour, by LAP, B-GLU and AP, respectively. Vmax values were further converted into nano- or micrograms of Carbon (LAP and B-GLU) or inorganic Phosphorus (AP) potentially released by enzyme hydrolysis, according to [3].

2.3 Statistical analysis

Statistical differences among epi-, meso- and bathypelagic layers were evaluated by one-way analysis of variance (ANOVA). A multivariate analysis was performed with the PRIMER 6 software, version 6Beta R6 (PRIMER-E Ltd) on normalised data elaborated according to Cluster analysis, using the Group average linkage method and Euclidean distance [12]. The hierarchical clustering into sample groups allowed to better represent the relationships linking the samples and analyse within each basin homogeneous patterns identified by a similarity value (S) working on a resemblance matrix.

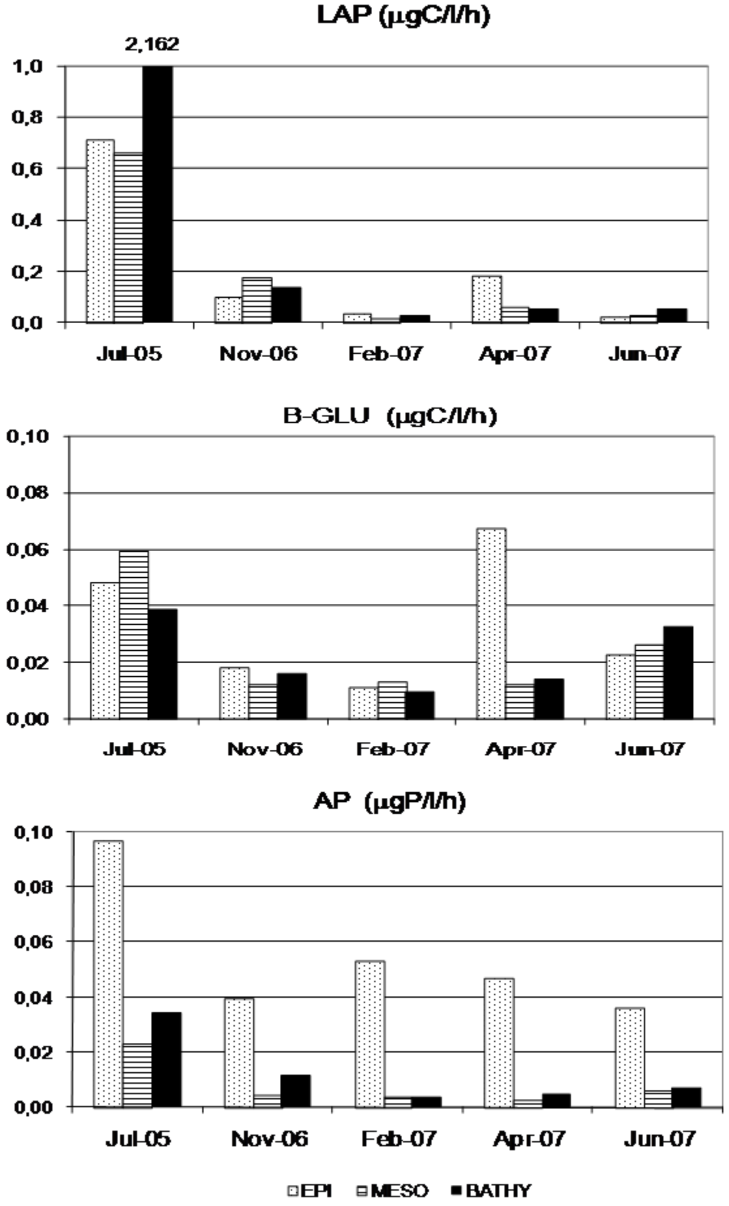

Figure 1: Mean enzyme activity values found in the Tyrrhenian Sea

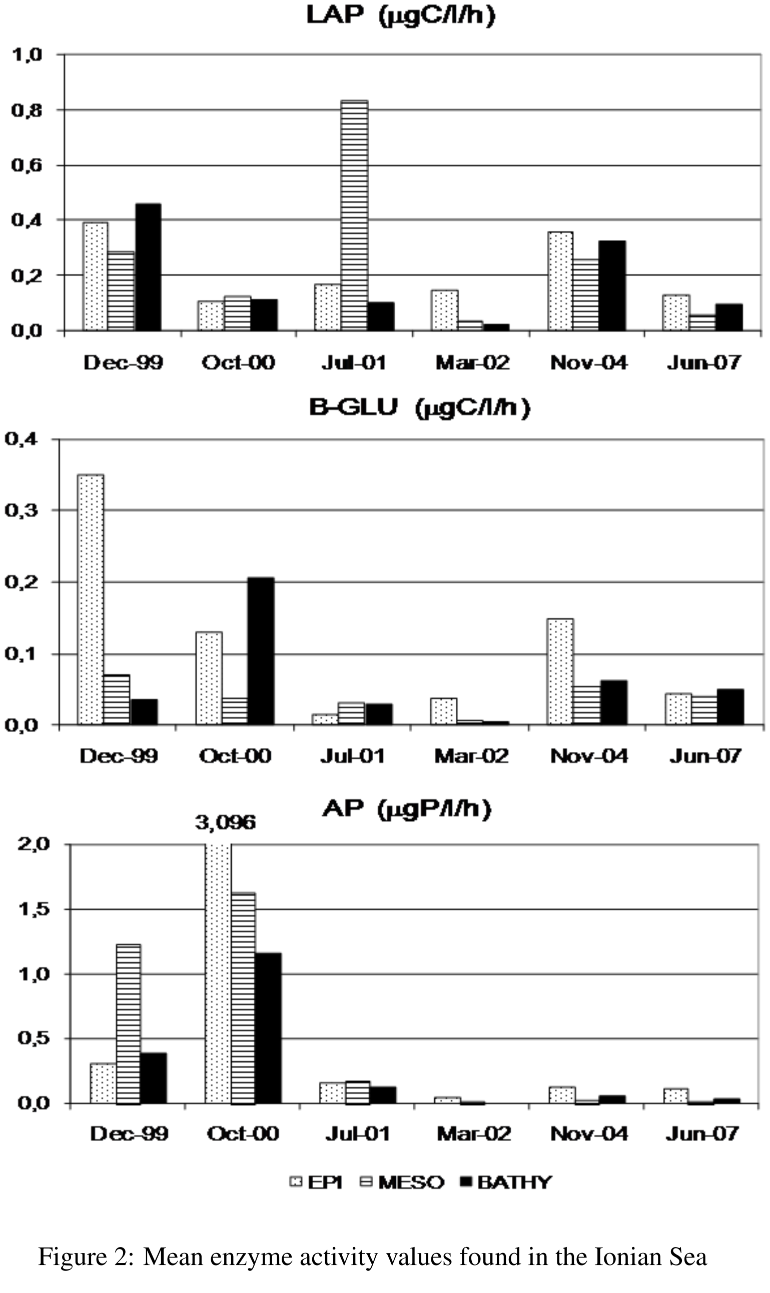

Figure 2: Mean enzyme activity values found in the Ionian Sea

3 Results

Results highlighted a broad variability in the studied microbial enzyme activity rates over different spatial and time scales. In the Tyrrhenian Sea, enzyme patterns were dominated by LAP, followed by AP and B-GLU. LAP values ranged from 0.018 ± 0.012 to 2.162 ± 1.03 μgC$\cdot$l$^{-1}\cdot$h^{-1}, which were found at the mesopelagic layer in Feb-07 and at the bathypelagic one in Jul-05, respectively. The highest LAP activity was detected in Jul-05 (Figure 1). The highest values were measured in epipelagic layers, excepting for Jul-05 and Nov-06 when peaks were found in bathy- and mesopelagic layers, respectively. ANOVA pointed out significant differences in LAP levels between epi- and bathypelagic layers (F = 8.27, $P < 0.05$) in Jul-05.

B-GLU values were comprised between 0.010 ± 0.003 and 0.068 ± 0.013 μgC$\cdot$l$^{-1}\cdot$h^{-1}, measured at the bathypelagic layer in Feb-07 and at the epipelagic one in Apr-07. Activity was generally high during productive periods (Jul-05, Apr-07 and Jun-07) (Figure 1). This enzyme prevailed in epipelagic layers in Apr-07, while during warm periods (Jul-05 and Jun-07) it appeared mostly concentrated at meso and bathypelagic layers, respectively. Significant variations of epi- versus meso- (F = 31.75, $P < 0.01$) and bathypelagic (F = 69.85, $P < 0.01$) layers were found in Apr-07.

AP values ranged from 0.003 ± 0.002 to 0.097 ± 0.077 μgP$\cdot$l$^{-1}\cdot$h^{-1}, measured at the mesopelagic layer in Apr-07 and at the epipelagic one in Jul-05, respectively. AP was always high at surface, probably due to phytoplankton contribution to its synthesis, with a peak in Jul-05 (Figure 1). Epipelagic AP levels were generally significantly different from meso- (F = 4.39, 7.41, $P < 0.05$ in Jul-05 and Apr-07, respectively) as well as from bathypelagic ones (F = 7.33, 22.59, $P < 0.01$, in the same periods, respectively).

In the Ionian Sea, enzyme activities were in the order LAP > AP > B-GLU, except for Dec-99 and Oct-00 when AP prevailed over LAP. LAP values ranged from 0.024 ± 0.021 to 0.836 ± 0.383 μgC$\cdot$l$^{-1}\cdot$h^{-1}, measured in Mar-02 at the bathypelagic layer and in Jul-01 at the mesopelagic layer, respectively. On average, the highest proteolytic activity was recorded in Dec-99, followed by Jul-01 and Nov-04, without clear seasonal trends (Figure 2). Concerning the vertical distribution, the highest enzyme activity rates were observed in intermediate waters during Oct-00 and Jul-01, in coincidence with water column stratification, and in deep waters during Dec-99. Conversely, in Mar-02, Nov-04 and Jun-07 the proteolytic activity appeared to be more concentrated in the surface layers. Significant variations were found comparing epi- versus meso- and bathypelagic layers in Dec-99 (F = 10.30, 28.41, $P < 0.01$, respectively) as well as in Oct-00 (F = 45.20, 52.70, $P < 0.01$, respectively).

B-GLU values were comprised between 0.004 ± 0.005 and 0.394 ± 0.143 μgC$\cdot$l$^{-1}\cdot$h^{-1}, which were detected in Mar-02 at the bathypelagic and in Dec-99 at the epipelagic layers, respectively. The highest activity was generally found in Dec-99 and Oct-00, followed by Nov-04, similarly to LAP (Figure 2). B-GLU activity levels were higher at epipelagic layers in Dec-99, Mar-02, and Nov-04, while in Oct-00 and Jul-01 at bathy- and mesopelagic layers respectively, probably due to water stratification that lead to the confinement of activity at the deepest waters. B-GLU was significantly higher at the epipelagic than at the bathypelagic layer in Mar-02 ($F = 5.34$, $P < 0.05$).

AP values ranged from 0.003 ± 0.002 to 3.096 ± 2.57 μgP$\cdot$l$^{-1} \cdot$ h^{-1}, measured in Mar-02 at the bathypelagic layer and in Oct-00 at the epipelagic layer, respectively. The highest AP levels were found in Oct-00 and in Dec-99, while in the other periods AP did not exceed 1.62 μgP$\cdot$l$^{-1} \cdot$ h^{-1} (Figure 2). AP prevailed generally at epipelagic layers; only in Dec-99 the highest values were recorded at the mesopelagic layer. Significant variations occurred in Oct-00 between epi- and bathypelagic layers ($F = 12.11$, $P < 0.01$).

Cluster analysis of enzyme values yielded the dendrograms reported in Figures 3 and 4, for the Tyrrhenian and Ionian Seas, respectively. In the Tyrrhenian Sea, LAP data showed a close reciprocal similarity along the whole column during Feb-07, Apr-07, Jun-07, grouping in a close cluster with S=0.06; a second homogeneous cluster (S=0.11) was found in Nov-06, whereas the value recorded in Jul-05 at the bathypelagic layer was as an isolated peak. B-GLU activity levels clustered together (S=0.26) over the whole column during Nov-06, Feb-07, Apr-07; similar patterns were observed during Jul-05 and Jun-07. AP dendrogram evidenced from Nov-06 to Jun-07 a distinct separation among epi-(S = 0.5) and meso-bathypelagic (S = 0.27) layers and a distinct peak in Jul-05 at the epipelagic layer (Figure 3).

In the Ionian Sea (Figure 4), LAP dendrogram showed a distinct peak in Jul-01 at the mesopelagic layer, and two clusters, grouping at S=0.43, consisting respectively of late autumn samples (Dec-99, Nov-04) and of samples from Mar-02, Jun-07, Oct-00 without any relationships to depth layers. B-GLU samples from Jun-07, Jul-01, Nov-04, mostly from meso- and bathypelagic depths, grouped together in a cluster with S = 0.27, while the Dec-99 sample from the epipelagos had high activity level similarly to Oct-00 and Nov-04 epipelagic samples. Concerning AP, samples from Mar-02, Jun-07, Jul-01, Nov-04 clustered in a single group with a close similarity level (S = 0.14), while AP levels in Oct-00 in the epipelagic layer were more similar to those measured in Dec-99 in the mesopelagic layer.

Comparing the Tyrrhenian to the Ionian enzyme activity rates, while LAP values fell in a similar order of magnitude in both the basins, AP and B-GLU were about 10 times lower in the Tyrrhenian than in the Ionian Sea. Cluster analysis of the full (Tyrrhenian + Ionian) dataset (not shown in figure) confirmed that, while no clear differentiation was possible according to LAP values, which peaked everywhere in summer, both B-GLU and AP were differently expressed between the two basins, showing higher activity in Jul-05 in the Tyrrhenian Sea and in late autumn (Dec-99, Oct-00) in the Ionian Sea.

4 Discussion

In marine environments, knowledge of the microbial metabolism and of its modulation played by environmental variables is still fragmentary; information on the deep sea is very scanty and predominantly collected in the open ocean [2, 13]. In the Mediterranean waters, only a few studies on the microbial functioning have been carried out to date [5, 14, 15, 16] and have recognised the importance of microbial processes in the biogeochemistry of meso- and bathy-pelagic processes [7, 17, 18, 16]. Enzyme rates obtained in the present study showed significant variations at the different spatial and temporal scales. On a spatial scale, higher AP and B-GLU values in the Ionian compared to the Tyrrhenian Sea could be related to the low availability of nutrients found in the Eastern Mediterranean Sea; this could stimulate microbial activities and organic matter recycling, as suggested by [19].

Along the water column, in both the Tyrrhenian and the Ionian Seas, the highest enzyme activities were often associated with epipelagic layers. This was generally true for AP, especially in the Tyrrhenian Sea, as underlined by Cluster analysis which pointed out that in this basin a spatial more than temporal discrimination occurred for this enzyme only. It is likely that the key role played by phytoplankton in the synthesis of AP accounted for this pattern [2]. Nevertheless, some sporadic peaks ("hot spots") of enzyme activities, particularly LAP and B-GLU, were detected also in meso- and bathypelagic layers probably due to the presence of different water masses. This result could agree with the availability of dissolved organic matter reported by [20] in deep Mediterranean waters and could support the hypothesis that the renewal of deep water following the Eastern Mediterranean Transient supplied deep waters with new organic matter not still degraded. LAP activity found at the bathypelagic layer indicated an active metabolism of the microbial community on proteic substrates also at the highest depths. Cluster analysis confirmed that on a temporal scale, in the Tyrrhenian basin, the highest LAP activity levels were found during warm season (Jul-05), suggesting a greater availability of labile matter, prone to microbial decomposition, during this productive period. In the Ionian Sea, both B-GLU and AP were particularly active during late autumn (Oct-00 and Dec-99) indicating the presence of high concentrations of carbohydrates and organic phosphates. Significant differences among water layers evidenced by ANOVA suggested that similar metabolic patterns took place in epi- and mesopelagic layers for LAP in the Tyrrhenian Sea and for B-GLU and AP in the Ionian Sea. The spatial and temporal variability of enzyme levels confirmed the dynamic capability of microorganisms to modify their enzyme profiles in order to adapt their metabolism to the available organic polymers, as observed by [8] and [14]. In conclusion, research until now performed has contributed to quantify the role of the microbial community within Carbon and Phosphorus fluxes; research prosecution, however, is still necessary to verify the observed trends and add more details for a more comprehensive view of environmental changes occurring in the Mediterranean scenario during the recent years.

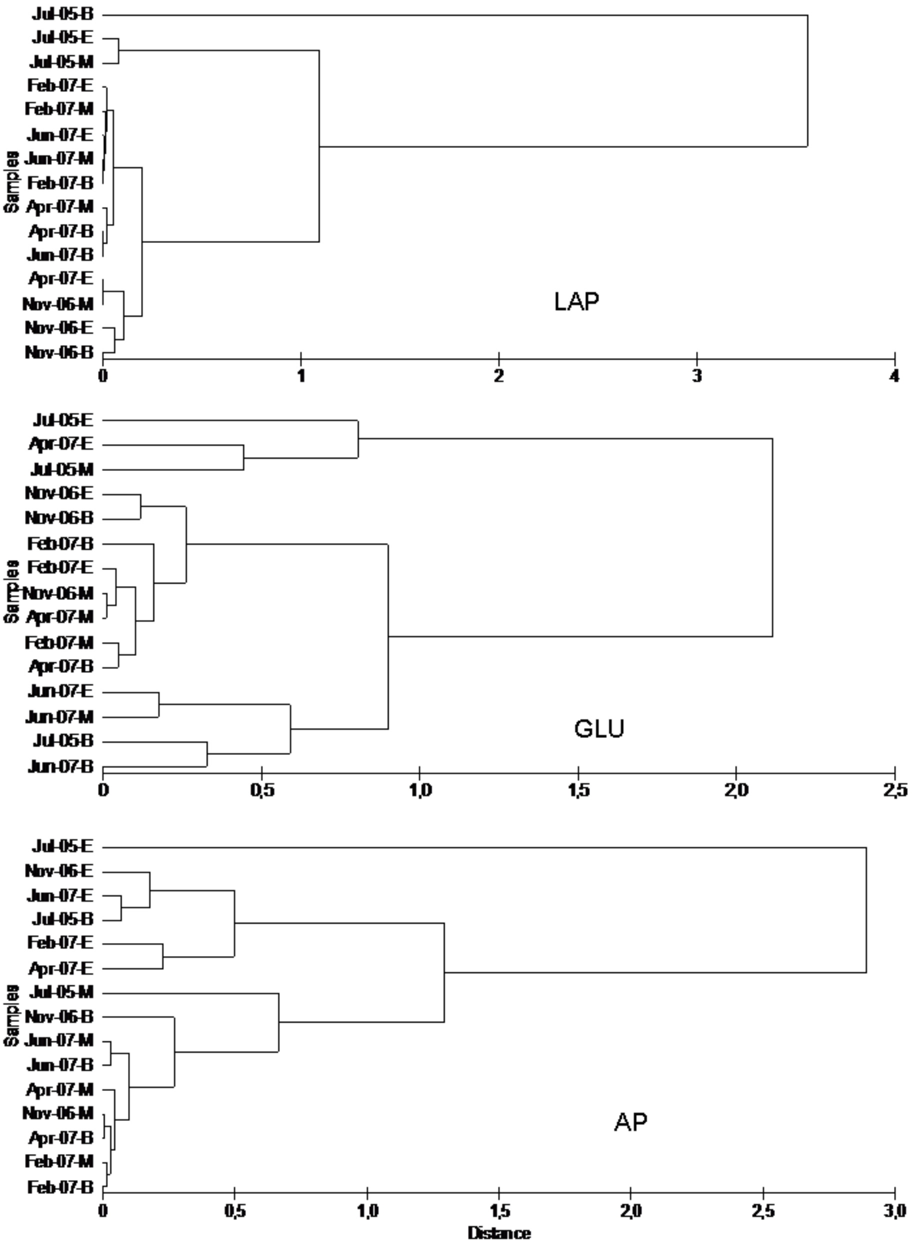

Figure 3: Cluster analysis of the enzyme data obtained in the Tyrrhenian Sea. E, epipelagic; M, mesopelagic; B, bathypelagic layer.

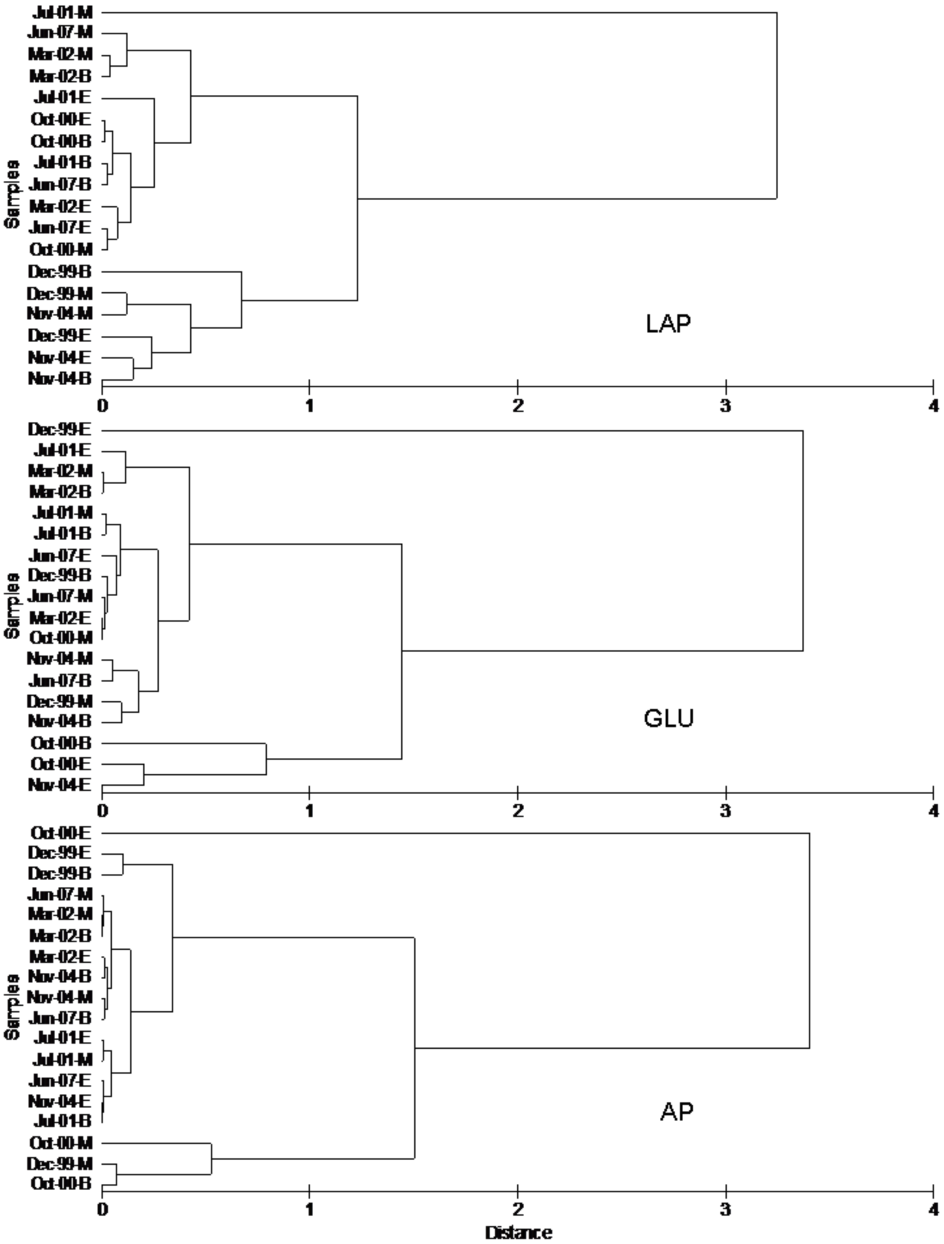

Figure 4: Cluster analysis of the enzyme data obtained in the Ionian Sea. E, epipelagic; M, mesopelagic; B, bathypelagic layer.

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The Impact of Plankton Communities over the Northern Adriatic Pelagic Ecosystem

G. Socal[1], F. Acri[1], M. Bastianini[1], F. Bernardi Aubry[1], F. Bianchi[1], E. Camatti[1], A. Conversi[2], A. De Lazzari[1], A. Pugnetti[1]
1, Institute of Marine Sciences, CNR, Venezia, Italy
2, Institute of Marine Sciences, CNR, Pozzuolo di Lerici (SP), Italy
giorgio.socal@ismar.cnr.it

Abstract

The research activity of ISMAR on plankton communities of the Northern Adriatic Sea is described here. The results derive from data that have been gathered in 40 years of oceanographic cruises. Spatial and temporal variations of abundance and species composition of phytoplankton and zooplankton communities have been studied in relation with meteorological forcings and hydrochemical conditions, e.g. water column structure, chlorophyll *a* concentrations and nutrient availability, with the aim of: i) highlighting the dominant scales of variability in biomass and species composition, ii) recognizing the possible relationships between environmental parameters, plankton biomass and specific changes, iii) identifying long-term signals of variability. The link between plankton communities and some environmental emergencies (mucilage events, discoloured waters, presence of potentially toxic algae) is also evaluated.

1 Introduction

In this paper we report and review the research activity of ISMAR in the study of phytoplankton and zooplankton communities of the Northern Adriatic Sea (NAS) during the last 40 years. This area is located between the Gulf of Trieste (46°N) and the imaginary line from Ancona, in Italy, to Zadar, in Croatia (approx. 44°N), and it is characterized by overall shallow depth (average = $\div$ 35 m). In the Mediterranean context, these features make the NAS as the northernmost area as well as the most extensive region of shallow water in the Mediterranean Sea: consequently, the hydrography of this basin is strongly affected by the atmospheric regime. The largest Italian river, the Po river, discharges its waters in this sub-basin (1918-2006 average flux = 1492 $\mathrm{m}^3 \cdot \mathrm{s}^{-1}$), after having crossed the most developed region in Italy in terms of inhabitants and industrial and agricultural activities. For this reason, the NAS is a site of intense biological activity and a good example of how the marine ecosystem copes with pollution, eutrophication, climate change and other human mediated activities [1]. Since the ‘1960s, ISMAR carried out several oceanographic cruises in this basin, in the framework of several national and international projects. Spatial and temporal variations of plankton community structures have been studied in relation to meteorological forcing and hydrochemical conditions, such as water column structure, nutrient availability and chlorophyll *a* concentrations, with special emphasis on the seasonal cycle of the main autotrophic and heterotrophic pelagic taxa. In this paper, the long-term available information about hydrology, chemistry and plankton communities of the NAS over the last 40 years is reported. The main aims are, first of all, to evidence the dominant scales of variability in biomass and species composition, recognizing the possible relationships between environmental parameters, plankton biomass and specific changes then, to identify long-term signals of plankton variability. Finally, the link between plankton communities and the main environmental emergencies (mucilage events, discoloured waters, presence of potentially toxic algae) is evaluated.

2 Spatial and seasonal variability of plankton communities

The vertical stability of the water column, from spring to late autumn, plays the main role in driving the species association and succession of plankton communities in the upper mixed and in the deep layers. Large abundance differences, sometimes by more than 1 order of magnitude, for both phytoplankton and zooplankton, are often observed during the stratification period from surface to the bottom, also in such a shallow water column (Figures 1 and 2). During the stratification period, the Po river discharge drives the marine salt content in the surrounding surface area, leading to salinities that may range from values below 35 to 38, in flood or low water conditions, respectively. The intermittent freshening of surface waters, coupled with high continental nutrient inputs, triggers the bloom of phytoplankton, with a general W-E decreasing gradient, from Italy to the Croatian coast [2, 3, 4]. Centric and pennate colonial diatoms belonging to the genera *Cerataulina, Chaetoceros, Leptocylindrus, Pseudo-nitzschia, Asterionellopsis* and small flagellates are generally dominant in the spring, summer and autumn communities. The extension of the Po river plume, which is regulated mainly by atmospheric factors (precipitation and wind regimes), modulates the seasonal growth of phytoplankton, leading to a marked interannual variability of biomass and availability for the grazers (Figure 3). In general, periods of intense phytoplankton biomass growth lead to intense swarms of pelagic microscopic grazers ($< 200\ \mu$m) - such as heterotrophic nanoflagellates ($<$ 20 μm), heterotrophic dinoflagellates, aloricated ciliats and tintinnids, copepods and cladocerans in the mesozooplankton assemblage ($> 200\ \mu$m). Low diversity and high biomass characterize the zooplankton populations in the coastal area, while in the offshore zone the zooplankton community is relatively stable, without predominance of single species [5, 6]. Unfortunately, there is little information on the smallest fraction of NAS zooplankton. A good analysis of the microzooplankton seasonal cycle has been realized by Fonda Umani et al. [7], who describe the main taxa recurring during the year; these communities are generally dominated by aloricate ciliates, showing spring-summer maxima. During the summer period, in the northernmost part of the basin mesozooplankton is dominated by strictly neritic species with the prevalence of a mixture of coastal species such as the copepod *Paracalanus parvus*, *Acartia clausi* and the cladoceran *Penilia avirostris* above the pycnocline, and in the deeper water by cold species as

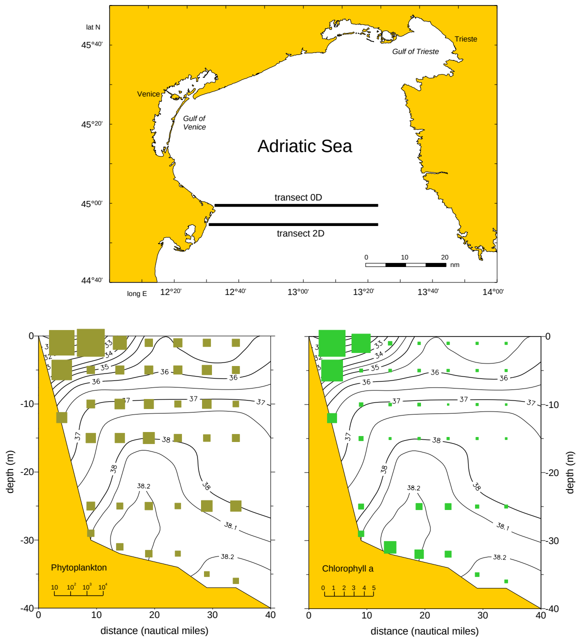

Figure 1: Adriatic Sea: study area (above). Summer vertical distribution (August 1979; below) of phytoplankton abundances (left, cell$*10^5$ l^{-1}) and chlorophyll *a* (right, $\mu\mathrm{g} \cdot \mathrm{l}^{-1}$) along transect 0D at 45°N. Symbols are proportional to the proposed magnitude and superimposed to the salinity distribution.

Pseudocalanus parvus and *Temora longicornis* [8, 9]. In the surface waters, the copepod *A. clausi* dominates most of the year, comprising at some points $> 80\%$ of the total biomass, followed by *P. avirostris*, which can account for $> 37\%$ in summer. However, in recent years the structure of the zooplankton community seems to have changed, with *P. parvus* beginning to overtake *A. clausi*, and with *P. avirostris* presence expanding in the entire northern basin [10, 11]. High Po river discharges may lead to intense phytoplankton blooms also at the end of their growing season, as in October-November 2000, when an impressive bloom of the pelagic diatom *Chaetoceros compressus* (up to 50 x 10^6 cells $\cdot \mathrm{l}^{-1}$) was observed all over the basin after an exceptional Po river flood (Oct-Nov average = 4976 $\mathrm{m}^3 \cdot \mathrm{s}^{-1}$ [12]) after this event an exceptional growth of the copepod *A. clausi* has been observed in the basin in the winter 2001 [6].

In winter, a regime of high vertical instability characterises the basin, with the presence of mixed waters offshore and of a western coastal front, with a partial thermohaline stratification. These features, together with low irradiances and temperatures, lead to a general decrease of phytoplankton biomass [13, 3]. In the offshore area, phytoplankton is frequently

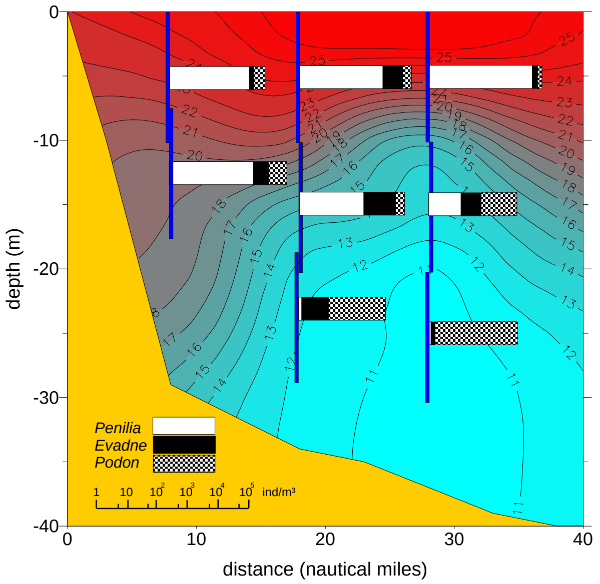

Figure 2: Summer vertical distribution (July 1973) of the cladocerans *Penilia*, *Evadne* and *Podon* in relationship to the temperature field at 44°55'N (transect 2D in fig. 1). The horizontal columns represent the abundance of the three genera.

dominated by the coccolitophorid *Emiliania huxleyi*. In late winter (February and March), when waters are calmer and the photoperiod lengthens, intense coastal blooms of the centric diatom *Skeletonema marinoi* (previously *S. costatum*), producing brown discoloration of waters, can be observed within the western coastal front [14, 15, 3]. In winter also microzooplankton and mesozooplankton are generally present in low abundances, with most homogeneous spatial distribution; the communities are dominated by aloricate ciliats [7] and by the copepods *Oithona, Clausocalanus, Calanus* and *Oncaea* (Figure 4 [16, 6]).

A detailed analysis of phytoplankton species composition both in nearshore and offshore waters (years 1992-2006 [17] shows that seasonal and spatial changes of abundance and biomass can be explained by environmental forcings (physical and chemical variables), whilst the species community structures is lead by the life cycles. Taxonomic composition, in fact, seems to be influenced by endogenous clock and phenology, rather than by spatial and temporal variations of abiotic parameters. Only quite recently, an analysis of the size spectrum of phytoplankton community has been carried out. In particular, a study of autotrophic picoplankton (< 2 μm, mainly represented in the NAS by the cyanobacteria *Synechococcus*) evidenced the importance of this size class, which may even dominate over nano- (2-20 μm) and microphytoplankton (> 20 μm). In addition, from summer to early autumn

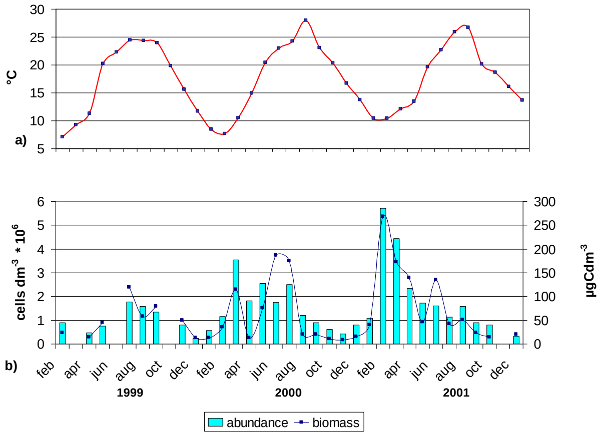

Figure 3: Temporal evolution of (a) surface temperature and (b) total phytoplankton abundance and biomass, 1999-2001: values represent the averages for the whole sampling area.

a shift from large to small cells is often observed in oligotrophic areas (characterized by high temperature and salinity and low nutrient concentration [18]). Pugnetti et al. [19] perform an analysis of the carbon partitioning in plankton biomass, demonstrating that nutrients, charged by the rivers, can impose a large interannual variability on the autotrophic/heterotrophic biomass ratio; when oligotrophy prevails, plankton community shows heterotrophic biomass (including bacteria) exceeding that of autotrophs, leading to a reversed trophic pyramid.

3 Long term trend of plankton communities

An analysis of the chlorophyll variations in the years from 1970 to 2007 at basin scale [20], with the help of satellite-derived information, demonstrates a generally declining trend of phytoplankton biomass concentration, reaching a decreasing value of about 0.11 μg l-1 per year in the last decade. The authors attribute this to a corresponding reduce in nutrient concentrations in the basin, indicating that the still common perception of the Adriatic Sea as a very eutrophic basin is no longer appropriate, at least for its northern part and in recent years. Through the analysis of time series, with regard to mesozooplankton we observe significant changes in community composition at the time scale of approx. 40 years, mostly due to a general decrease of *Acartia clausi*, as dominant species, being replaced by *Paracalanus parvus*. When compared to past records, *Penilia avirostris* swarms appear to have extended their temporal occurrence too. These changes might be related to the observed general increase of the average sea water temperature and the reduction of phosphate in the NAS [21, 6, 10]. Copepod time series

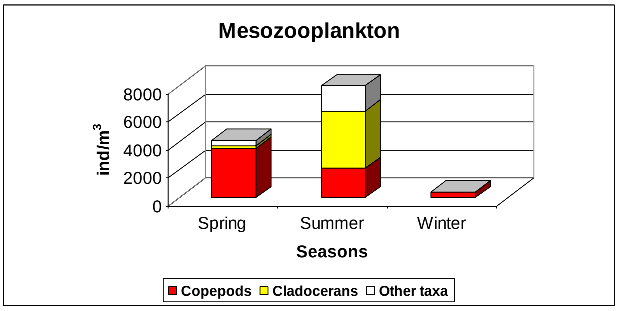

Figure 4: Seasonal averages of abundance of the main taxonomic groups of mesozooplankton. Data collected during PRISMA 1 project in the Northern Adriatic Sea (1995-1996).

in the Gulf of Trieste and environmental variables has been analysed by Conversi et al. [11]: the authors chose 1987 as boundary year, based on a review of the Mediterranean circulation and on the analysis of the winter SST. They showed that the whole copepod community in the area underwent a substantial transformation after that year. This included an approximate doubling in abundance, mostly due to the increase of smaller species (*Oithona, Oncaea*), changes in the phenology of the majority of the species, the decline of cold water species (in particular *Pseudocalanus elongatus*), the appearance of a new species (*Diaixis pigmaea*), and the rise of species found commonly in the southern basin of the Adriatic Sea (i.e., *P. parvus*). The Ionian gyre reversal of 1987, and the consequent augmented input of the North Atlantic surface waters in the Adriatic Sea, coupled with the general warming of the area, were hypothesized as possible explanations for this alteration in the copepod community.

4 Plankton communities and environmental emergencies

Recently a multidisciplinary oceanographic project was oriented towards the study of the mucilage occurrence in the Adriatic [22] many authors evidenced that the mucilage has a planktonic origin, although the mechanism by which the mucilage is formed is not fully ascertained. It has been suggested that mucilage would be due to aggregation of marine snow, formed after phytoplankton blooms, occurred in strong and prolonged termohaline stratification of the water column. Some authors have hypothesized a relationship between the mucilage event and unusually high Dissolved Organic Carbon (DOC) or colloidal matter accumulations [23, 24], although some components of Particulate Organic Carbon (POC), like transparent exopolymeric particles (TEP) and other submicron particles, have also been suggested to play a role in the mucus aggregation process [25, 26, 27, 28]. Phytoplankton species produce different mucous exter-

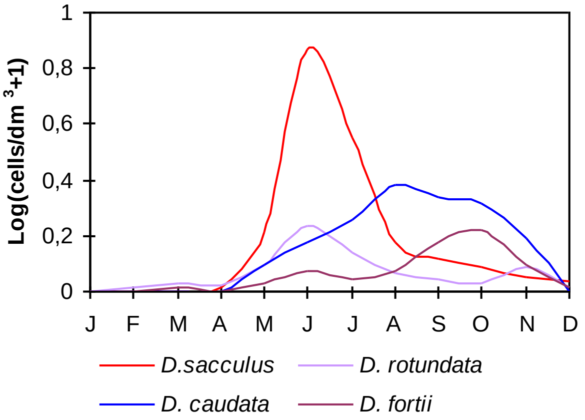

Figure 5: Annual cycle of the chief species of the dinoflagellate *Dinophysis* in the western coastal area of the NAS (1990-1996).

nal layers, which have different ecological and biological functions. If this production, in the form of cellular exudates, is in excess the mucous is spread in the water column like excretion. The loose of coupling between phytoplankton primary production and bacteria during mucilage events has been demonstrated by Fonda Umani et al.[29]: after the authors the larger fractions of produced Carbon flows in other path respect to grazing, sedimentation and storage in the water column thus Organic Carbon in excess derived form accumulated slow to degrade dissolved organic matter. An analysis of phytoplankton communities has been made over a large spatial-temporal scale in the basin in order to evaluate species composition in relation to mucilage formation [30]. The results demonstrated that the species composition inside the aggregates was similar to that observed in the water column outside of them; moreover, the phytoplankton species composition, (mainly diatoms) during the mucilage-event was comparable to that observed in summer without extensive mucilage occurrence. Conversely other authors [31], observed that during the mucilaginous formations in Adriatic, cells of the dinoflagellate *Goniaulax fragilis* were first present in seawater samples about 20 days before their appearance. Microscopic observations of mucilage samples provided evidence that *G. fragilis* was always present within the polysaccharidic material, and that it was more abundant in the earliest stages of the phenomenon. The definition of the role of this taxon in mucilage formation is still under discussion. Mucilage aggregates have different impact over zooplankton communities, acting in some ways as inhibitors for naupliar copepod populations and for grazing activity [7]. This is supported by grazing experiments with *Temora longicornis* and *Pseudocalanus elongatus*, which demonstrated that copepod feeding was inhibited by diatom exudates, precursors of mucilage formation [32].

Ecological monitoring has also been an

important tool for the evaluation of the potentially toxic algae in the basin. Dinoflagellates represent the taxonomic group with more frequent cause history of poisoning for the man in the world. In the Adriatic Sea the first episodes of poisoning by algal biotoxins through mussels was described in summer 1989 in the coastal area of Emilia and Romagna [33]. These events had been attributed to the release of okadaic acid by the dinoflagellate *Dinophysis*, a toxin able to produce Diarrheic Shellfish Poisoning (DSP). Fortunately these episodes were not so frequent in the basin in the last years; in fact our data, gathered in coastal and offshore systems, highlight that, especially from the 90's, the frequency of "red tides" by dinoflagellates was very rare. The growth and the diffusion of taxa of this phytoplankton macrogroup is generally hindered in those areas, characterised by high hydrodynamic variability as the NAS, especially northward of the Po river [15]. Studies on the seasonal dynamics of *Dinophysis* have been carried out in the coastal NAS [34, 35]. Results highlight the optimal period of growth for each *Dinophysis* species (Figure 5) and confirm that the growth of *Dinophysis* is favoured by stratification and low turbulence, induced by hydrodynamic calm. Conversely, illness by other toxins produced by dinoflagellates, such as *Alexandrium* and by the diatoms, such as *Pseudo-nitzschia* group, are still now not yet recorded up to now. However, when looking at long term data, it appears that the red tides and mucilage events have alternated during the decades. Mucilages have been noted in the beginning of the century (1903, 1905, 1920, 1930, 1949, possibly 1951), and, after disappearing for almost 40 years, they reappeared in 1988 and continued in 1989, 1991, 1997, 2000, 2002, 2004 and 2007. Red tides events on the other hand started in 1968 and affected the northern Adriatic each year, until 1987. After the reappearing of mucilages in 1988, there was another red tides season only in 1990 [10]. This alternance is possibly linked to a general change that affected the entire basin at the end of the 1980s, including biologic communities, marine circulation, and climate.

5 Conclusions

The work above highlights on one side the complexity of the NAS, and the sensitivity of the plankton component to climate change and anthropogenic impacts. As seen in other systems, circulation/climate may have a stronger effect in shaping planktonic communities than food sources. For example, while chlorophyll has generally decreased over the period from 1970 to 2007 at basin scale [20], the mesozooplankton abundance from 1970 to 2005 in the Gulf of Trieste has quasi doubled [11]. Temperature changes and associated changes in phenology may be very relevant. Therefore results underlines that a correct analysis of plankton communities can represent a diagnostic tool for the definition of the health of a marine ecosystem. The case study of the NAS is important because it involves extremely high in time and space biomass variations and represents a nucleus from which starts the one of the most relevant pulses for trophism in the Mediterranean. Finally these results also highlight the importance of long time series for discerning local anthropogenic impacts from large scale climate impacts.

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Impact of an Acoustic Stimulus on the Motility and Blood Parameters of European Sea Bass (*Dicentrarchus labrax* L.) and Gilthead Sea Bream (*Sparus aurata* L.)

G. Buscaino[1], F. Filiciotto[1], G. Buffa[1], A. Bellante[1], V. Di Stefano[1], A. Assenza[2], F. Fazio[2], G. Caola[2], S. Aronica[1], C. Buscaino[1]
1, Institute for Coastal Marine Environment, CNR, Capo Granitola (TP), Italy
2, Department of Experimental and Applied Biotechnology, University of Messina, Messina, Italy
giuseppa.buscaino@cnr.it

Abstract

The physiological responses of fish to underwater noise are poorly understood and further information is needed to evaluate any possible negative effects of sound exposure. We exposed European sea bass and gilthead sea bream to a 0.1–1 kHz linear sweep (150 dBrms re 1 μPa). This band frequency is perceptible by many species of fish and is mainly produced by vessel traffic. We assessed the noise-induced motility reaction (analysing the movements) and the haematological responses (measuring blood lactate, and haematocrit levels). The noise exposure produced a significant increase in motility as well as an increase in lactate and haematocrit levels in sea bream and sea bass. A linear correlation between blood parameters and motility in fish exposed to the noise was observed. The acoustic stimulus produced intense muscle activity.

1 Introduction

The impact of human activity on marine habitats can produce adaptive alterations and other significant changes in animals [1, 2, 3]. In recent years, many studies have been carried out with the aim of evaluating the effects of anthropogenic acoustic disturbance on marine organisms [4, 5, 6], thus increasing the awareness of the damage done to animals exposed to human related underwater sounds [7, 8]. These sounds are associated with shipping, seismic surveys, sonar, recreational boating and many other anthropogenic sources that are known to induce several types of responses in fishes [9, 10, 2, 11, 12, 13, 14]. Pacific herring (*Harengus pallasi*) exhibited alarm responses in reaction to motorboat noise [13]. Engås et al. [10] found that seismic shooting has effects on the local abundance and distribution of Gadus morhua and haddock (*Melanogrammus aeglefinus*). Smith et al. [14] examined the short- and long-term effects of increased ambient noise on the behaviour and hearing of goldfish (*Carassius auratus*). They noted that goldfish exhibited an initial startle response with a rapid burst of erratic swimming followed by general increased swimming activity with the onset of an experimental noise (bandwidth ranging from 0.1 to 10 kHz at 160–170 dB re 1 microPa total sound pressure level). Kastelein et al. [15] observed a behaviour response of sea bass exposed to pure tone signals ranging between 0.1 and 0.7 kHz at 0–30 dB above the hearing thresholds. Since acoustic signals in the 100–500 Hz band are detected by many species of fish [16] and there is an increase in this low-frequency ambient noise as a result of increased international shipping [17], it is safe to assume that these noises are having an impact on the welfare of many fish species. While the effects of such anthropogenic sounds on marine mammals have been described [2, 18, 7, 8, 19], the impact of underwater noise on marine fish is not understood sufficiently. Further information is needed to evaluate or predict any negative effects [3]. Previous studies have shown that acoustic stimulation can affect fish behaviour, but the physiological consequences have hardly been studied. Some studies have shown that acoustic stimulation can produce metabolic changes in fish. [14] pointed out a significant modification of plasma cortisol and glucose levels in goldfish after exposure to white noise. [4] demonstrated variations in cortisol, glucose, lactate, AMP, ADP, ATP and cAMP levels in different tissues of sea bass, indicating a typical primary and secondary stress response to air gun detonations. Wysocki et al., [20] indicated that ship noise constitutes a potential stressor for European freshwater fishes. The present study aims to investigate the motility and haematological responses of European sea bass and gilthead sea bream exposed to an experimental acoustic stimulus in a low-frequency range using analyses of movement, blood lactate and haematocrit values. The correlation between movement and blood parameters was also investigated.

2 Materials and methods

2.1 Study animals

The experiment was carried out from July to September 2008 at the Istituto per l'Ambiente Marino Costiero of Consiglio Nazionale delle Ricerche (IAMC-CNR) – Laboratories of Capo Granitola (Trapani, Italy) using 14 sub-adult European sea bass (*Dicentrarchus labrax*) weighing 189.4 ± 80 g with body lengths of 26.2 ± 3.3 cm, and 14 sub-adult gilthead sea bream (*Sparus aurata*) weighing 172.6 ± 23.7 g with body lengths of 22.9 ± 0.9 cm. Three months before the beginning of the experiment, upon arrival at the laboratories from the marine fish farm of Trappeto (Palermo, Italy), fish were placed in circular tanks (diameter: 3 m, depth: 1 m, volume: 5000 L) at a low stock density (5 $kg{\cdot}m^3$) with recirculated and filtered seawater. Fish were exposed to the natural photoperiod and fed daily with commercial dry pellets. Feeding was stopped at least 48 h before the experiment.

2.2 Acoustic stimulus

Most fish are able to detect sound and the range of hearing is from 100 to 500 Hz [16]. Keeping this in mind, as well as the fact that acoustic energy produced by vessel traffic is more intense at low frequencies [17], it was decided that an acoustic stimulus with a frequency band of 0.1–1 kHz would be used. A 1-second linear sweep was used to cover the frequency band. The linear sweep was repeated for 10 min without pause. The signals, generated

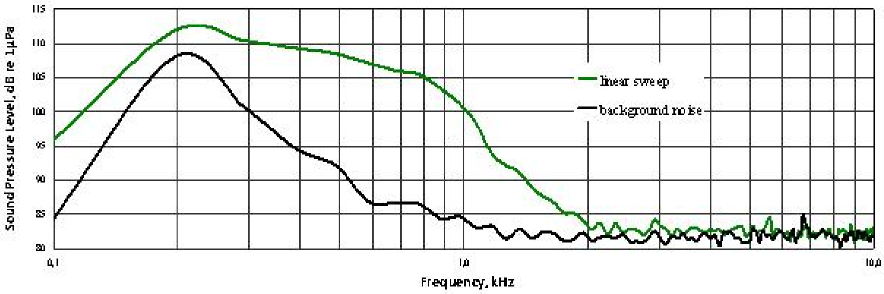


Figure 1: Mean power spectrum of sweep signal and background noise. The sampling frequency of the signal was 100 kHz. The size of Fast Fourier Transform (FFT) was 1024 points and the window type was cosine tapered.

by a waveform generator (Model 33220A, Agilent Technologies, Santa Clara, CA, United States), were amplified (Model PA-4000 Inkel, Chonan City, Korea) and emitted using an underwater moving coil loudspeaker (Model UW30, Lubell, Columbus, Ohio, USA) with a 100 Hz–10 kHz rated frequency response.

The sound pressure level [21] of the emitted signal was measured using an omni-directional calibrated hydrophone (TC4034, Reson, Slangerup, Denmark) positioned inside the cage (1.5 m deep) 5.5 m from the underwater speaker. Signals were pre-amplified (VP1000, Reson, Slangerup, Denmark) and were recorded using a DAQ card (Ni DAQCard-6062E, National Instruments, United States) using a sampling frequency of 100 kHz. Digital signals obtained were elaborated with a routine procedure developed by the Interdisciplinary Group of Oceanography (GIO at CNR-IAMC, Capo Granitola, Italy) using LabView rel. 7.1 (National Instruments, United States). The maximum sound pressure level of a single sweep was 150 dBrms re 1 μPa. The mean power spectrum and the spectrogram of 5 s of emitted signal are shown in Figures 1 and 2.

2.3 Experimental procedure

During the experimental phase, sea bass (n=14) and sea bream (n=14) were randomly assigned to control (n=7 sea bass; n=7 sea bream) and test (n=7 sea bass; n=7 sea bream) groups. For each test, a single fish was transported to an experimental sea cage (see Figure 3) located in a circular natural harbour (with a diameter of about 200 m and a depth of 3 m) and left there to acclimate. A research cabin was placed 8 m away from the sea cage. The cabin housed the sound generator, as well as video and sound recording equipment. One hour later, the specimen was recorded (both audio and video) for 10 min with an acoustic acquisition system and two underwater video cameras (model RE-BCC6L, DSE, Italy) mounted outside the cage. One camera was mounted on the middle of the cage and the other camera was mounted on top of the cage (Figure 3) so that the entire cage was visible. During video and sound recording, individuals in the test group

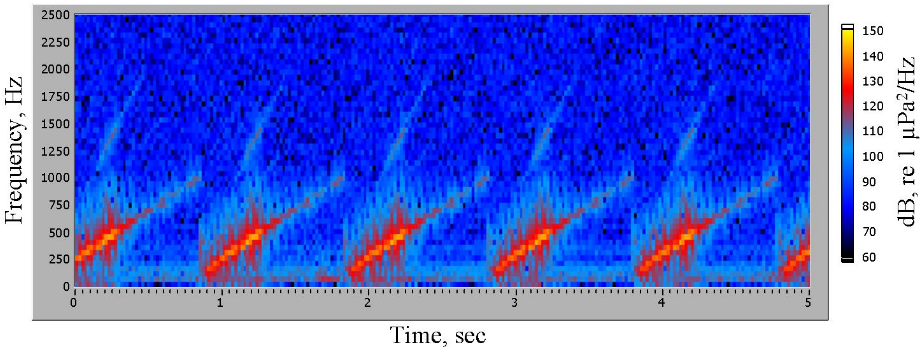


Figure 2: Spectrogram of the sweep signal. Time in seconds and frequencies in Hertz are shown along the x-axis and y-axis, respectively. Amplitude was measured in dB (re 1 μPa) using a colour scale. The sampling frequency of the signal was 100 kHz, the size of FFT was 2048 points and the window type was cosine tapered.

were exposed to an acoustic stimulus while members of the control group had no stimulus applied. The fish were then captured with a net to take blood samples. After this, the fish were transferred into a small tank and then released after recovery. The lactate and haematocrit levels were measured in the fish blood samples, while fish motility was evaluated using the video recordings (see Section 2.5). Blood parameters and motility data recorded in the control and test group of sea bass and sea bream were statistically analysed to assess possible differences between the groups. Moreover, a statistical correlation was applied to the total number of movements and blood parameters. The entire work presented here complied with current regulations regarding animal experimentation in Italy.

2.4 Blood sampling procedure and chemical analyses

A standardized handling procedure for each sea bass and sea bream was applied in order to standardize the potential stress produced during the blood sampling. After 10 min of audio and video recording, the fish was captured with a net from the experimental cage and immediately anesthetized with 2-phenoxyethanol (0.4 ml L-1) in a 10-litre tank. Fish reached stage V of anaesthesia [22] within 1–2 min after which they were weighed and measured. Later, blood samples were collected from the caudal vein (using a 2.5 ml syringe with a 22 G x 1 ½” needle) for the immediate assessment of lactate on whole blood with a portable blood lactate analyser (Accusport, Boehringer Mannheim, Germany). The time between capture and blood collection was less than 5 min. A Select-A-Fuge Model 24 blood micro haematocrit centrifuge (Bio-Dynamics, Inc., Indianapolis, United States) running at 3600 rpm for 5 min was used to assess the haematocrit value.

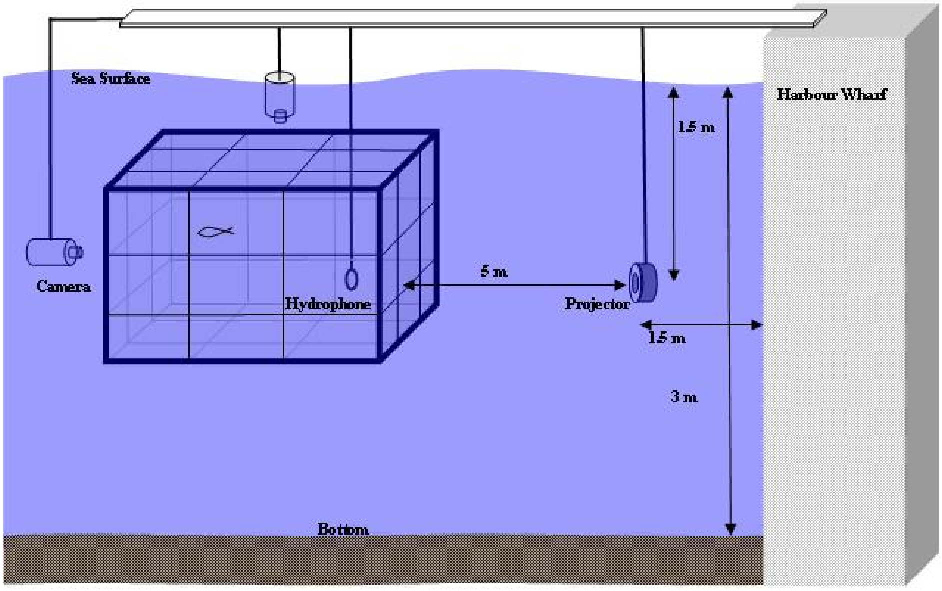


Figure 3: A schematic view of the sea cage with two underwater video cameras, a hydrophone inside the cage and the underwater speaker.

2.5 Motility sampling procedure

Two grids were affixed on the sides of the sea cage monitored by the cameras, each comprising nine regions, to analyse the movements of fish along the three axes of space (see Figure 3). The images from the two cameras were synchronized and fish movements were analysed from the video recordings. The transit of the specimen from one region to another region of the grid was recorded.

A focal animal sampling technique modified from Altmann [23] was used to analyse the images in slow motion. For each minute of sampling, we recorded a value (as sum of the movement across the x, y and z axes) for a total of 10 values for each specimen.

2.6 Statistical analysis

An unpaired t-test was used to determine significant differences in blood lactate and haematocrit levels as well as differences in movement (as the total movement during each minute of sampling for all specimens) between the control groups and the test groups. A linear regression model ($y = a + bx$) was applied to the total movement during the 10 min of sampling and the values for the blood parameters (lactate and haematocrit) of each fish in order to determine the degree of correlation in the control and test groups. A $P < 0.05$ was considered statistically significant.

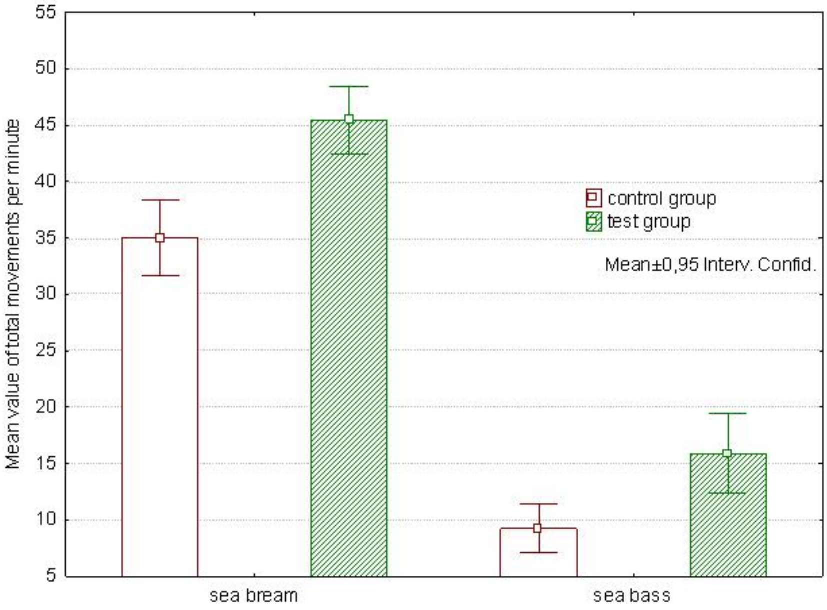


Figure 4: Mean values (±interval of confidence, p=0.05) of total movement per minute for sea bream and sea bass.

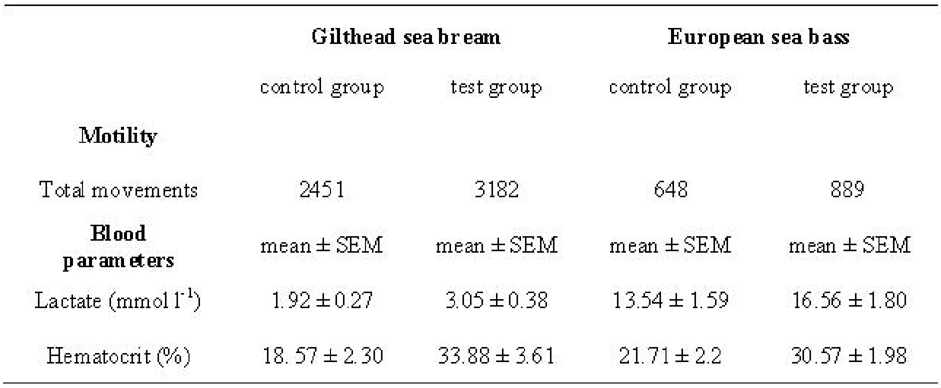
3 Results

3.1 Motility

The results obtained during the motility sampling procedure showed that the amount of movement observed in the test groups of sea bream and sea bass were significantly higher ($P < 0.0001$ and $P < 0.001$, respectively) than those of the control groups. The total movement for the control and test groups of sea bream and sea bass are shown in Table 1 while the mean values of total movement per minute and the statistical significance are shown in Figure 4.

3.2 Blood parameters

In sea bream, blood lactate exhibited significantly increased values ($P < 0.05$) in the control group compared to the test group (difference of 1.13 (mmol·l^{-1}). In sea bass, blood lactate showed significantly higher levels ($P < 0.01$) in the test group. In both species, significantly higher levels of haematocrit were recorded for the test group compared with the control group (sea bream: $P < 0.01$; sea bass: $P < 0.05$), with an increase of 15.3% and 8.9% for sea bream and sea bass, respectively. Mean blood lactate and haematocrit levels of sea bream and sea bass are shown in Table 1, while the mean values and the standard error are shown in Figure 5.



	Gilthead sea bream		European sea bass	
	control group	test group	control group	test group
Motility				
Total movements	2451	3182	648	889
Blood parameters	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM
Lactate (mmol l^{-1})	1.92 ± 0.27	3.05 ± 0.38	13.54 ± 1.59	16.56 ± 1.80
Hematocrit (%)	18. 57 ± 2.30	33.88 ± 3.61	21.71 ± 2.2	30.57 ± 1.98

Table 1: Total movement and means ± standard error of the mean (SEM) for blood lactate and haematocrit levels in the control and test groups of gilthead sea bream and European sea bass.

3.3 Correlation between movements and blood parameters

In the control groups of both species, the application of a linear regression showed no significant correlation between the values for total movement and the values for the blood parameters. The total value of the movement for the sea bream and sea bass test groups had a significant correlations with the values of blood lactate and haematocrit levels (Figure 6).

4 Discussion

The present study was conducted in a confined space, so that the obtained fish responses may be different to those observed in the wild. The analysis of sea bream and sea bass motility showed increased swimming activity, indicating a disturbance due to the acoustic stimulus. The blood parameters corroborated the motility observations for both species. The blood parameter changes and motility responses reflected an intense metabolic activity involving white muscle anaerobic fibres during the acoustic exposure. The muscle metabolism of fish has been thoroughly investigated in the past [24, 25, 26]. Fish, like other vertebrates, have several types of muscle fibre with different properties. Two fibre types are most important in locomotion: red or slow oxidative fibres (type IIb; low power system) and white or fast glycolytic fibres (type Ia capacity-limited, high-power system). Aerobic red muscle is used for routine swimming while anaerobic white muscle is used for bursts of activity (sprints and faststart Bennett and Licht, [27, 28, 29]. Anaerobic pathways can also fuel intermediate or prolonged swims that can be sustained for few minutes, but these pathways eventually tire the fish [30, 25]. In fact, white muscle glycogen reserves can be depleted by 50% in only 2 min of forced activity [28]. The capacity for anaerobic energy production has been estimated from decreases in substrate reserves and end products accumulation with a conversion of glucose to lactate followed by stimulation of pyruvate metabolism and oxygen debt [28, 31, 32, 33, 29]. Lactate produced in the myotome is released into the circulatory system following bursts of anaerobic activity. In the present study, the statistically significant responses in motility and blood lactate observed in both sea bass and sea bream exposed to the acoustic stimulus indicate increased muscle activity using anaerobic Ia fibres. Furthermore, the increase of metabolic muscle activity implies a higher demand for oxygen, which is increased by increasing the respiratory rate. During this adaptive response, fish experience the haemopoietic activity of the spleen that encourages the production of red blood cells for oxygen transport [34]. This condition leads to an increase in the levels of blood corpuscular components such haematocrit, one of the most reliable indexes. This increase was also found in the present study (Figure 5). The significant correlation between the movement values and the selected blood parameters (Figure 6) supports what has been described above.

5 Conclusions

In conclusion, the results from this study showed that anthropogenic noise at low frequencies can influence the swimming activity of fish. Moreover, many previous studies have shown that muscle activity rates can be a very large part of the fish energy budget [35, 36]. Consequently, increased swimming activity and the associated metabolic costs could compromise other biological activities, such as food acquisition, regulation due to environmental perturbation, migration and reproduction. Banner and Hyatt [37] and Lagardère [38] observed a drastic reduction of egg survival and reproductive and growth rates in farmed fish species exposed to high sound levels. The present study shows the relationship between behaviour and haematological parameters in relation to noise exposure. It also shows that the use of different study approaches (physiological or behavioural) can lead to similar results. Moreover, our results indicate that our real-time field assays were appropriate for measuring the physiological impact of the acoustic stimulus in sea bass and sea bream. Although this short-term noise experiment showed an increase in fish motility, lactate and haematocrit values, it would be useful to carry out future tests with a similar experimental procedure on other fish species using longer-term noise exposure to better understand the impact of anthropogenic noise on fish behaviour and physiology.

6 Acknowledgments

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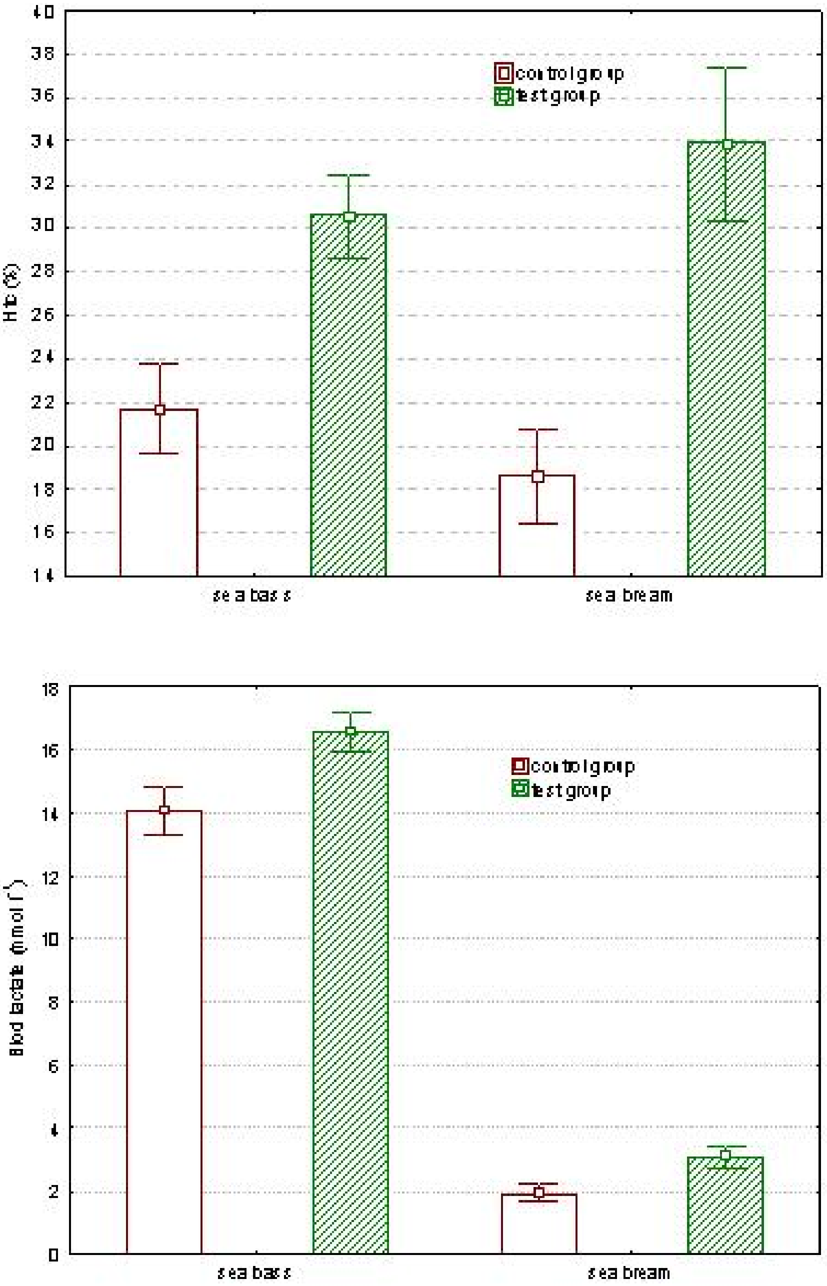

Figure 5: Mean values (±standard error of the mean) of haematocrit levels and blood lactate for sea bream and sea bass.

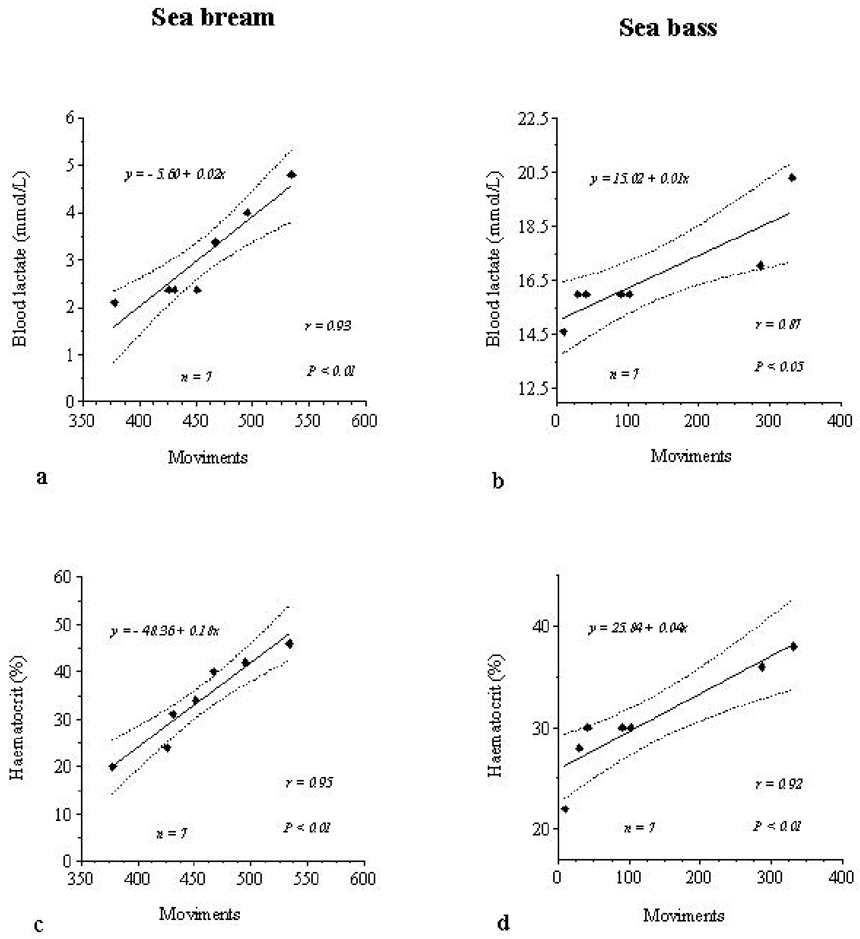

Figure 6: Linear regression between the total number of movements and blood lactate (mmol·l^{-1}) (a) and haematocrit levels (%) (b) in the gilthead sea bream test group; (c) and (d) are the same measurements for the European sea bass test group. The dashed lines delimit the 95% confidence interval.

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Acoustic Characterization of Middle Trophic Level Species in the Ross Sea Ecosystem (Antarctica)

I. Leonori, A. De Felice, I. Biagiotti, F. Campanella, M. Azzali
Institute of Marine Sciences, CNR, Ancona, Italy
i.leonori@ismar.cnr.it

Abstract

By far the largest components of the Ross Sea Middle Trophic Level are the oceanic (*E. superba*) and the shelf (*E. crystallorophias*) krill species. In spite of their abundance and key role in Ross Sea trophic chain, they have received very little attention from the scientific community before the six large scale acoustic surveys conducted during the Italian expeditions to Antarctica of 1989-90, 1994, 1997-98, 2000, 2003-04. The region sampled acoustically is roughly a rectangle of 131000 nm^2, 64% of the Ross Sea. A three frequency method, adequate to the biological and environmental situation of the Ross Sea, much more complex than that of Atlantic sector areas, was developed. The estimation of *E. superba* biomass was around 2.5 millions of tons in Nov. 1994, around 2 millions of tons in Dec. 1997 and 1 million of tons in Jan. 2000. The decreasing of *E. superba* biomass in the Ross Sea from Nov. to Feb. occurred in parallel with the movement northwards of the polynya ice edge. This suggests that *E. superba* habitat enlarges to the Pacific Ocean, and portions of this population of the Ross Sea spread into the Pacific waters, beginning from the second half of Dec. On the other hand the total biomass of *E. crystallorophias* was rather stable ($\sim$200000 t) in the five surveys. It suggests that ice krill population is segregated within the Ross Sea.

1 Introduction

Ross Sea is the last marine ecosystem not yet exploited by man. It is covered by ice for nearly nine months in a year and the distribution, abundance and behaviour of all the species in this region are regulated only by natural factors. The sea ice expands from late February onwards, and retreats from late October. Both processes start near the front of the Ross Ice Shelf. The water properties and circulation in the Ross Sea are well known from extensive surveys over the last few decades.

The circulation separates the Ross Sea in two regions: (1) the inshore region with depth < 500 m and extension around 30% of the entire region, it is dominated by a wind driven gyre [1, 2]; (2) the offshore region that is dominated by processes in the wider Southern Ocean. This circulation means that Ross Sea is somewhat separated from the rest of the Southern Ocean. The Ross Sea is bounded to the North by the 3000 m depth around 69° S latitude and 160° W longitude. The extension of the total region is calculated in 205000 nm^2. The area of this study is the East part of the

Ross Sea that is around 64% (i.e. 131000 nm^2) of the entire region (Figure 1).

The trophic chain in the Ross Sea is relatively simpler than that in the ecosystems at lower latitudes. By far the largest components of the Ross Sea Middle Trophic Level are the oceanic species of krill, *Euphausia superba*, and the shelf species of krill, *Euphausia crystallorophias*.

The two krill species are closely related genetically [3, 4] and similar in behaviour [5] and are probably in competition. They have similar feeding appendages [6], and both feed on phytoplankton [7], though they may have different food preferences.

The current scientific opinion is that *E. superba* prefers turbulent environment habitats such as continental slopes, where marine currents mix, and it is seldom found in shallow waters or close to ice, such as the Ross Ice shelf, in which the smaller *E. crystallorophias* predominates [8, 9, 10].

However, many important questions remain. What are the boundaries of the two krill populations? How are the biomasses of the two krill populations distributed within their boundaries, and how great is their degree of overlap? What are the spatial and temporal relationships between the distributions of the two krill populations and that of both sea ice and phytoplankton? The importance of dispersion and dispersion rate in the interactions of two competing species in spatially heterogeneous environments is well known [11].

The aim of this study is to answer some of those questions and thus to gain a deeper understanding on mechanisms that allow the coexistence of the two krill species in an area where the physical, spatial–temporal relationships between two euphausiid species, chemical and biological parameters are particularly variable due to the ice dynamics [12]. This problem is important not only under an ecological point of view, but also for the future management of krill fisheries in the Ross Sea.

Until 1989, the scientific opinion was that the Ross Sea was inhabited almost exclusively by *E. crystallorophias* [9]. The six acoustic surveys carried out within the PNRA projects from 1989 to 2004 have shown that the most abundant krill species is in fact *E. superba* [13], with an average biomass of about 2 million tons, which is about 10 – 15 times that of *E. crystallorophias*. Both species are eaten by predators, but only *E. superba* is used for human food. There are no fisheries for *E. superba* at present in the Ross Sea, but this could change in the near future, altering the strategies of coexistence between the two krill species with completely unforeseeable effects.

The spatial scale chosen for this study is the region of the Ross Sea where the environmental gradients have their maximum values from November to January. This paper firstly describes the methods used for the acoustic surveys, then presents data on the spatial distributions of krill, focusing on key differences between the two krill species, and finally provides a possible mechanism which allows the coexistence of these two species in the Ross Sea [14, 15].

2 Material and Methods

The data presented in this paper are related to the region of the Ross Sea (Figure 1) where the temporal variability in the water column associated with ice-edge melting and recession is particularly strong [12]. The region sampled acoustically is roughly a rectangle delimited by latitude 69° 30' and 78° 06' S (516 nm; 955 km) and by

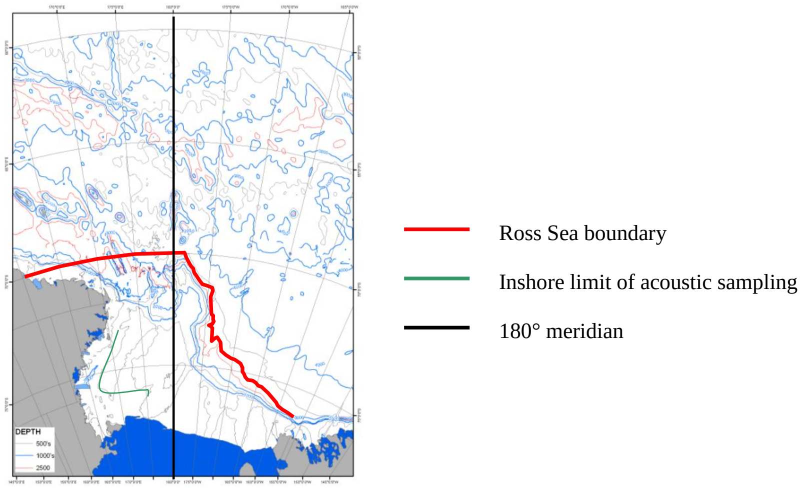

Figure 1: Study area.

longitude 164° 30’ E and 175° 30’ W (extending 248 nm, 460 km, at the southern end, and 411 nm, 762 km, at the northern end). It was surveyed acoustically six times in different conditions of ice cover, in 1989, twice in 1994, and then in 1997, 2000 and 2004.

Data on this region come from four large-scale surveys carried out from 1994 to 2000 in very different ice coverage situations (from 5% to 90%). The first survey was conducted from 9 November to 10 December 1994, behind an icebreaker, in conditions of almost 100% ice cover (Figure 2a). The second survey was carried out from 17 to 28 December 1994 in conditions of partial ice cover (Figure 2b). In both surveys, acoustic data were collected using a BioSonics 102 scientific echosounder, operating at 38 and 120 kHz, and a dual beam echosignal processor (BioSonics ESP), running on a personal computer [13].

The 1997 survey was conducted from 7 December to 5 January in conditions of partial ice-cover (20-30%, Figure 2c), the 2000 survey from 16 January to 7 February in almost ice-free waters (ice-cover 5%, Figure 2d) [14] and the 2004 survey from 28 December to 27 January with 40-50% ice-cover. In the last three expeditions, acoustic measurements were carried out using a Simrad three-frequency echosounder (38 and 120 kHz split-beam transducers and 200 kHz single beam transducer). Data were recorded and processed on an HP9000/715 workstation with BI500 software.

The three-frequency method was applied for the identification and separation of *E. superba* and *E. crystallorophias* swarms [16]. On the basis of this method, the probability of correct classification of an unknown swarm is estimated at > 0.90. About 91% of the *E. superba* swarms and 97% of *E. crystallorophias* swarms sam-

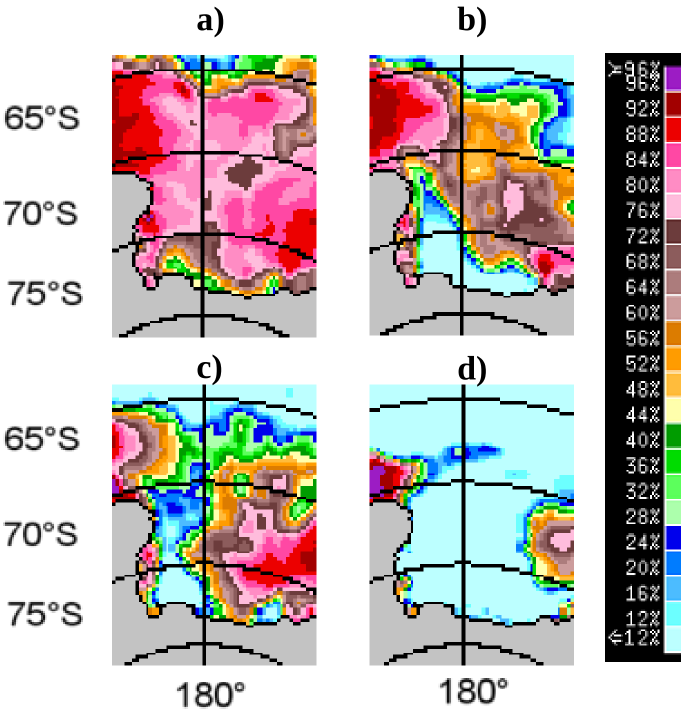

Figure 2: Sea-ice concentrations in November 1994 (a), December 1994 (b), December 1997 (c), and January 2000 (d), respectively. Satellite images representing ice-coverage in the months of the acoustic surveys were downloaded from the National Snow and Ice Data Center.

pled by the net during the last expeditions were identified correctly by this method.

The mean length of the two species was determined from the trawls data. Hauls were carried out along the routes, using a 5 m^2 Plankton Hamburg Net (HPN). The number and position of the hauls were different in these four expeditions because of the difference in ice cover. During the two 1994 surveys, 23 hauls each of 60 min duration were carried out: eight in the first survey and 15 in the second survey. During the 1997 and 2000 surveys, 35 hauls of 60 min duration and 63 hauls of 30 min duration were conducted, respectively [17, 18]. At each sampling site, euphausiids were identified and counted. If the catch was large, the mean length of each species was determined from a random sub-sample of 100 individuals. Otherwise, the mean lengths were determined from measurements of all the individuals.

The biomass of the two krill species was calculated at 120 kHz from the mean

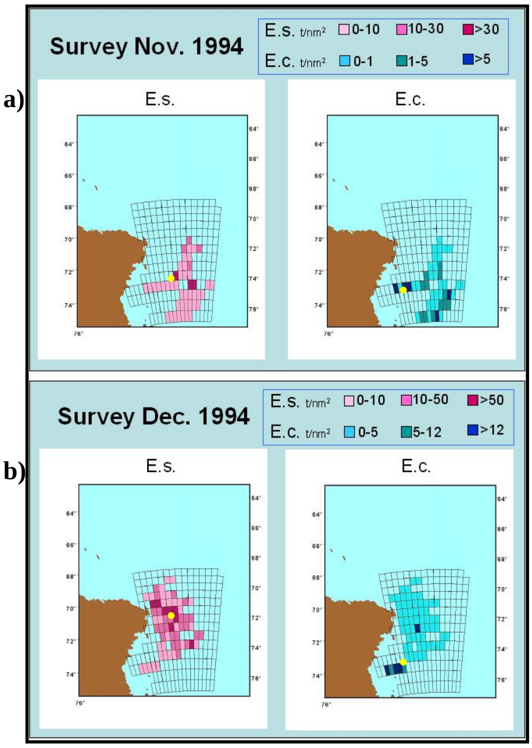

Figure 3: Spatial distribution of *E. superba* and *E. crystallorophias* during the four surveys carried out in November 1994 (a), December 1994 (b). The yellow circle in each map indicates the biomass centroid.

volume backscattering strength, using the weight/length relationships of each species [19] and the backscattering cross-sectional area of krill.

The geographic distribution of the biomass was estimated for each species using the Elementary Statistical Sampling Rectangle (ESSR) method of MacLennan and Simmonds [20]. The surveyed area was transformed into a lattice of rectangles of constant area (600 square nautical miles or about 2058 km^2), spaced 1° in longitude and with variable intervals in latitude (Figures 3, 4). The area under examination was filled with 220 rectangles. The number of sampled rectangles was 53 (23%) in November 1994, 62 (27%) in December 1994, 66 (29%) in December 1997, and 101 (44%) in January 2000. The distance travelled in each rectangle was 46 – 65 km (20 – 30 nm). It is assumed that the measured krill densities in each rectangle are representative of the rectangle within which they were collected. An aggregate of rectangles containing at least 90% of the total biomass of one krill species was

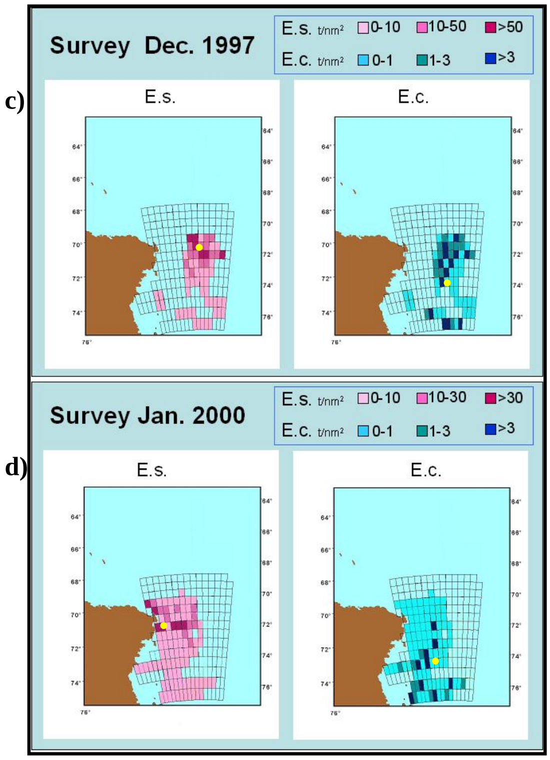

Figure 4: Spatial distribution of *E. superba* and *E. crystallorophias* during the four surveys carried out in December 1997 (c) and January 2000 (d). The yellow circle in each map indicates the biomass centroid.

called the domain of that species. The southern and northern borders of the domain were called species boundaries.

Two types of measurements were calculated from the sampled rectangles in the lattice [21]:

- the positions of the centroids were calculated to represent the spatial distributions of the biomass;
- the distances, and the change in distances, between the centroid positions were calculated to represent the difference in the spatial biomass distribution between the two species over time.

The centroid is the point of balance of the biomasses in the surveyed area. It has two components (Xc, Yc), denoting the column and row positions of balance of the biomasses. The two numbers are expressible by:

$$X_c = \left(\sum_j \sum_i xiBi,j \right) /B;$$

$$Y_c = \left(\sum_i \sum_j y_j B_{i,j} \right) /B;$$

$$i = 1, 2, \cdots, 20(column);$$
$$j = 1, 2, \cdots, 14, (row),$$

where B$_{i,j}$ is the biomass in the rectangle (i, j), B is the total biomass in the surveyed area, and (x$_i$, y$_j$) are the coordinates of the centre of the rectangle (i, j).

The centroid is determined only by the mutual locations of the rectangles and their biomasses. When the set of biomasses is in motion, the centroid is also in motion. Therefore, the spatial biomass patterns and movements can be studied using the centroid techniques.

In this paper, the coordinates of the centroids are expressed in terms of longitude (x-axis, increasing from 164°30' E and 175°30' W) and latitude (y-axis increasing from 69°30' S (0 km) and 78°06' S (955 km)), while the distances and the changes of distances in the distribution of centroids are expressed in terms of kilometres. Three distances are calculated: along latitude, along longitude, and in total.

3 Results

The geographical distribution and biomass density (t/nm^2) of the two euphausiid species in the seasons and years are shown in:

- Figure 3a: late spring (November 1994) when polynya ice edge is in the southern part of the Ross Sea;
- Figure 3b and 4c: early summer (December 1994 and 1997), when polynya ice edge is moving north;
- Figure 4d: full summer (January 2000) when polynya and Pacific waters mix.

3.1 November 1994

The spatial distribution of the biomass of the two krill species in November 1994 is shown in Figure 3a. The total biomass of *E. superba* was estimated in 2.4 million tonnes (mean density: 21.9 tkm^{-2}), which is almost 11 times higher than the biomass of *E. crystallorophias* (0.2 million tonnes; mean density: 1.8 tkm^{-2}). More than 95% of *E. superba* biomass was distributed between latitude 71°00' S (northern species boundary) and 75° 08' S (southern species boundary) and longitude 171° E and 177° 30' W. Three rectangles had high concentrations of *E. superba*, containing 77.6% of the total *E. superba* biomass while representing only 5.7% of the surveyed area. The centroid of *E. superba* biomass (latitude 74°09.6' S longitude 175°53' E) was 108 km north of its southern species boundary and close to the three rectangles of highest concentrations. *E. crystallorophias* mainly occurred in the areas south of latitude 74°28.6' S (northern species boundary). This area included 58.5% of the surveyed region and contained 92.1% of the *E. crystallorophias* biomass. The centroid of the *E. crystallorophias* population (latitude 75°08.1' S longitude 170°38.4' E) was close to its northern border. It was 108 km south and 147 km east of the centroid of the *E. superba* population. Even though the domains of the two krill species slightly overlapped, their centroids were separated by 185 km. In November 1994, the catch of both species comprised only adults. From all samples, the average *E. superba* length was 42.51 mm TL (range 42.4 – 45.0 mm), and the average *E. crystallorophias* length was 25.58 mm TL (range 25 – 30 mm).

3.2 December 1994

The average densities of both krill species found in December 1994 were approximately the same as those 1 month before. However, their spatial patterns, as well as the extension (33%) and distribution of ice cover, had changed dramatically (Figures 2b and Fig. 3b). The rectangles containing high *E. superba* concentrations had increased in number from three to 14, decreasing in average density, and moved to the north-west. The domain of *E. superba*, containing about 96% of the biomass, was delimited by latitude 74°28' S (southern species boundary), 70°30' S (northern species boundary) and by longitude 170°30' E, 178° W. The centroid of *E. superba* biomass was located in the centre of the domain and surrounded by rectangles of high concentrations (latitude 72°22.3' S; longitude 174°52' E). The *E. crystallorophias* biomass was found mainly between latitude 72°39.2' S (northern species boundary) and 75°49.4' S (southern species boundary). This indicates that the domain of *E. crystallorophias* had extended northward and that its degree of overlap with the southern side of *E. superba* domain had increased concomitant with a reduction in ice cover. However, the centroid of *E. crystallorophias* biomass (latitude 74°48.2' S and longitude 170°30' E) had moved very slightly with respect to the previous period, although its distance from the *E. superba* centroid had increased to about 297 km. In December 1994, the average *E. superba* length was similar to that found 1 month earlier (42.36 mm TL), but the range of length distribution (34.9 – 46.8 mm) had increased due to the presence of juvenile krill. However, the number of small krill was too low to identify any juvenile or sub-adult age group. The average length of *E. crystallorophias* of 26.8 mm was similar to that in November, but the number of individuals caught was too low to calculate even the size range.

3.3 December 1997

The spatial distributions of the biomass of the two krill species in December 1997 is given in Figure 4c. The ice cover (21%) was less extensive and had a different distribution to that in December 1994. The total biomass of *E. superba* was estimated to be 2.2 million tonnes (average density 16.3 tkm^{-2}). It was 11 times higher than the *E. crystallorophias* biomass of about 0.2 million tonnes (mean density of 1.4 tkm^{-2}). The krill population sampled in this survey was characterized by a consistent number of juveniles of both species [17]. The average *E. superba* length in December 1997 was 36.63 mm TL (13.5% lower than in December 1994). The juveniles of *E. superba* (19.24 mm TL) represented 19.2% of the net samples. They were found between latitude 73° and 75° S and longitude 171°30' and 173°30' E, within the domain of the *E. crystallorophias* population, and between latitude 71° and 72° S and longitude 176°30' and 178°30' E, in the continental slope. The average length of *E. crystallorophias* was 17.02 mm TL (35.70% lower than in December 1994). The juveniles of *E. crystallorophias* represented 30% of the net samples. They were found both in areas very close to those with juveniles of *E. superba* and in a small area, in front of Terranova Bay, between latitude 75° and 75°30' S and longitude 164°30' and 165°30' E. The areas containing more than 90% of total *E. superba* biomass extended in latitude from 71°00' S (northern species boundary) to 73°50' S (southern species boundary) and in longitude from 171° to 179° E. There were eight rectangles of *E. superba* concentrations, all situated north of latitude 72°39' S, which represented 12% of the sampled area and contained 69% of the total *E. superba* biomass. The centroid of *E. superba* biomass (latitude 72°05.3' S; longitude 175°18' E) was shifted slightly to the northeast of that obtained in December 1994. Over half (57%) of the *E. crystallorophias* biomass was found between latitude 71° and 73° S, mixed with *E. superba* biomass. The remaining 43% was distributed between 73° and 77° S, in rectangles with very few *E. superba*. Although the domains of the two krill populations overlapped to a large extent in this survey, the centroids were still far apart. The *E. crystallorophias* centroid was found 196 km to the southeast of the *E. superba* centroid. Comparison between these results and those obtained under similar ice conditions in December 1994 indicates that the southern boundary of *E. superba* biomass moved northward in December 1997, but its centroid remained similar to that found in 1994. In contrast, in December 1997, the northern *E. crystallorophias* boundary was much further north, the biomass centroid had moved to the northeast, and the portion of *E. crystallorophias* biomass found within the *E. superba* domain was much higher than in December 1994. It is possible that krill distribution is influenced by age structure, and the consistent number of juveniles of both species, found in December 1997, had facilitated their mixing.

3.4 January 2000

Figure 4d represents the spatial distributions of the two krill populations in January 2000, when the waters of Ross Sea were virtually ice-free (Figure 2d). The total *E. superba* biomass was estimated to be 1.2 million tonnes (average density 11 tkm^{-2}), 8.6 times higher than *E. crystallorophias* biomass of 0.14 million tonnes (average density 0.7 tkm^{-2}). In ice-free conditions, the domain of *E. superba* comprising 93.4% of the biomass was distributed in an area between latitude 70° S (northern species boundary) and 73°14' S (southern species boundary). There were seven rectangles of high *E. superba* concentrations representing 6.9% of the sampled area and containing 49.7% of the total *E. superba* biomass. The centroid of *E. superba* biomass (latitude 71°47.6' S; longitude 173°18' E) was found at the most north-western position of all previous surveys. The domain of *E. crystallorophias*, containing more than 90% of the biomass, was delimited by latitude 72°39' S (northern species boundary) and 77°18' S (southern species boundary). The areas south of latitude 73°14' S were dominated by the *E. crystallorophias* population. They contained 87.2% of *E. crystallorophias* biomass and only 6.6% of *E. superba* biomass. The centroid of *E. crystallorophias* biomass (latitude 74°28.6' S; longitude 174°30' E) was located more than 300 km south of the *E. superba* centroid. This is the largest distance between centroids in all the surveys, albeit similar to the distance in December 1994. It seems that in ice-free conditions, the *E. superba* biomass distribution may extend northwards beyond the edge of the Ross Sea in parallel with the progression and melting of the sea-ice. In contrast, most of the *E. crystallorophias* biomass remains confined within the southern part of the Ross Sea in all ice-cover situations. The average *E. superba* length in January 2000 was 44.8 mm TL (range 32.2 – 54.5 mm), but the number of small krill was too low to identify any juvenile or sub-adult age group. The average length of *E. crystallorophias* was 20.4 mm TL (range 7.9 – 40.41 mm). The number of juveniles represented 35.9%, and the number of sub-adults 31.5% of all the catches [18].

4 Conclusions

The results of the acoustic surveys are the following:

1. Biomass level. In average the biomass density of *E. superba* is around an order of magnitude higher than that of *E. crystallorophias*.
2. Biomass distribution. The northern and north-western areas of the Ross Sea are particularly suitable for the *E. superba* population, the southern and south-western areas are more suitable for the *E. crystallorophias* population, while in the central area of the Ross Sea there is a variation in the two species related with ice cover and perhaps with their age structure. This general distribution seems consistent with that of phytoplanktonic communities of the Ross Sea, with diatoms dominating to the north and west and Phaeocystis to the south along the ice shelf. This distribution is probably also reflected in the different diets of the two krill species.
3. Biomass dynamics. The progressive movement northward of the ice edge from November to January seems to influence the apparent movement of both krill species. The centroid of *E. superba* biomass moves markedly northward from lat. 74° S (November) to the Ross Sea border (January: around 71° S) [22]. Also the centroid of *E. crystallorophias* moves Northward from lat. 75°, but it does not go beyond 73° S. This suggests that the spatial extent of the *E. superba* population may enlarge up to the Pacific Ocean and spread into the open ocean waters beginning from January, while *E. crystallorophias* population seems to be segregated within the Ross Sea.

The acoustic surveys have produced some evidence on the relation between the dynamics of krill biomass distribution and ice cover and on the spatial segregation of the two krill species.

The progressive movement northward of the ice edge from November to January seems to influence the apparent movement of both krill species as measured by their biomass centroids. However, the centroid of the *E. superba* biomass moved differently with respect to that of the *E. crystallorophias* biomass. The *E. superba* centroid moved almost 265 km from latitude 74° 09' S to an area close to the Ross Sea border (around 71° 41' S). The *E. crystallorophias* centroid moved northeast, had a maximum displacement of 141 km to the north, and did not extend north of 73° 50' S. This suggests that portions of the Ross Sea population of *E. superba* may spread into the open ocean waters of the Southern Ocean beginning in January. On the other hand, the *E. crystallorophias* population seems to be confined within the Ross Sea.

All the results seem to indicate that the two krill populations occupy only partially overlapping domains, move at different spatial scales, and react differently to ice cover. Thus, it seems that the two krill populations generally tend to be confined to separate areas and to avoid substantial mixing through migration. This mechanism is probably activated by some external biological and physical factors caused by ice melting and retreating, but may also include some active internal factors such as inter-specific competition and age effects.

However, the acoustic surveys in the Ross Sea occurred in different years, seasons and environmental conditions that may have introduced some bias into the estimation of biomasses and their spatial distributions. Further experimental and theoretical work is necessary to understand the physical, biological, and behavioural factors that govern the spatial distributions of the two krill species, how those distributions influence the trophic level above and are influenced by the trophic level below, and how much the fluctuations of the biomasses and their spatial distributions depend on seasons, years, environmental conditions and unusual events.

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The Development of Bottlenose Dolphin Sonar System During the First Months of Life, the Role of the Mother and the Acoustic Interactions with the other Community Members

M. Azzali, I. Leonori, I. Biagiotti
Institute of Marine Sciences, CNR, Ancona, Italy
i.leonori@an.ismar.cnr.it

Abstract

The birth of a dolphin in captivity is a rare occasion to study the learning and the development process of the sophisticated sonar system that these marine mammals use. In order to investigate the development of bottlenose dolphin sonar system during the first months of life, the role of the mother in the learning process and the acoustic interactions with other community members, ISMAR carried out a study on five calves (three females and two males) in three different pools from 1997 to 2005. The aims of the study were to point out: a) the time necessary for a calf to learn the use of biosonar, b) the role played by the mother and the other community members in this learning process, and c) the possible acoustical changes of the community in response to the entry of a new calf. Our observations indicate dolphin calves' inability to emit high frequency sounds during their first month of life. The five studied calves preferred to use low frequency pulses with a predominant peak around 50 kHz, unlike the other adult dolphins, that generally presented a more marked peak around 100 kHz. As known, low frequencies allow only a rough perception of the external world, while high frequencies make an extremely detailed analysis. The new born calves were acoustically influenced first by the mother and then by the father and the other juveniles of the group. At the same manner, all members of all the communities seemed to dynamically participate in the calves' learning phase and their acoustic behaviour was affected by the presence of the new entries. After some months the calves played a central role within the communities and the other members tended to modify the acoustic features of their biosonar adapting it to that of the calves.

1 Introduction

Dolphins have an unusual perceptual system: echolocation. When dolphins echolocate, they emit high-frequency sounds (>20 kHz), called clicks or sonar signals, which are echoed back to them by objects of their environment. Echolocation is foreign to humans: we lack neural networks necessary to understand what is going on in the brains of echolocating dolphins and intuition can provide only a partial account of this sensory system. On the contrary dolphins are masters in using the language of echoes: they can extract from echoes information about many different objects features, which even the sharpest sight will never be able to perceive. For example dolphins gather data about the material from which an object is made [1, 2], object wall thickness [3], object size and distance [4], object three-dimensional shape [5, 6] and object aspect-dependent shape [7]. It is general opinion that dolphins use sonar signals only for representing objects of their environment, while for social interactions they use low-frequency sounds ($<$20 kHz), called vocalizations. However several authors have supported the idea that sonar signals may be an additional and sophisticated method of communication among members of the same community [8, 9, 10]. A fact that suggests there is an additional function for clicks other than echolocation is that dolphins use clicks also when the objects of their environments are well known and echolocation information are not needed. These clicks, unlike echo locating ones, have a very high repetition rate that does not follow the two-way transit (TWT) time rule (i.e. the interclick intervals of echolocating dolphins depend on and increase with the distance of target, see [11]) and in some social situations are emitted in couples [10]. Double clicks and clicks with high repetition rate are not believed to have an echolocation function, according to current sonar theory. In fact such click trains were detected during courtship, mother-infant interaction, discipline, exhibition, play, aggression, fear, danger when sonar use seems to have no utility at all. The threshold of dolphin's somatic pressure receptors was estimated by Kolchin an Bel'kovich [12] around 160-170 dB re 1μPa, well below (40-50 dB) the sound pressure of sonar signals, ranging between 228 and 210 dB re 1μPa [10]. The genital and mammary regions are the areas most sensitive to sound pressure, followed by the upper rostrum, lower jaw, forehead, flukes and pectoral and dorsal fins. So, when a dolphin is hit by a click train emitted by another dolphin, it receives not only auditory information but also tactile sensations. It is very probable that acoustic and tactile information are intimately related in dolphins and if one cue is partially masked the other cue can complete it. This suggests that a dolphin can transfer through a packet of clicks its emotive state (as danger, aggressiveness, courtship, fear, agonistic or playful state) into another individual, who must have enough knowledge about the structure of clicks and the environmental context for assigning the correct emotional interpretation to the tactile-acoustical message.

Some other aspects of the existing knowledge on echolocation signals may have peculiarities and functions not yet understood.

An hydrophone lowered among a dolphin group picks up only chaotic fluctuations in the pressure of the medium. However each echolocating dolphin can recognize, in this complex network of sounds, its own clicks and the returned echoes so as we can easily attend to the voice of a single person, even while many people around us are simultaneously speaking. Therefore both sonar signals and echoes must carry also information concerning the identity of the echolocating dolphin [8], as for us the voice identifies a person.

In a group only some dolphins echolocate, the others move silently, but behave synchronously with the echolocating ones. Experiments in controlled environment [13] demonstrated that passive listening dolphins can identify the object that their neighbours are echo-locating by eavesdropping on its echoes, so imagining the same auditory scene and sharing the same knowledge. Shared knowledge enhances group effectiveness and might be one of the mechanisms that allows dolphins in groups to cooperate closely in hunting the prey or in defending themselves from enemies. If it so, each dolphin must be capable of recognising a dolphin of its group by click features and of deciphering perceptual information contained in the continuously varying click echoes, even if distorted by noise or at times obliterated. This mechanism is basically similar to that of human speech perception, except it seems to be used by dolphins mostly in the immediate context of events. A human listener, as a dolphin, converts with little conscious effort the continuously varying acoustic stimulus produced by a speaker into an internal representation of a sequence of events, even in presence of large distortions in the signal. This appear to be so because the listener has an extensive knowledge of both the speaker's language and slang and the speech context. If the substrate of understanding echoes by passive listening dolphins and speech by humans is similar, then it is of crucial importance for a young dolphin to learn both the language of echoes and the individual acoustic characteristics of each member of its group.

One could speculate if dolphins could use sonar signals as metaphors of a state, action or thing out of the context of events, to transfer or evoke perceptual experiences of past events into other individuals of the same group and influence their behaviour. We know that various social mammals use metaphorical signals as their most advanced mean of communication [14]. For example the howling of wild dogs, occurring before the hunt [15] may be metaphors of the chase, but without the prey present. It was demonstrated that dolphins that have been given human language training can respond to symbols representing various parts of speech [16]. Therefore dolphins, who live in a foggy, vast and open environment as ocean and then need subtle forms of acoustic signals to assure social cohesion in their complex societies based on mutual altruism, have certainly enough cognitive capabilities to learn metaphorical signals and perhaps to use them for manipulating the relationships among members of the same group or for preparing a future event. However at moment there is no experimental evidence that dolphins use echolocation out of event context, although very little is known about how dolphins use echolocation in their natural environment.

In conclusion information carried by sonar signals may regard, in order of increasing levels of complexity: 1) the objects of dolphin's environment; 2) dolphin emotional state; 3) objects that dolphin's neighbours are echolocating; 4) things, state and action out of the context of events.

The acoustic signal is characterized in each instant only by one quantity the pressure on the auditory apparatus. How can a mono-dimensional quantity so poor as the pressure carry so many types and pieces of information? Only learning and a complex .brain properly programmed from birth can allow to perceive a dimensionality much higher than that strictly physical in very rapid pressure amplitude fluctuations that occur within short (some tenths of microseconds),and often weak and distorted echoes. Dolphins have the most highly specialized auditory systems known in any animal group, including humans and produce and process the sounds with a peculiar mechanisms not yet understood fully. Young dolphins, animals with one of the longest period of dependence on mother ($\approx$ 4-6 years), learn during this period large part of their acoustic behaviour. Ethologists and their opponents have attempted to clarify in many ways the dichotomy "nature/nurture" or "instinct/learning" in dolphins with contrasting conclusions. Instead of debating or taking up extreme positions about whether the acoustic ability of dolphins is predominately innate or learned, the question that here we attempt to answer is how does this ability develop in young dolphins.

The optimal strategy should be to study how the acoustic behaviour develops in an infant dolphin free swimming in the sea together with its mother and adults. Unfortunately, if the experiments are carried out in natural conditions on wild dolphins, the techniques which are needed still present enormous difficulties and uncertainties. It is a big problem even to establish how many dolphins are echolocating and which dolphin is producing the recorded signals, because dolphins produce sounds without moving any muscle and remaining quite imperturbable. To our knowledge systematic measurements in wild on the development of echolocation in young dolphins have never been done. On other hand the birth of a dolphin in captivity is a rare event. We were fortunate to have occasions to study the learning and the development process in five young dolphins, born in three different facilities and from four different mothers. The aim of this work is to point out: a) the possible existence of an infant language, not linked to external factors, included calf mothers and their communities; b) the time necessary for a calf to learn the use of biosonar; c) the role played by the mother and the other community members in this learning process.

2 Material and methods

The study of the infant language regarded five calves and their mothers, held in three different facilities. They are Dafne, born on 26^{th} September 1992 in Cattolica Delphinarium from Bonnie and Clide; Blue born on 26^{th} June 1997 from Beta and Speedy and Rocco born on 27^{th} September 1999 from Alfa and Speedy housed in Rimini Delphinarium, Nau born on 28^{th} October 2001 and Tango born 31^{st} March 2005 both generated by Betty and Robin and housed in Palablu of Gardaland (VR). The data presented in this work are based on weekly 1 hour long recording sessions from birth through 15^{th} postnatal week and then every two or three weeks until 20^{th} or 26^{th} week of age of the calves, depending on years and the pools. Sonar signals were recorded using a Brüel & Kjær Type 8105 hydrophone, a charge amplifier (Type 2626 Brüel & Kjær) and a custom-built wide band (30 Hz – 300 kHz) analogical recorder (a Sony U-Matic video recorder, in which the video band was adapted to work as an audio broadband). The hydrophone was placed 2 m below the water surface and 3 m away from the pool wall. The signals produced by dolphins were monitored on a HP 54520-A digital recorder. The comments of a skilled researcher, who was observing both the monitor and the animals and especially which dolphin was pointing towards the hydrophone, were contextually recorded into the low band ($<$ 20kHz) channel of the same recorder. Unidentified, uncertain and not well shaped signals were discarded, the others were digitalized at an effective sampling rate of 5.12 μs and processed using Matlab m-file language [8]. In each session, six adimensional statistic parameters were extracted from the sets of signals emitted by each dolphin (generally the mother and its offspring). They are: asymmetry coefficient ($X_1 = a_3$), kurtosis coefficient ($X_2 = a_4$), mean centroid of the time domain waveform s(t) related to standard deviation ($X_3 = C_t / \sigma_t$); mean Gabor timewidth related to standard deviation ($X_4 = T_G / \sigma_T$); mean centroid of the frequency spectrum S(f) related to standard deviation ($X_5 = C_f / \sigma_f$); mean Gabor bandwidth related to standard deviation ($X_6 = B_G / \sigma_F$). See Blahut [17] for definitions and formula regarding Gabor timewidth T_G and Gabor bandwidth B_G. The Gabor timewidth is linked to Gabor bandwidth by: $T_G * B_G \geq 1/4\ \pi$, with equality only if s(t) is a Gaussian pulse. The number of information-bearing parameters in the echoes increases with the width of the Gabor bandwidth (B_G). Therefore the parameter $X_6 = B_G / \sigma_F$ can be considered as an indicator of the expected informative content of the returned echo. The barycentre of the frequency spectrum C_f is very near to the frequency of maximum power of the spectrum, that represents the fundamental frequency of the emitted signal . Therefore the parameter $X_5 = C_f / \sigma_f$ gives a measure of the characteristic "intonation" of the signal emitted by a dolphin. The six parameters were used to calculate, by means of cluster analysis, the similarities among the ten dolphins (the five calves and their mothers) for each period. We have represented each set of signals emitted by a dolphin during a session as a data point in a space with six dimensions (X_n , n = 1, 2,. . . 6). Therefore a data point represents the mean acoustic behaviour of a dolphin. We used as measures of acoustic dissimilarity (or similarity) between dolphins the Euclidean distances between two data points:

$$D_{i,j} = \left[\sum_{n=1..6} (X_{ni} - X_{nj})^2 \right]^{1/2}$$

where X_i and X_j are two different data points (e.g. a mother and its offspring or two different calves/mothers or the same dolphin tested in two different periods, and $D_{i,j}$ is a measure of the acoustic similarity (or dissimilarity) between them (similarity = k / $D_{i,j}$; dissimilarity = k $*$ $D_{i,j}$). The nearest distances among a group of dolphins were calculated using hierarchical clustering method. Initially every data point is considered as a separate cluster. The matrix of the distances is calculated. For example if the dolphins under examination are N (i.e. the five calves and their mothers give N=10), the number of inter point distances is: N(N-1)/2. In the next stage the two most similar points are combined to form a cluster. The new matrix of distances is calculated. This merging process is continued, reducing at each step the number of cluster by one, till when all the points (e.g. dolphins) are assigned to one cluster.

3 Results

3.1 Have infant dolphins a their own talk?

The acoustic development of the five infant dolphins can best be described dividing the 15 weeks of observation in three periods: the first period from birth to the 9^{th} postnatal week; the second period from the 10^{th} to the 12^{th} postnatal week and the third from the 13^{th} to the 15^{th} postnatal week. During these 15 weeks, and mostly in the first 9 weeks, great part of

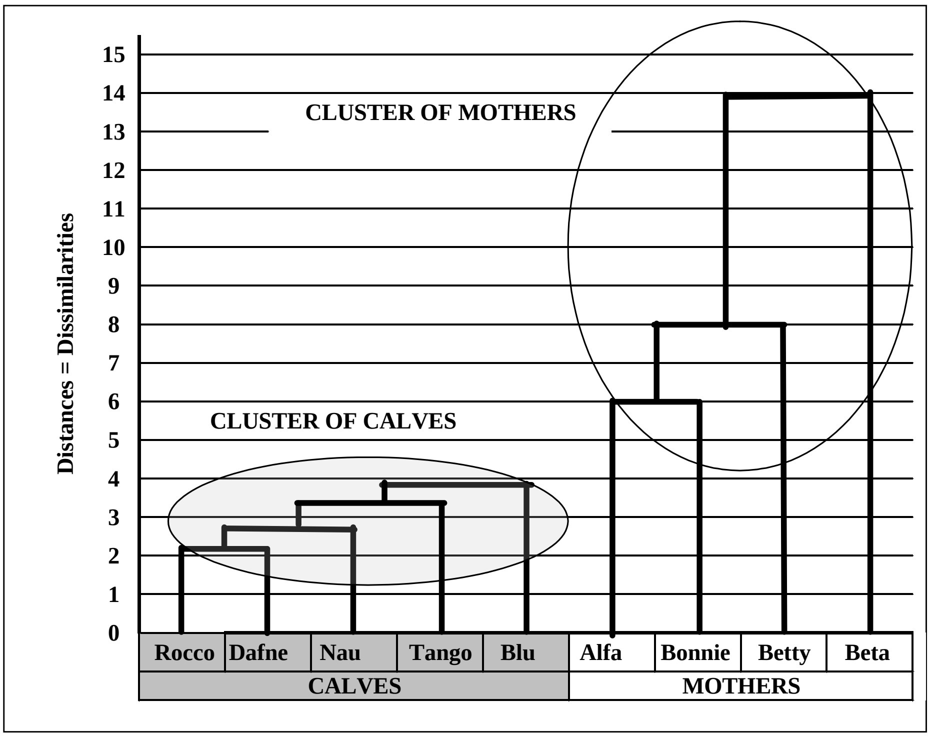

Figure 1: Dendrogram for five calves, and their mothers concerning the period from the 1^{st} to the 9^{th} postnatal week. It shows two distinct clusters: the cluster of calves that have dissimilarities between 2 and 4 and the cluster of their mother. (X_5, X_6, X_7, X_8) that have dissimilarities between 6 and 14.

the acoustic signals emitted by infants and analysed with cluster analysis may be defined pseudo echolocation signals (low frequency poorly structured waveforms).

In the first period of observation , from birth to the 9^{th} postnatal week, the threshold level splits the data set into two clearly distinct clusters (Figure 1):

1. the cluster of calves, where the acoustic dissimilarities among the individuals range from 2.2 to 3.8 (mean dissimilarity = 3).
2. the cluster of the mothers, where the acoustic dissimilarities among individuals range from 6 to 14 (mean dissimilarity = 10).

In the second period, from the 10^{th} to the 12^{th} postnatal week, the mean distance among the calves increased to 10.1 and in the third period, from the 13^{th} to the 15^{th} postnatal week, it increased further up to 16.7.

Table 1 shows the changes in the dissimilarity of a calf versus its own mother in the three periods under examination. In the first period the dissimilarities between calf and its mother were in average almost 3 times greater than the dissimilar-

Calves vs. mothers	weeks		Distances = Dissimilarities			
			Mothers			
	from	to	Bonnie	Beta	Alfa	Betty
Dafne vs. Bonnie	I	IX	6.1			
	X	XII	4.2			
	XIII	XV	18.2			
	mean (I - XV week)		**9.5**			
Blu vs. Beta	I	IX		14.0		
	X	XII		8.5		
	XIII	XV		2.8		
	mean (I - XV week)			**8.4**		
Rocco vs. Alfa	I	IX			5.4	
	X	XII			2.8	
	XIII	XV			7.8	
	mean (I - XV week)				**5.3**	
Nau vs. Betty	I	IX				6.2
	X	XII				4.1
	XIII	XV				4.8
	mean (I - XV week)					**5.0**
Tango vs. Betty	I	IX				8.0
	X	XII				1.9
	XIII	XV				18.5
	mean (I - XV week)					**9.5**

Table 1: Changes of acoustic dissimilarity of a calf versus its own mother in the periods under examination. The mean dissimilarity in the entire period is in bold.

ities among calves in the same period: they ranged from 5.4 (Rocco-Alfa) to 14 (Blu-Beta). However in the second period the dissimilarities calf-mother were found around 54% lower than in the first period. They ranged from 1.9 (Tango-Betty) to 8.5 (Blu-Beta). Surprisingly in the third period these dissimilarities again increased remarkably even respect to the first period for all the pair calf-mother, except Blu-Beta. They ranged from 4.1 (Nau-Betty) to 18.5 (Tango-Betty). On the contrary the dissimilarity Blu-Beta decreased further up to 2.8. It seems that the infant Blu needed more time than the other calves to develop its own acoustic repertoire. The close similarity among calves' signals from the birth to around the 9th postnatal week suggests that infant dolphins have a their own acoustic repertoire, not depending on genetic or external factors. However the increasing dissimilarities from the 10th postnatal week even between calves belonging to the same community (Rocco and Blu; Nau and Teide) and the changing of dissimilarities of each calf versus its own mother indicate that each calf develops a its own acoustic repertoire, and this acoustic development is influenced not only by its mother, but also by other factors as genetic factors and perhaps experience. Similar results were found by Iannessa [18] in her dissertation on the acoustic evolution of four calves of *Tursiops truncatus*.

3.2 How much time need a calf to learn the use of biosonar?

The infant Blue, from birth to the 15^{th} postnatal week, emitted no clicks, but only short, low frequency and little modulated signals. Her first echolocation pulse was recorded approximately 16 weeks after her birth [19], while Rocco, housed in the same Rimini Delphinarium, needed about a half time ($\approx$ 7 weeks) to learn the use of the biosonar.

The time necessary for the infant named Nau to learn the use of biosonar was investigated in detail with weekly observations and recordings from its birth to the 26^{th} postnatal week [20]. See Figure 2 for a summary of the results. In this period the calf and its mother resided separately from the other dolphins of Palablu. The infant from birth to 10^{th} postnatal week emitted no clicks, but only low frequency waveforms similar to those of Blue, and it swam prevalently in resting position. From 11 to 20 weeks Nau was able to produce clicks with only one peak at 50 kHz (Figure 2B and 2C) . At 21 weeks Nau produced more structured waveforms with two peaks at 40 and 60 kHz (Figure 2D). This trend seems to reflect the parallel improvement in the motor control from the young dolphin, observed in the same period.

The isolation of Nau from the community could have delayed its acoustic and motor development. However Tango, who also resided in Palablu and as Nau remained for six months from its birth only with its mother and Nau was particularly precocious: it emitted its first sonar pulses 7 weeks after its birth [21].

The first echolocation pulse of Daphne, housed in Cattolica Delphinarium, was recorded approximately 9 weeks after her birth [19].

In conclusion the studied calves needed from 7 to 16 weeks to produce their first sonar pulse. These observations find a confirmation in Lindhard [22] that illustrated the dolphin calves' inability to emit high frequency sounds (>20 kHz) during their first month of life. Moreover, all studied calves preferred to use pulses with few cycles and a predominant peak around 50 kHz, unlike the adult dolphins, that generally presented a more marked peak around 90-100 kHz. As known, low frequencies allow only a rough perception of the external world, while high frequencies make an extremely detailed analysis. It is unclear if these first simple signals with poorly structured spectra were emitted by the dolphins intentionally to obtain environmental information or in imitation on mother and if they were related with the changes of mother-infant swimming positions occurring at these ages.

With growth, calves produced more structured waveforms (bimodal spectra) showing several cycles, but at 26 weeks from birth the signals emitted by the calves were yet lacking of frequency components above 100 kHz. The bimodal clicks became more frequent with the longer departures of the calves from their mothers. This seems to suggest that young dolphins intentionally use more structured signals to move in the pool without maternal aid [23].

3.3 The role of the mother

It is difficult to analyse the role the mother plays in providing echolocation model and instructions to its offspring. For example since when the calf Nau emitted its first clicks (11 week), Betty seemed to simplify her own typical clicks and to adapt them to the acoustic characteristics of Nau (Figure 2C). A similar acoustic behaviour was observed in Alfa and Betty. It is interesting to speculate if this may reflect the intention of mothers to share the same environmental information with their offsprings or provide them a model to imitate. The trend of acoustic dissimilarities mother calf showed in Table 1 seems to indicate that the mother plays an important role in providing echolocation models to its offspring mostly between the 10^{th} to the 12^{th} postnatal week. Finally it is unclear the function of the two almost simultaneous pulse emissions from mother – infant, recorded without any significant changes in all our experiments. These signals may have been used by the two dolphins to maintain acoustic contact or the mother may have produced standard echolocation signals as models, expecting that its offspring could imitate them.

3.4 Possible acoustical changes of the community in response to the entry of a new calf

The study on the possible acoustical changes of the community in response to the entry of a new calf was done in Palablu on the calf Nau. The calf resided separately from the other two adult dolphins of the community and lived only with its mother for six months. The calf and its mother were reintegrated, first gradually (May-June 2002) then completely (since July 2002), into the community. Calgiago, in her dissertation [24], has found a progressive decrease of the acoustical distances, measured with the cluster analysis, between the calf and the other members of the community, and mostly between the calf and its father. This decrease seems due not only to changes in the characteristics of the calf click structure, but also to acoustical changes of the community after the entry of the calf in it.

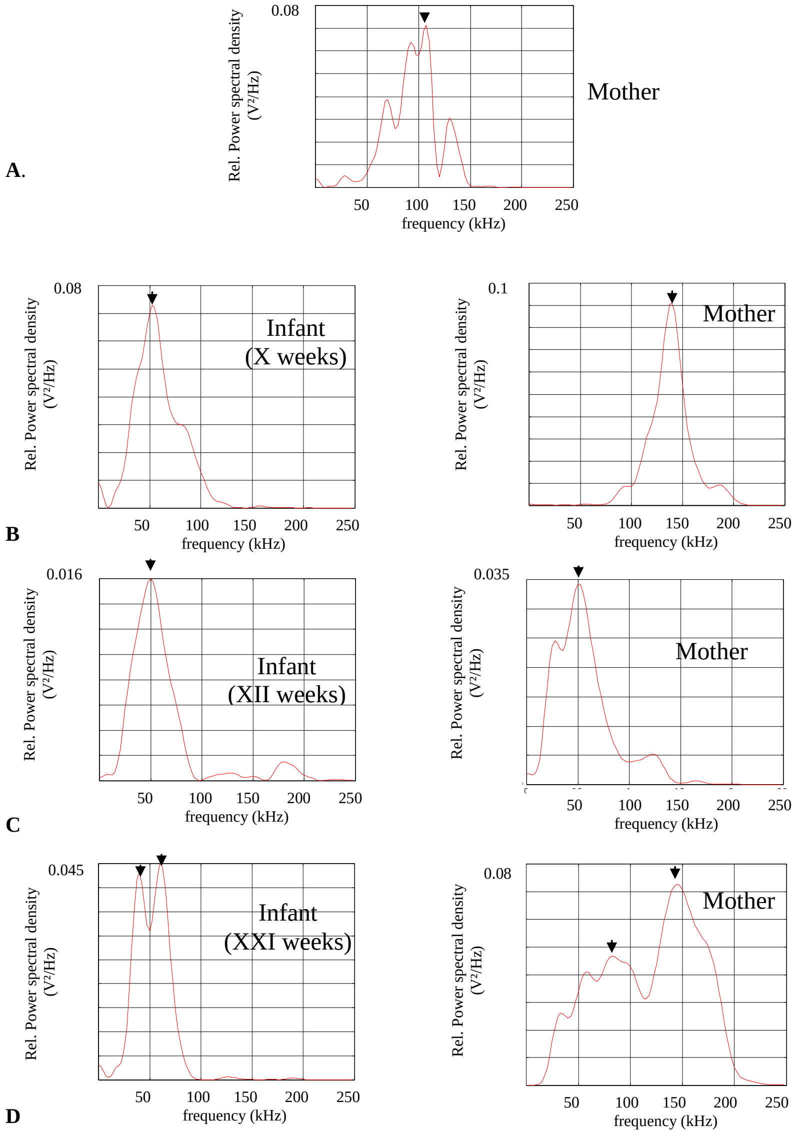

Figure 2: A) An average spectrum of Betty produced when its offspring was II weeks old (Nov.: 2001). In B), C), and D) typical spectra produced by Betty and Nau at the end of the three periods of observations. At the end of the second period C) the spectra of Betty and of its offspring were very similar.

4 Conclusion

Although the acoustics of the bottlenose dolphin's echolocation system is well researched, the studies on the development of echolocation in young dolphins has received much less focus.

Our observations, done in controlled experiments, indicate that:

- Infant dolphins till about 9^{th} postnatal week from birth seem to have a their own acoustic repertoire, not depending on genetic or external factors (Figure 1).
- From the 10^{th} postnatal week the dissimilarities among calves' signals increased progressively. Then since the 10^{th} postnatal week each calf seems to develop a its own acoustic repertoire.
- The five studied calves needed from 7 to 15 weeks from birth to produce their first sonar pulses.
- The same calves till the 26^{th} postnatal week used low frequency pulses with a predominant peak around 50 kHz, unlike the other adult dolphins, that generally presented the most marked peak around 100 kHz.
- With growth the acoustic dissimilarities calf-mother first decreased remarkably (second period) then (third period) tended to increase again (except Blu-Beta, Table 1). This trend could indicate that the mother plays an important role in providing echolocation models to its offspring mostly around the X-XII post-natal week. Figure 2C shows that when the calf Nau was able to emit sonar signals, her mother seemed to adapt some features of its typical signals to those of Nau. It may reflect the intention of mother to share the same environmental information with its offspring or provide a model to imitate. Moreover it is unclear the function of the two almost simultaneous pulse emissions from mother – infant, recorded without any significant changes in all our experiments.
- The calf Nau studied in Palablu of Gardaland who lived only with its mother for the first six months, when reintegrated in its community was acoustically influenced mostly by the father and then by the other members of the group. On the other hand, the acoustic behaviour of all members of the community seemed to be affected by the presence of the new entry.

Although the experiments in controlled conditions are of great importance, we must consider, as future research, similar measurements in the wild. Other critical areas for future research are the social, passive and metaphorical uses of echolocation both in captivity and in the wild.

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Ecotoxicology and Ecology of Ionian Sea Species *Gammarus aequicauda* (Martynov, 1931): a Review

F. Biandolino, E. Prato
Institute for Coastal Marine Environment, CNR, Taranto, Italy
francesca.biandolino@iamc.cnr.it

Abstract

The application of an organism in ecotoxicological testing requires some knowledge of basic ecological aspect. This review covers the general topics of habitat, life history, trophic relathionships and ecotoxicology of the Ionian sea species *Gammarus aequicauda*. The information here reported further highlights the ecological relevance of this species in coastal ecosystem, considering its wide distribution and abundance, its role as a consumer and as a prey and its sensitivity to toxicants. The paper evidences the necessity of further studies on this interesting and ecologically important species.

1 Introduction

The protection of an estuarine or marine habitat from damage due to contaminants requires an understanding of both the sensitivity of invertebrates to contaminants and their ecological requirements. Sediment bioassays are a convenient and appropriate way of evaluating this sensitivity and also have the advantage of reflecting the bioavailable fraction of a contaminant, which can be very different from the total amount determined by chemical analysis [1]. They are recommended along with other methodologies to obtain information on the ecological impact of contaminated sediments [2].

This review pretends to be a first contribution to compile and organize scattered and diverse data about biological, ecological and ecotoxicological aspects of *G. aequicauda*, which is one of the most important amphipod species along Ionian coastal systems. We have found a great potential in this amphipod species for ecotoxicological research, namely for testing estuarine and marine sediment toxicity. However to proceed with this research has been necessary to have a background database on ecological aspects. 5-year study was conducted on the life history of the amphipod *G. aequicauda* from the point of view of its biology and ecology in one of the most important basins of the Southern Italy, Mar Piccolo of Taranto.

2 Ecology

Gammarus aequicauda (Martynov, 1931), (Crustacea, Amphipoda), is an euryhaline and euryvalent species very common in warm temperate waters, widely distributed along the Mediterranean and Black Sea coasts [3]. It is a characteristic inhabitant of the intertidal zone of shallow marine coastal waters, although it is found most frequently and in abundance in brackish waters with freshwater influence (lagoons, estuaries, river mouths), under stones or among algae. It lives overall between macroalgae such as *Chaetomorpha linum* and *Ulva* ssp. These formations seem to be advantageous for the amphipod, because it can find plenty of food here and protect it against predation [4, 5].

G. aequicauda is a recognized macrophagus, omnivores species that feeds on macroalgae, detritus, vascular plants, epiphyte sand, epifaunal species, which populate the macrophyte communities. These crustaceans constitute a significant group in terms of energy flux in the Mar Piccolo ecosystem but the ecofunctional and trophodynamic roles are not known. Concerning this aspect Biandolino and Prato [6] analysed the lipid and fatty acid composition of *G. aequicauda* from Mar Piccolo basin, in order to elucidate the spectrum and variety of its food source. Often the feeding type cannot be deduced with certainty from the stomach contents, because it doesn't always represent the food that is effectively digested [7]. The importance of lipid and fatty acid composition as trophic markers has been demonstrated in various studies [8, 9]. Therefore a fatty acid approach can be useful to explore habitat preferences, feeding strategies and food sources.

Five FAs characterized the fatty acid profile of *G. aequicauda*, namely the palmitic acid, 16:0, the oleic acids 18:1 (n-9), 18:(1n-7), the eicosapentaenoic acid 20:5(n-3) and arachidonic acid 20:4(n-6) and a small amount of docosahexaenoic acid 22:6(n-3). These results, together with high values of the biomarker ratio 20:5(n-3)/22:6(n-3) and a low ratio 18:1(n-9)/18:1(n-7), suggested a contributionn of diatoms in the diet of *G. aequicauda*. A further contribution is given by macroalgae for the presence of fatty acid 20:4(n-6) while the fatty acid 18:1(n-9) emphasized its carnivorous habits.

This amphipod often roams in the sediment, picking up pieces of decaying materials and in muddy sediments *G. aequicauda* burrows into the sediment surface. Its intimate contact with the bottom sediment and overlying waters for extended periods of its life cycle can determine adverse effects in the presence of contaminated sediments. From our observation *G. aequicauda* is a food source for a diverse range of vertebrate and macroinvertebrate predators. In addition in our sampling location in Mar Piccolo basin, we observed frequently shorebirds feeding among the macroalgae cover, where *G. aequicauda* is collected.

2.1 Life history

G. aequicauda from Mar Piccolo of Taranto shows a clear seasonal variation in abundance with a maximum in spring-summer and a minimum in autumn- winter. This evidences that, in spite of the low autumn–winter abundance, this amphipod species is present throughout the year in Mar Piccolo. The number of individuals is affected by its life cycle, although the predation by fish and large invertebrates is also an important factor controlling the amphipod population structure, which can regulate the seasonal pattern of their abundance [10, 11, 4]. The seasonal change in the abundance of this amphipod is also significantly linked with fluctuations of environmental variables. Indeed the temperature and pH positively affect the abundance of *G. aequicauda*. No relationship is found with salinity and oxygen [4].

G. aequicauda reproduces continuously in the Mar Piccolo estuary, as can be concluded by the presence of ovigerous females throughout the year. This is observed also in other populations of this species, studied by Greze [12], Janssen et al. [13], Porcu and Tagliasacchi Masala [14] and Kevrekidis and Koukouras [15]. Two generations each year are recorded: juveniles born in the spring, grow, mature, and become reproductively active during the summer months, giving rise to the next input of recruits in autumn. Although our observations from laboratory cultures indicate that the population probably produces several generations per year [16]. Therefore, coupled field and culture data indicate that *G. aequicauda* population of the Mar Piccolo estuary reproduces several times in the year and has a multivoltine life cycle, that is, produces several generations per year.

The sex ratio (number of males/number of females) vary considerably over the year with the mean value of 1.12 (± 0.1 standard error), however, females were more abundant than males during winter months.

Regarding size-class structure, the population of *G. aequicauda* consists in spring of adult individuals and few juveniles. Size differences between males and females occur throughout the year because the males grow faster than females and reach a longer size.

In the summer months most of the last generation seem to die and the population is mainly constituted by juveniles and small individuals. This tendency is kept relatively constant during early autumn until December being mainly made up of small-sized adults.

The recruitment quantification, particularly of the animals with total length (TL) < 2 mm is hard to do, since these animal's densities show pronounced variations between monthly replicates. The presence of juveniles throughout the year evidences a continuous recruitment. Although in the period spring-summer the number of juveniles is too small to account for adults of the next months. This may be a sampling deficiency, as mentioned by Anger [17] for other small-sized amphipod species. An increase in juveniles number occurs in autumn–winter. Furthermore, it is possible that, the decrease in the adult population in autumn-winter months maybe due to some migration of adults between different habitat types related to the breeding periods [18, 10]. The size of the brood is positively correlated to the cephalic length of the female. Indeed in summer and early autumn is registered the lowest fecundity when the mean ovigerous females size is the smallest. *G. aequicauda* females become ovigerous when they reach a size of about 5mm, producing more than 20 eggs only when females exceed 8mm.

2.2 *Gammarus aequicauda* rearing in the laboratory at different environmental variables

On the basis of field data alone, it is difficult to come to any conclusion about the processes governing the life history traits. A combination of field and laboratory experiments may provide adequate estimates and improve our understanding of population dynamics and biological traits that can be difficult to follow in the field. Findings from this study have implications in developments of ecotoxicological testing with *G. aequicauda*. In order to fully understand the effects of pollutants in acute and especially chronic toxicity investigations, *G. aequicauda*'s life history in the laboratory under fixed conditions of temperature and salinity (18°C-36‰) was studied. Thus embryonic development, fecundity, growth strategies, and development of juveniles produced from precopula pairs were determined [16]. These aspects may be highly important for the interpretation of data from ecotoxicological studies and for defining strategies for chronic and long-term toxicity tests. In a chronic toxicity test the organisms are exposed to chemicals for a long period of its lifetime. These data have been subsequently used as a basis for evaluating the toxicant effects on the life history traits of this species.

G. aequicauda, cultured at 18°C and 36‰ spend in precopula an average time of 1-3 days, shorter than a mean time of 4 (range of 1-7) reported for *Hyalella azteca* observed by Shuhaimi and Pascoe [19]. Males (both small and large) usually prefer to enter into precopula with females smaller than themselves. The mean duration of embryonic development is 6.25 ± 0.97 (range of 5-8) days.

Brood size increases with body size of female and the highest number of juveniles was released by a female with a head length of 1.25 mm (corresponding to 10.9 mm of total length). A linear correlation between mean brood size and female size is a common feature in amphipods, e.g., *G. locusta* [20], *Orchestia gammarellus* [21], and *Echinogammarus marinus*[22]. Larger females generally carry more eggs than smaller ones because of the greater body length, as the marsupium capacity is proportional to body size [23].

In *G. aequicauda*, as in other amphipods, development is direct, and newly hatched juveniles possess all the structures typical of the adult. The reared females of *G. aequicauda* produce broods similar to those produced by females collected in the field [4]. In the laboratory, it was observed that females could produce at least 3 consecutive broods. The growth of individuals was found to be continuous throughout life under our laboratory conditions; nevertheless, growth rates were lower in the early weeks. The maximum body size attained by individuals was higher than the body size attained by organisms in the field [4]. In the Baltic Sea, Kolding and Fenchel [24], studying life cycle of gammarid amphipods, observed that the growth rate decreases when they mature and begin to reproduce. It is possible that favorable conditions (an ad libitum food supply, stable rearing conditions, absence of predators) might well benefit individual growth rates. The mortality was relatively high during first 6 weeks of life, followed by a decrease during their remaining life span. The total lifespan of the animals was estimated to be 145 ± 2 days.

The sex of individuals was determined after 30-37 days, and precopulatory pairing behavior began after 44 days. The number of precopula pairs reached a maximum at 84-88 days, corresponding to 46% of the existing population (except for newborns).

The advantages of laboratory-cultured populations are that individuals are close to a normal physiological state, are capable to grow and reproduce in captivity and are of known ages. In addition a laboratory toxicity test requires a large number of organisms, and field populations often exhibits significant variations in abundance, thus experiments may be interrupted because test organisms from the field are unavailable. Test organisms used in chronic toxicity test should grow and reproduce freely under laboratory conditions and have a high reproductive potential and a short life cycle [25]. In this study, *G. aequicauda* met these requirements, thus the effects of contaminants upon the growth and reproduction of this species can be evaluated.

But since an estuary is a partially enclosed entity characterised by fluctuating environmental conditions, estuarine amphipods must be able to adapt functionally to dynamic environmental variables. Therefore, when amphipods are exposed to temperature and salinity changes, they can obtain optimal performance by altering either their behaviour or their physiology, such as growth and reproduction, when the variations are over a sufficiently long period [26]. Since no background information was found in the literature about the *G. aequicauda* life cycle under different temperature and salinity laboratory conditions, the effect of these two environmental variables on *G. aequicauda* life history was investigated, with the aim of establishing the best combinations of temperatures and salinities for chronic toxicity testing [27].

Three salinities (10, 20 and 36 ‰) and three temperatures (10, 18 and 24°C) were used, combined in nine different treatments. These conditions were selected according to the range of temperatures (7–35 °C) and salinities (10–36 ‰) registered annually along the Ionian coast, where the natural population of *G. aequicauda* lives.

Compared with 10°C, the life cycle at 18°C and 24°C was characterised by a shorter brood development time, quicker sexual differentiation, faster individual growth, shorter lifespan and higher population growth rate. In particular the greatest time required for the embryonic development was 12±0.94 days at 10°C and 36 ‰. Brood development time in amphipods decreases with increasing water temperature. In general, the highest number of juveniles released by *G. aequicauda* females was observed at 10°C and 18°C and the lowest at 24°C, at all salinities tested. These results were consistent with the field findings, when *G. aequicauda* females produced smaller broods, during summer months (the warmest months) [4]. This is probably due to the increased metabolic maintenance costs, at higher water temperature, which allow a decreased allocation energy for reproduction, reducing the capability to produce eggs [28].

Individual growth is a fundamental biological process that often has a strong influence on fitness. Variation in growth patterns will affect the lifespan and size at maturity. A fast growth period can be beneficial in a chronic toxicity test, because the test should comprise the entire life cycle of the organism, but, on the other hand, the long exposure duration and personnel time would involve increased costs [26]. In our study *G. aequicauda* reared at the lowest temperatures 10°C and 18°C grew more slowly than those reared at 24°C. The highest growth rates were observed at 24°C at all salinities tested and the lowest at 10°C and 36‰ and this fact is not surprising because is known to occur in the natural population of amphipods [29]. Individuals reared at 24°C had a shorter lifespan (ranging from 98 to 105 days) and have reached a smaller size, while individuals kept at 10°C also having lower growth rates, exhibited the largest size because of their longer lifespan (ranging from 182 to 210 d). Although the maximum body size attained by individuals under laboratory conditions was larger than the body size attained by organisms found in the field [4].

The sex of males and females could be confirmed between 35 and 56 days after hatching for individuals kept at 10°C, and between 28 and 35 days for individuals at 24°C. By comparing life cycle traits of *G. aequicauda* at the conditions (18°C-36 ‰) previously investigated with different temperatures and salinities of this study, we can confirm the use of a temperature of 18°C in the toxicity test. Regarding salinities, those below 36 ‰ can be used in chronic toxicity tests with *G. aequicauda*, but providing a gradual acclimation. Under these conditions the life cycle was shortened to about 6–9 weeks, without lowering the amphipod's performance. Although at 24°C the life cycle has been even briefer, *G. aequicauda* showed a low fecundity.

3 Ecotoxicology

Amphipods are frequently chosen as bioindicators in acute marine and estuarine sediment toxicity tests [30, 31] because of their high sensitivity [32] and because their population densities are known to decline along pollution gradients in the field [33].
G. aequicauda as a potential test species for use in the sediment toxicity assessment was evaluated , since this is an endemic species in the Mediterranean and fulfils many of properties [34]. *G. aequicauda* showed to be tolerant to different salinities and temperatures, although it prefers a range of 18 to 20°C and 15 – 36 ‰. This species has shown to be tolerant to different grain size showing a high survival (91%) in a range of sediment types from 0.5 to 100% fine fraction [5]. The effect of organism density is one of the factors to consider when developing toxicity tests. Prato and Biandolino [5] found a highest survival values with 5 and 10 individuals/dm^{2}density.
The effect of the diet type on the survival of this amphipod over a 28-day period showed that it does not like a dried diet, but this species can be conveniently cultured with a fresh natural diet [5, 6].

3.1 Sensitivity to contaminants

Studies performed included acute and chronic exposure to contaminants in the water, spiked and field-contaminated sediments [5, 35, 36, 37, 38, 39, 40]. The sensitivity of *G. aequicauda* to references toxicants is used to assess the condition of the test organism. This positive control is a routine procedure for toxicity testing. In order to evaluate the sensitivity of *G. aequicauda*, a water-only toxicity test was conducted with different reference toxicants: cadmium, copper and mercury. The 96-h LC50 values (95% confidence limits) for *G. aequicauda* were respectively of 0.80±0.42 mgCd/L, 1.08±0.5 mgCu/L and 0.01±0.005 mgHg/L. These results evidenced a high sensitivity to reference toxicants.
In addition this amphipod has shown to be sensitive to mixture toxicant, indicating a synergic effect for Cu-Cd combination and an antagonistic effect for Hg-Cu and Hg-Cd [37].
In acute 10-d sediment toxicity *G. aequicauda* has shown to be sensitive to sediments from contaminated sites for which mortality was very high compared with control sediment. In a previous study Prato et al. [38] found that that temperature and season of collection influence the sensitivity of *G. aequicauda* to cadmium. The 96h-LC50 values recorded, ranged from 1.50 mgCd/L in winter at 10°C to 0.10 mgCd/L in spring and summer at 25°C. The integration of the results from this series of tests has culminated in the definition of an experimental protocol to conduct acute sediment toxicity test with the amphipod *G. aequicauda* [5].
Another aspect to consider before undertaking the ecotoxicological studies is the knowledge of the physiological status of

test organism, necessary for a correct interpretation of bioassays. Because of its potential as an indicator of physiological status, the lipid content of test organisms was measured in seasonal experiments by Prato and Biandolino [38]. This study showed that lipid content had a significant effect on the sensitivity of *G. aequicauda*. The highest LC50 values appeared with an increase of total lipids. While algal biomass and temperature have not had a significant influence upon sensitivity. This information will be used to improve the interpretation of ecotoxicological test results with *G. aequicauda*.

This review confirms the importance and ecological relevance of *G. aequicauda* in Mar Piccolo basin and its sensitivity to environmental disturbance and toxicants, which makes it a relevant species for environmnental and ecotoxicological studies. The short life cycle of *G. aequicauda*, its amenability to experimental investigation, and its sensitivity to pollutants make the species an ideal laboratory organism for the assessment of toxicity [41, 5].

Nevertheless thought this review many aspect of this species'ecology were highlighted, showing that further research devoted to this species is required.

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The Effect of Heavy Metals on Benthic Foraminifera in the Gulf of Gela (Sicilian Channel)

M. Musco[1], A. Cuttitta[1], S. Bonomo[1], G. Tranchida[1], A. Mazzola[2], S. Vizzini[2], C. Tramati[2], A. Bonanno[1], B. Patti[1], G. Basilone[1]
1, Institute for Coastal Marine Environment, CNR, Capo Granitola (TP), Italy
2, Department of Ecology, University of Palermo, Italy
m.musco@iamc.cnr.it

Abstract

Benthic foraminifera are very sensitive to oceanographic parameter variations, to which they rapidly response through changes in the abundance and/or richness of their associations and in morphological variations of their shell. Many studies have used benthic foraminifera as bioindicators. We investigated recent benthic foraminiferal assemblages in the Gulf of Gela (Sicilian Channel, Mediterranean Sea), examining their relative abundance, specific richness and morphological abnormalities, to highlight a possible correlation with oceanographic features and the concentration of heavy metals in sediments. Sediment was sampled in summer 2006 using box corers in four stations at increasing distances from a point-source of industrial pollution. We performed hydrological characterization of the study area, grain size analysis and heavy metal determinations in sediments, and quantitative and qualitative analyses of benthic foraminifera. Heavy metal analyses highlighted very high mercury and cadmium concentrations in the station nearest the pollution source, in which the Dominance index showed the minimum of population density. In conclusion, a positive correlation seems to exist between the abundance of foraminifera and the concentration of cadmium and mercury in the sediment.

1 Introduction

The Gulf of Gela is located in the southern coast of the island. Since 1962 the Petrochemical Industry has been active in the middle of the gulf, close to the city of Gela, where about five million tons of crude oil per year are refined, to produce LPG, gasoline, diesel and pet-coke.
At the end of the refining process, many highly dangerous substances, which include mercury, cadmium, arsenic, etc, are released in the marine environment as waste products.
The other main pollutants of the area come from the massive presence of greenhouses on the coastal dunes of the Gela's Plain [1].

Benthic foraminifera are one of the groups of marine microorganisms often used as bioindicators of pollution in marine ecosystems. These marine protozoa can live in different microhabitats and many of them have a mineralized shell.
The peculiarity of benthic foraminifera is their sensitivity to environmental variation, to which they rapidly respond through

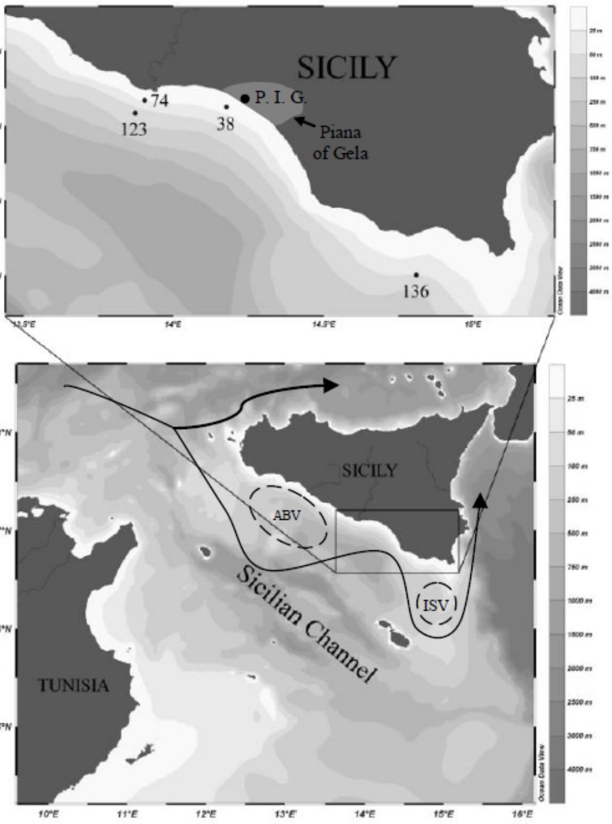


Figure 1: The Gulf of Gela and the sampling stations. P.I.G.: Petrochemical Industry of Gela; AIS: Atlantic Ionian Stream; ABV and ISV: Adventure Bank and Ionian Slope Vortexes.

changes in the abundance and/or richness of their associations and in morphological variations of their shell.

Studies on the effects of pollution on benthic foraminifera began in the second half of the last century: the innovative works by Zalesny [2], Resig [3] and Watkins [4] were followed by several other studies that used benthic foraminifera as indicators of marine pollution, particularly in coastal areas [5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]. Similar works were conducted in Italy in coastal marine areas and in transition environments [17, 18, 19, 20]. There are different ways to use benthic foraminifera as bioindicators of pollution: Frontalini & Coccioni [21], Samir & El-Din [15], and Triantaphillou et al. [22] corroborated the use of shell deformation as biomarker of heavy metals pollution, while Bergin et al. [23] and Geslin et al. [16] showed the correspondence between distribution of benthic foraminifera and the concentration of heavy metals in sediment. The princi-

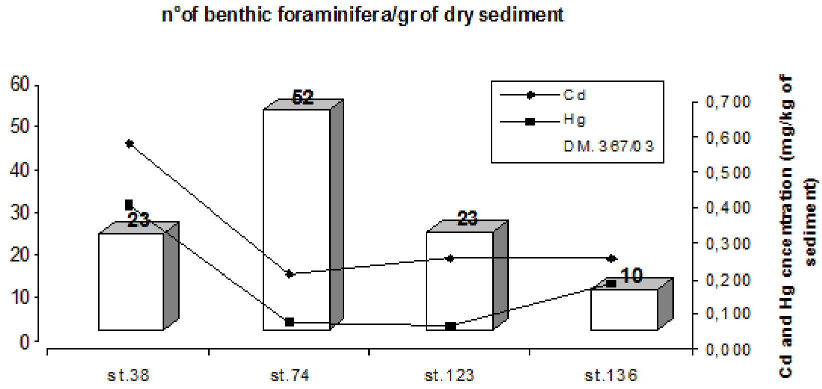


Figure 2: Density of benthic foraminifera (n° per gram of dry sediment) and Cd and Hg concentrations in the four stations compared with the Italian law limit.

pal objective of this work was to investigate the use of benthic foraminifera as bioindicators of heavy metal pollution in the Continental Shelf of Sicily.

2 Materials and Methods

Recent benthic foraminiferal assemblages were investigated in the Gulf of Gela (Sicilian Channel, Mediterranean Sea). Sediment was sampled during the oceanographic cruise “Bansic’06”, in summer 2006, by box corers in four stations (st. 38, st. 74, st. 123, st. 136). These stations were chosen for their oceanographic features and distance from pollution sources. The most relevant oceanographic factor in the Sicilian Channel is represented from the Atlantic Ionian Stream (AIS), a strong shallow current which runs along the southern Sicilian coast to the Levantine Basin, in west-east direction. Along its way it creates two cyclonic vortexes, inducing transportation of nutrients and elements, and favouring the instauration of upwelling currents ([24] – Figure 1). Relatively to this current’s flow, the stations 74 and 123 were chosen as control areas, while the stations 38 and 136 as possible impacted areas. The bathymetric range of the sampled stations is included between -10m of the station 38, and -84m of the station 136. Sediment samples were immediately frozen at -20°C; chemical and biological analyses were performed only on the first 2 cm of sediment, corresponding to a relatively recent benthic foraminiferal fauna (average sedimentation rate in the sampled area: 0.28 cm$\cdot$yr^{-1} – [25]).

Marine environment was investigated under three different aspects: sediment analysis, hydrological analysis and biological analysis, and possible correlation between biotic and abiotic feature were then explored.

We performed hydrological characterization (pressure, temperature, conductibility, oxygen, light transmission and fluorescence) using a multiparametric probe CTD

SBE 911plus (Underwater Unit Deck Unit) of the SEA-BIRD ELECTRONICS Inc.

Grain size analysis was carried out through a Laser particle sizer (Analysette 22 Fritsch), and heavy metal determinations (Cd, Cr, Cu, Ni, Pb, Hg, As, V) were conducted on the $<63\ \mu$m sediment fraction through two steps: sediment mineralization (US EPA 3052/96 - hotplate digestion technique) and ICP-OES (Inductively Couplet Plasma-Optical Emission Spectrometer) analysis (US EPA 6010B/96 protocols).

Benthic foraminifera were identified and counted under a microscope 60-110X, following the taxonomy proposed by Loeblich & Tappan [26, 27].

3 Results and Discussion

Temperature, salinity and dissolved oxygen trend, resulted from the hydrological analysis, and the pelitic-sand grain characterization showed values according to distance from the coast and bottom. In the station 38, closest to the coast, heavy metal analyses highlighted mercury and cadmium concentrations higher than those established by the national law (Figure 2). Almost constant concentrations were found for nickel in all the stations, while a peak of lead was highlighted in the station 136. In general, most heavy metals showed a slight increase from the coast to the open sea (Figure 3), probably due to the AIS. A cluster analysis of heavy metals in sediment showed a similarity between the stations 123 - 136, and 38 – 74 (Figure 4). This data suggest a possible influence of the heavy metals distribution by the oceanographic patterns (AIS).

Seventy-three benthic foraminifera species were identified. In the stations 38 a predominance of the epiphaunal species *Ammonia beccarii* and *Elphidium crispum* was found, while in the open-sea stations the infaunal species *Bulimina aculeata* and *Bolivina catanensis* were dominant. Species distribution seems greatly influenced by the ecological, oceanographic and bathymetric condition of the sampling area. In the Q-mode cluster analysis *Ammonia* spp. is grouped with *Bulimina* spp., *Bolivina* spp. and *Valvulineria* spp., which prefer low oxygen environment (Figure 5). This grouping, based on the relative abundance of the genus in the different stations, well highlighted the stress-tolerant nature of *Ammonia* spp. [28, 29, 30, 31], which dominated the majority of the assemblages, also in low oxygen environment.

The number of individuals per gram of dry sediment followed a coast-wide negative gradient, made exception for the station 38 that presented an inferior value to the attended one. This trend is highlighted also by diversity index: Dominance index, evaluated for benthic foraminiferal assemblages, showed a low population density in the station 38, which presented the highest mercury and cadmium levels, while the Shannon index was influenced by the oceanographic conditions (Figure 2, Table 1).

Morphological analysis performed on shells of benthic foraminifera showed only 4 malformed individuals of the epiphaunal species *Ammonia beccarii* and *Elphidium crispum*, in the coastal stations 38 and 74 (Figure 6).

According to Boltovskoy et al. [32] and Geslin et al. [16], these malformations, resulting less than 1% of the total number of individuals observed, are probably due to the natural morphological adaptation to the substrate.

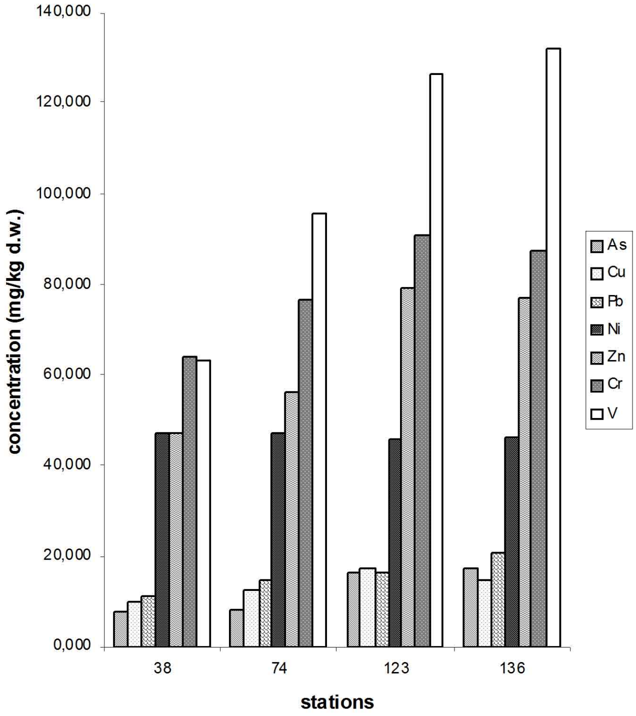
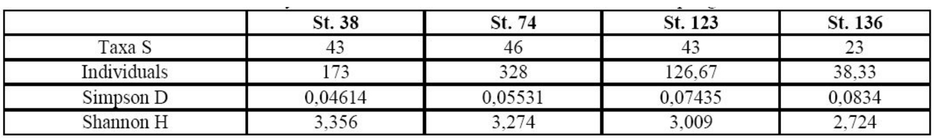


Figure 3: Heavy metal concentration (mg/kg d.w.) in the four sampling stations.



	St. 38	St. 74	St. 123	St. 136
Taxa S	43	46	43	23
Individuals	173	328	126,67	38,33
Simpson D	0,04614	0,05531	0,07435	0,0834
Shannon H	3,356	3,274	3,009	2,724

Table 1: Diversity indexes values of benthic foraminifera in the sampling stations.

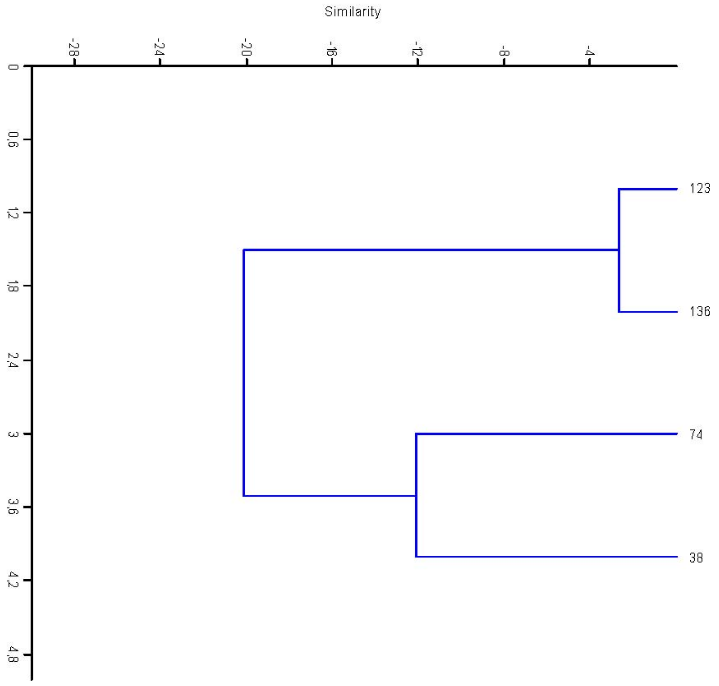


Figure 4: R-mode cluster analysis of heavy metals in the four sampling stations.

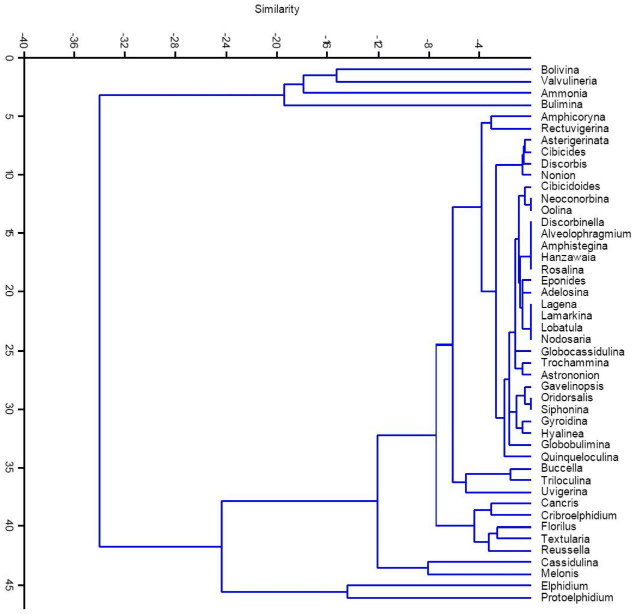


Figure 5: Q-mode cluster analysis of benthic foraminifera genera in the sampling stations.

Figure 6: Malformed specimens of *A. beccarii* (A,C) and *E. crispum* (B) in the stations 74 (A, B) and 38 (C), and the respectively normal specimens (D and E).

4 Conclusions

Considering the results obtained in this study, it seems that the high concentration of the more toxic heavy metals (i.e. mercury and cadmium) in the sediment, has a negative influence on the abundance of benthic foraminifera, while distribution and genera richness, as well as shell malformations, seem to be due to natural (oceanographic, ecological and bathymetric) condition.

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A 20-Year Time-Series Analysis of Carbon Oxidation Rates in the Dark Waters of the Ionian Sea: the Microbial Response to the Global Changes

M. Azzaro, R. La Ferla
Institute for Coastal Marine Environment, CNR, Messina, Italy
maurizio.azzaro@iamc.cnr.it

Abstract

In the marine ecosystem the biological pump controls the export of biogenic carbon from the surface layers down to the depth. In brief, through photosynthesis processes in the euphotic zone, this mechanism transforms the carbon dioxide into organic matter, the latter sinks to the waters below and at the same time is consumed by respiration with the production of metabolic CO_2. However, during the formation of dense waters, a significant quantity of organic products is conveyed from the surface within the water mass and remineralized inside it, thereby altering the normal flow of the biological pump.

In 1990 a transitional climatic event (EMT, Eastern Mediterranean Transient) has changed not only the physical characteristics of the Mediterranean Sea but the microbiological, too. The organic load advected from Aegean Sea has fuelled the deep-sea biota that has utilized most of the oxygen carried by the new water masses for the biological oxidation. In this study the evolution of carbon dioxide production rates in the deep Ionian Sea, before and during the Eastern Mediterranean Transient (EMT), is reported. The check of the carbon oxidation evolution has shown that EMT event has constituted a natural test for a different approach to the oceanic carbon sequestration. Biogeochemical implications of accelerated rates of oxygen consumption and then of CO_2 sequestration in the Ionian Sea will be analysed and discussed in the text.

1 Introduction

During the last 150 years, the global increase in the consumption of fossil fuels together with the reduced capacity to remove CO_2 due to deforestation, have caused a dramatic input of carbon dioxide into the atmosphere [1]. The atmospheric carbon dioxide levels increased by nearly 40%, from preindustrial levels to 2007 [2] and this increase, is at least an order of magnitude faster than has occurred for millions of years [3]. The resulting enhancement of the natural Greenhouse Effect is tempered by oceanic uptake by sequestration of CO_2 in the deep oceanic waters, which accounts for nearly a third of anthropogenic carbon added to the atmosphere [4, 5]. In fact, topical studies have supported the significant role of the oceanic biological pump [6, 7] and continental shelf pump [8] with respect to the atmospheric CO_2 build-up (Figure 1).

Option to store carbon dioxide in the ocean is a potential climate change mitigation that is currently receiving an increasing level of attention within business, academic and policy communities [9, 10, 11]. In the ocean the main strategies currently studied are: the enhancement of the net oceanic uptake from the atmosphere by fertilization of phytoplankton with micro- or macronutrients and the direct injection of a relatively pure CO_2 steam to ocean depths great than 1000 m [12, 13]. The long term effectiveness and potential environmental consequences of ocean sequestration by either sequestration strategies are still unknown [14].

In this paper a mechanism of ocean sequestration is reported, born studying the evolution of the carbon dioxide production rates in the deep Ionian Sea, pre- and during the Eastern Mediterranean Transient (EMT). Such event has dramatically modified the pre-existent thermohaline circulation and rapidly replaced about 20 % of the waters below 1200 m horizon by younger waters of Aegean origin (Cretan Sea Overflow Water, CSOW). The CSOW is characterized by higher oxygen levels, lower nutrient concentrations, high chlorofluorocarbon (CFC-12) and ^{137}Cs values and higher DOC estimates with respect to the previously Eastern Mediterranean deep waters [15, 16, 17]. The organic load advected from Aegean Sea has fuelled the deep-sea biota that has utilized most of oxygen carried by the new water masses for biological oxidation. The check of the carbon oxidation evolution has showed that EMT event constituted a natural test for a different approach to the ocean sequestration. Biogeochemical implications of accelerated rates of oxygen consumption and then of CO_2 sequestration in the Ionian Sea will be analysed and discussed in the text.

2 Materials and Methods

The carbon dioxide production rates (CDPR) analyzed in the present work derive from different multidisciplinary field studies carried out in the period 1995-2008 on the Italian R/V Urania. Details on the surveys and water sampling procedures were already described by [18, 19] and [20, 21]. CDPR data are derived by Electron Transport System assay (ETS) performed according to the tetrazolium reduction technique [22]. The ETS assay allows an estimate of the maximum velocity (V_{max}) of the dehydrogenases transferring electrons from their physiological substrates (NADH, NADPH, and succinate) to a terminal electron acceptor (O_2) through their associated electron transfer system. The ETS was corrected for *in situ* temperature with the Arrhenius equation using a value for the activation energy of 15.8 kcal/mol [23]. The ETS below 200 m depth was converted into Oxygen Utilisation Rates (OUR) and in CDPR using the current conversion factors [6, 24].

3 Results and Discussion

In Figure 2, we report the evolution of remineralization rates (as CDPR) in the Ionian Sea derived from both [25] and [26] data, as well as from our direct estimates of CDPR. Estimate of [25] was made considering an oxygen and nutrient balance while estimate of [26] was obtained using a model to estimate oxygen consumption by fitting simulated oxygen distributions. The biological consumption is related to the remineralization of carbon and nutrients, thus under steady-state conditions OUR measures new primary production.

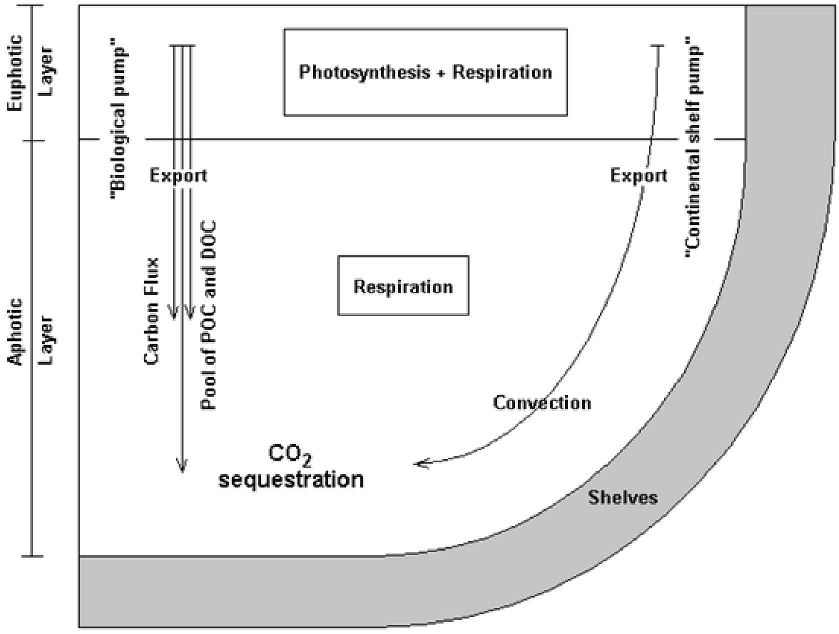

Figure 1: Simplified scheme of CO2 sequestration by Biological pump and Continental shelf pump.

Bethoux [25] estimated a CDPR of 33.1 mg C $m^{-2}d^{-1}$ (pre-1987), projected a 3 % per year rise in new primary production due to a year increase in nutrients terrestrial discharges and predicted anoxia in the deep Eastern basin in a few tens years. Such prevision was of course rather academic, not only because of the uncertainty of the current and future nutrient supply, but also since, as mentioned, the circulation of the sea, and hence the distribution of properties determining local consumption, have lately undergone profound changes. The remineralization estimated by ([26]) for 1987 (43.8 mg C m^{-2} d^{-1}) was slightly higher than those by [25] for pre-1987 and both are referred to the pre-existent thermohaline circulation before EMT event. Our estimates based on the ETS assay in 1995 for the Ionian Sea, still not affected by EMT, result twice higher than Bethoux projection for 1995 (42 mg C m^{-2} d^{-1}). After the EMT event, the remineralization rate became considerably higher. Part of the striking difference between our values and previous estimates could be due to the several approaches utilized, but the increase since 1995 until to 1999 is really remarkable. These data (in 1998, 226.7 mg C m^{-2} d^{-1} in 1999, 349.9 mg C m^{-2} d^{-1}) would even worsen the Bethoux's previsional trend and would then suggest that the EMT should even shorten the time before the occurrence of anoxia. However, recent experimental observations have shown a new scenario, since a drastic reduction of remineralisation rates was monitored in the Ionian Sea from 2001 to 2008 and in the latter year the

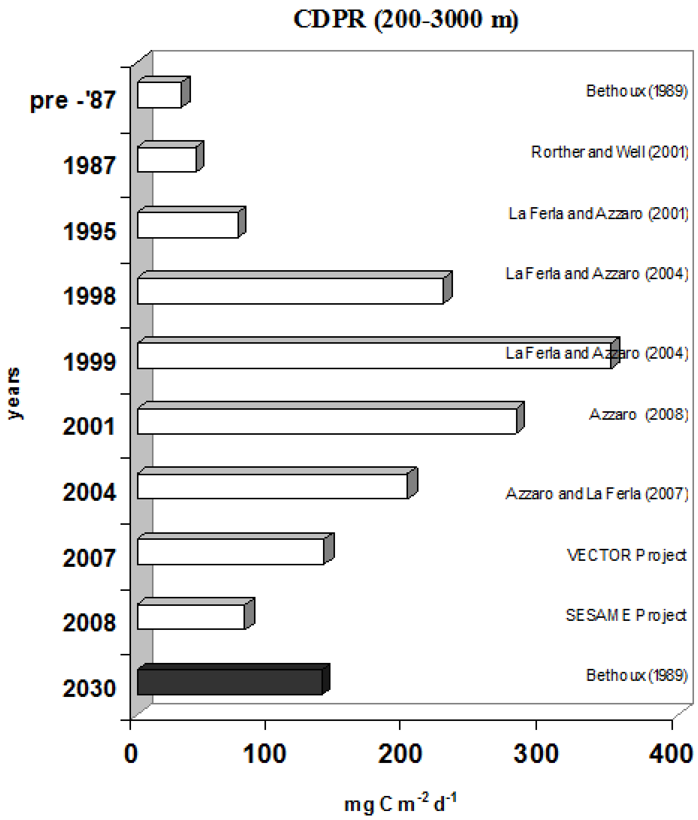

Figure 2: Remineralization rates determined in the Ionian Sea from data antecedent 1987 until to 2008 (white horizontal bars), and projection at 2030 (black horizontal bar).

CDPR were comparable to those measured with the same method in the pre-Transient state. So the anoxia has been averted for now for the following reasons: 1) the increase in deep oxygen utilization depended much less on a possible increase of export production [27] than on the labile carbon entrained in the newly formed deep waters [17]; 2) the higher respiration rates measured for a decade, were indeed associated with water masses that, being very recently formed, had a higher value of pre-formed oxygen. Therefore, the large spike in respiration has been compensated, up to now, by the larger amount of oxygen carried by the new water masses [28, 29, 30]. However, we cannot rule out the possibility of anoxia in the Eastern Mediteranean Sea, because is in act a significant increase in nutrient inputs in the basin, as hypothesized by [25]. Certainly the EMT event should also be relevant in studies on the occurrence of anoxia in past climates which led to the well-known Mediterranean sapropels [31]. Together with the probable anoxia another key of lecture of EMT event is the accelerated carbon dioxide sequestration registered in the deep water of Ionian Sea. One contributing factor to the accelerated deep-water CDPR might be the untypically high temperature in the deep Mediterranean Sea (> 12 °C) that is not a limit to the microbial metabolism. Precedent studies has already evidenced the importance of carbon sequestration in the deepest with respect to the shallowest layers in the Mediterranean Sea in comparison to oceans [32, 18].

The unusual CO_2 sequestration registered during EMT event highlighted that deep basins do not constitute only an inert sinks of carbon dioxide but could act quickly in carbon sequestration mitigating effect of the climate changes on regional scale.

Extension on a global scale of the EMT experiment is conceptually feasible but need at least the control of three main factors: water temperature, available organic substrates, oxygen levels. Deep-water temperature in the oceans is lower than in the Mediterranean Sea and could affect the microbial carbon sequestration for about 40 % [30]. It is well known that the majority of dissolved organic matter in the deep ocean is refractory, then a natural event (e.g. EMT) or anthropogenic activity should consider the injection of labile easily oxidable DOC. During the EMT event, pre-formed oxygen was driven by CSOW in the Eastern Mediterranean depth. Hence a constant balance between oxidable organic substrate and oxygen level must be maintain with the aim of avoiding anoxia.

4 Conclusions

The occurrence of changing patterns in microbial biogeochemistry has emphasized the Mediterranean vulnerability to climate changes, confirming that the export of biogenic carbon is partially driven by the biological pump and enhanced by lateral advection.

The EMT event resulted a particular case of continental shelf pump that has significantly affected the deep respiration in the Ionan Sea. Nevertheless the occurrence of anoxia in a shorter time than Bethoux prevision is removed by the larger amount of oxygen carried by the younger Aegean water masses.

In the context of ocean sequestration, the EMT resulted a natural test which focused the possible role of oceanic depths in mitigating the climate changes and then contributing to dump atmospheric CO_2 build-up.

5 Acknowledgements

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Settlement and Habitat of Juvenile European Spiny Lobster *Palinurus Elephas* (Fabricius, 1787) (Crustacea Decapoda) in the North Western Sicilian Coasts

M. Gristina[1], G. Garofalo[2], F. Fiorentino[1]
1, Institute for Coastal Marine Environment, CNR, Mazara del Vallo (TP), Italy
2, Institute for Applied Mathematics "Mauro Picone", CNR, Roma, Italy
michele.gristina@iamc.cnr.it

Abstract

The ecological and commercial value of species of the genera Palinuridae is now widely recognized throughout the European continent. These species present a complex life cycle, with slow growth rate and a high size at maturity. As a consequence, these species are particularly susceptible to fishing pressure and the management and conservation of this resource requires specific attention. *P. elephas* is considered a vulnerable species mainly due to the high age-dependent mortality of adults and sub-adults individuals, which consequently reduces fishing recruitment and that might lead to an ecological bottleneck for this species limiting population growth.
With these notions in mind, this study aimed to identify the settlement stage of *Palinurus elephas* and to describe the preferred microhabitat for juveniles (Fabricius, 1787), along the north-western coast of Sicily (Isola delle Femmine – Palermo). Furthermore, on the basis of field observations, we tested in a controlled environment, the geometric features of the substrate needed for *P. elephas* settlement, This was done to propose suitable artificial structures that might favor or facilitate the settlement of *P. elephas* and that might protect juveniles during their benthic life stage as well as reducing natural mortality.

1 Introduction

Due to their important commercial value, lobsters represent one of the main target for artisanal fisheries and, even if catches are sporadic and reduced, this fishery has been active over the years. Thus, almost all *P. elephas* populations in the Mediterranean Sea are evidently overexploited [1, 2]. Despite its important economical and ecological value, the ecology of *P. elephas* stocks presents large knowledge gaps and very few attempts have been made to achieve a sustainable exploitation of this resource. Lobsters are generally characterized by peculiar life cycle which involves a long pelagic larval stage (from 5 to 12 months depending on the species), with two development stages (namely Phlyllosoma and Puerulus), a juvenile benthic stage (post-puerulus) and an adult benthic stage. In addition, lobsters present a slow growth rate and they reach a late size at maturity (4/5years) [3]. Consequently, lobsters are particularly susceptible to fishing activities that are not managed or regulated.

Furthermore, the high natural mortality experienced in the post-pueruli stage reduces dramatically recruitment, hence creating a bottleneck which prevents population growth.

Experiments carried out in situ and in the laboratory on Palinuridae, both in the tropical and temperate regions, demonstrated the importance of appropriate artificial substrates to increase larval settlement and for the survival of juveniles. However, at present such experiments are not available for *P. elephas*. Only recently Diaz et al. [4] provided some indications on habitat preference by *P. elephas* in the Spanish Mediterranean.

This study aims at filling the present gaps on the ecology of *P. elephas* and to provide new insights on the biology and ecology of the juvenile's stage of this species.

Specifically, this study has the following objectives:

1. to identify the season during which occurs the transition from the pelagic stage (phyllosoma) to the benthic stage (post-puerulus)
2. to describe the features of the preferred habitat of *P. elephas* juveniles (substrate type, shape and slope)
3. to assess the depth range where the highest number of juveniles is recorded.

2 Methods

Field Studies - Evaluation of habitat characteristics and settlement period for *P. elephas* A visual census was carried out on *P. elephas* juveniles in the two study areas (Isola and Barcarello). Visual census was done by scuba diving every 15 days between May and October 2005 and after a short break during the winter (November 2005 to February 2006), scuba activities were resumed (March to November 2006). For each area, sampling was performed at four depth ranges (5-10, 10-15, 15-20 and 20-25m). At each depth range, each of the two divers carried out 2 transect for 5 minutes, with a interval of ½ a minute in between.

Thus, for each depth range, 4 transects were run, of 5 mins. each, for an approximate area of 25 m^2. data was expressed in n of individuals/m2 and average for the 4 transects were obtained. To identify the preferred substrate for *P. elephas* juveniles, when an individual was found divers recorded slope and orientation of refuge, type of refuge, distance from the bottom, habitat type (rocks, cliffs, detritus). We compared the preferred schelter features by the log-likelihood ratio test for goodness of fit (G-test) to reveal any positional bias. The shelter preference of lobsters was compared to expected values from a Poisson distribution by the G-test [5] with Williams' correction.

3 Mesocosm studies

Individuals used in the experiment were captured with seine nets from local fishermen, handled and transported carefully to the laboratory facility as soon as possible after landing. For the experiment, we used a number of seawater tanks with a recirculation system (water flow 56±7,3 l min-1), with a water residency time of 12 hours.

Temperature and salinity in the seawater system was monitored daily during the whole length of the experiment. Temperature and salinity values never differed among tanks (17,9 – 18,3 °C; 37,5 ppt). Each individual of *P. elephas* was measured prior to be introduced in the tanks; carapace length (LC), width (LargC), total

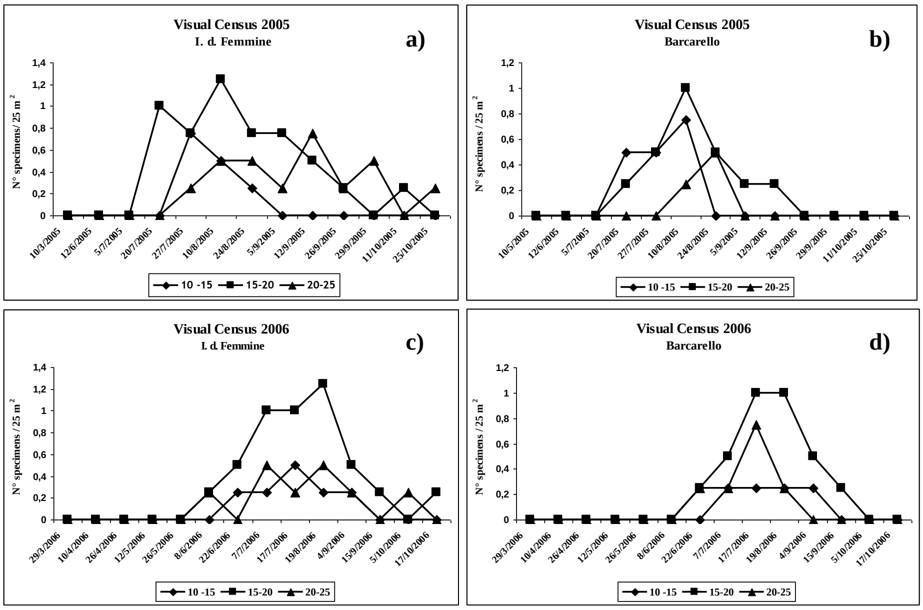

Figure 1: Juvenile density for each site, over years an at each depth range.

length (LT) and sex were recorded. A small tag with a serial number was applied under the skin to identify the animal. Lobsters used in the experiment were fed daily using frozen *Mytilus edulis*, Parapenaeus longirostris e Boops boops. Prior to the beginning of the experiment, individuals were acclimated to the tank system for at least 48h. Each individual was only used once for each experiment. Using different experimental set-up we allowed to test:

1. Substrate slope.
   Preference for substrate slope was evaluated by offering each individual a choice of two different substrate slopes. Two artificial structures were built using identical calcarenitic bricks (40 cm x 40 cm x 40 cm). The first structure was a regular parellelepiped (40 x 40 x 40 cm); the second resembled an inverted cone (40 x 40 x 28 cm) to mimic a wall with a 35 degree slope. Each structure had 6 circular openings (three 4.5 cm wide and 15 cm deep, three 6 cm wide and 15 cm deep) and a small rectangular crevice (10 cm high, 30 cm wide and 25 cm deep). The dens were sufficiently large for all the individuals used in the experiment. Six independent replicates were performed for each combination of substrate slope and arena. Each replicate was made up of three independent observations and the response variable was the percentage of substrate slope selection. A total of 72 lobsters (33 male and 39 female) were tested, of 24 to 54 mm CL (mean 41.8 ± 7.5 SD). G-test [5] with Williams' correction was performed to test for differences between the observed number of

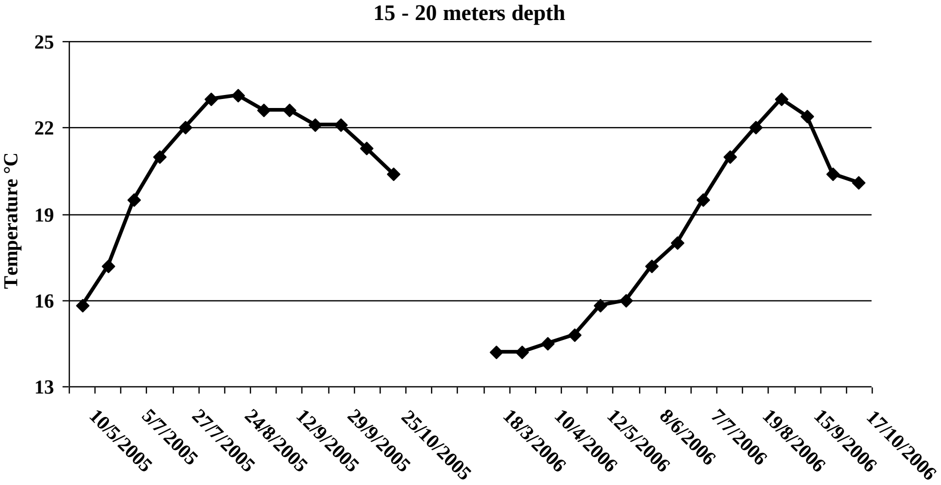

Figure 2: Temperature (°C) variations over the study period.

and expected values from a Poisson distribution.

2. Lobster preference for shelter size.

This was evaluated by presenting each individual with a choice of five different shelter sizes (5.0; 6.0; 7.5; 9.0; 12.0 cm in diameter). The experiment was carried out under two different conditions. In the first, lobsters chose a shelter without external pressure, in the second a potential predator (textitOctopus vulgaris) was introduced into the arena. To allow emission of chemical cue [6] but to avoid active predation on the lobster, the octopus was held in a small lobster pot in the centre of the experimental arena. A total of 36 lobsters (16 male and 20 female) (18 with and 18 without predator) and 18 octopuses of similar size were used. Lobsters ranged from 21 to 54 mm CL (mean 41.7 ± 8.4 SD) and octopuses from 982.51 to 1114.08 g total wet weight (mean 982.51 ± 114.08 SD). The relationship between lobster carapace length and shelter diameter, in either the presence or absence of a predator, was modeled using least squares linear regression.

3. Lobster vulnerability to octopus predation.

To assess lobster vulnerability (the probability that a lobster is attacked and killed by textitOctopus vulgaris) in artificial structures with different slopes (vertical or horizontal), live juvenile lobsters were tethered [7, 8] on different substrate slopes and exposed to a potential predator (textitOctopus vulgaris). Lobsters were tethered with monofilament tied around the cephalotorax between the fourth and fifth pairs of walking legs. The opposite end of the monofilament was tied to an eye fixed to the artificial calcarenitic structure (60 cm x 60 cm x 60 cm), allowing about 20 cm of movement. In this way specimens could move along one surface (either vertical or horizontal) but move-

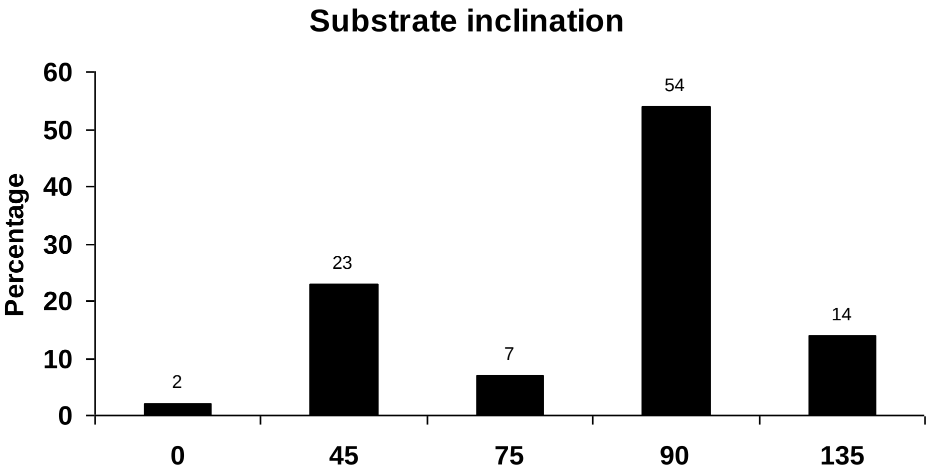

Figure 3: Number of juveniles associated with different substrate inclinations.

ment along the other surfaces was restricted. For each test two lobsters of similar size were chosen (± 1.0 mm CL), randomly assigned and tethered to one of the two structures.

One hour after the lobsters were tethered to the artificial structures one octopus was released in the centre of the arena.

A total of 30 lobsters and 6 octopus of similar size were used. Lobsters ranged from 28 to 63 mm CL (mean 41.3 ± 5.1 SD) and octopuses from 524 to 879 g total wet weight (mean 743.20 ± 110.67 SD). Thus, after each trial, the octopus used were put back into the storage tank from where twenty-four others were then randomly selected for further trials until the end of the experiment. These procedure reduced the chance of using the same predator in the same treatment more than once.

The proportion of surviving lobsters between the treatments was determined using G-test [5] with Williams' correction assuming that the octopus had the same probability of choosing the prey (0.5).

4 Results

Field Studies - Evaluation of habitat characteristics and settlement period for *P. elephas*. A total of 54 sampling replicates were conducted over the whole study period: 162 *P. elephas* individuals were collected, 119 were juveniles (LC $<$ 44.5 mm) and 43 were sub-adults and/or adults. Both in 2006 and 2007, recruitment began between June and August, reaching a peak between the second half of July and the beginning of August. A higher number of individuals was collected in Isola delle Femmine (74) compared to Barcarello (45). Figures 1a, b, c, d illustrate recruitment data over depth ranges. The figures do not include the shallow depth range (5-10m) as individuals were not found at this depth.

The temporal trend of recruitment appears

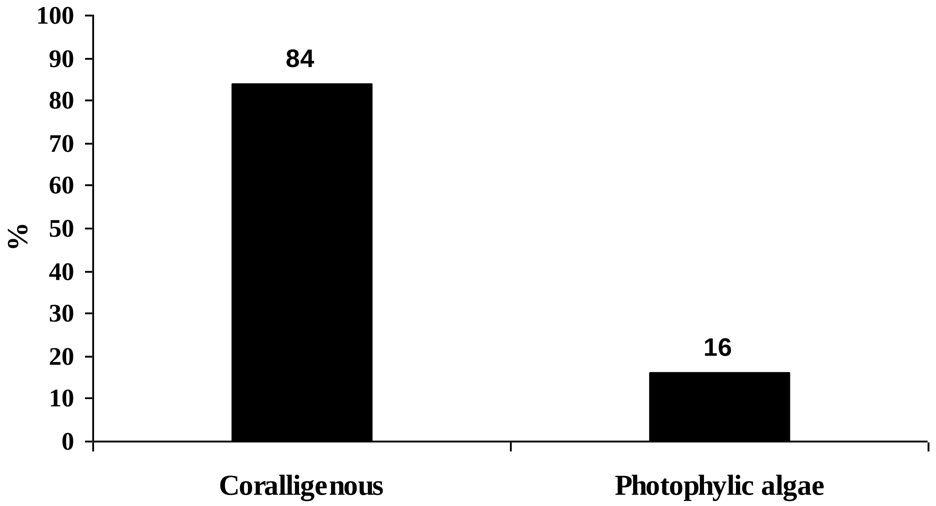

Figure 4: Number of juveniles associated with coralligenous and photophylic algal formations.

similar for the three depth ranges for both years. However, we found a higher number of individuals between 15 and 20m depth. At this depth, recruitment started in June and ended in September, although new recruits were censed till late October. Between 10 and 15m depth, recruitment shows a peak between July and August, but occurs over a shorter time frame compared to the deeper strata. After the beginning of September, we did not find any individuals at this depth.

At 20-25 m depth, a similar trend is reported, although recruitment presented a higher temporal and spatial variability. Figure 2 reports temperature variations over time at a depth comprised between 20-25m. It can be noted that recruitment peaks match temperature increase for both years (2005 and 2006). Water temperature increased in late spring to reach the warmest peak in July/August (23 °C).

P. elephas juveniles show distinct habitat preferences. The relationship between number of recruits and substrate slope is reported in Figure 3. More than 50% of the individuals (64) was recorded on vertical slopes (90°), while 23% (39) was found on slopes at 45° and only 14% of the individuals (16) was found on sub-horizontal slopes (135°). Over 84% of the sampled individuals was found to be associated with coralligenous formations (Figure 4). Algae, encrusting sponges and bryozoans are the most common organisms found in lobster habitats. Only 16% of the recruits (19) was associated with photophylic algae formations, such as textitCystoseira spp. and *Sargassum vulgaris*.

Figure 10 highlights how 77% of *P. elephas* juveniles prefers holes (with general dimensions similar to carapace length and width), while 23% prefers crevices that are significantly larges than individuals size (Figure 5). While previous work reported the utilisation of holes created

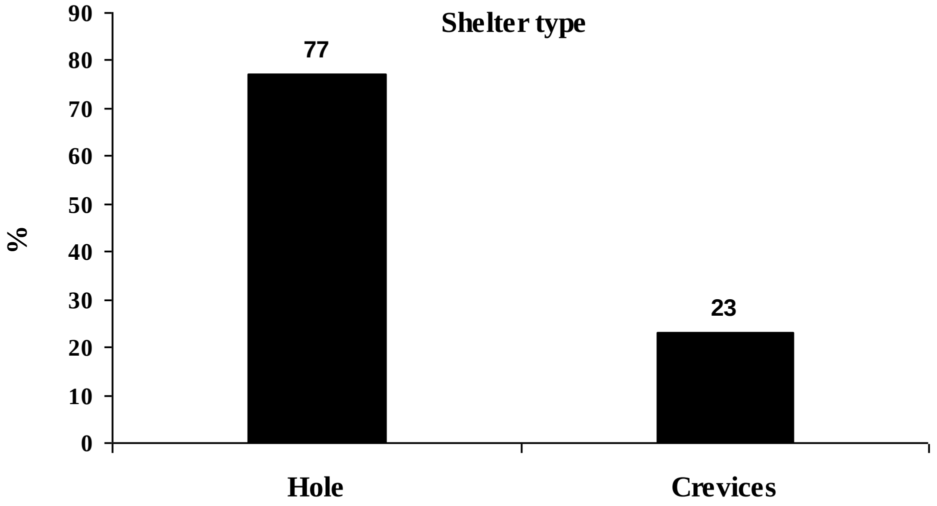

Figure 5: Number of juveniles associated with different types of refugia (hole vs crevices).

by textitLithophaga lithophaga by *P. elephas*, we did not find such association in this study. Most holes created by textitLithophaga lithophaga in the habitat we sampled are found in shallow water (0-5m), where no individuals of *P. elephas* were present.

5 Mesocosm studies

1. Substrate slope.
   A total of 40 individuals were used for this experiment. For these individuals, carapace length (LC) ranged between 19mm and 54mm, while width variations ranged between 14 and 40mm. 70% of the animals (28) showed a clear preference for a steeper slope (45°), while only 30% (12) preferred a vertical slope (Figure 6). Considering a probably of 0.5 that each individual will choose a slope rather than another, a G-test with Williams (n = <200) correction showed a significant preference of a 45° slope to the vertical slope (90°) (G = 6.481, d.f. = 1, P = 0.025).
2. Lobster preference for shelter size.
   A total of 36 individuals were used for this experiment. For these individuals, carapace length (LC) ranged between 21mm and 54mm. the experiment was conducted in two different steps. In the first step, we allowed individuals to choose refugia without other stimuli; in the second step we introduced a predator (textitOctopus vulgaris). The first step involved the use of 18 individuals, of these 17 were found within a hole at the end of the experiment. The outliner was not included in the analysis.
   Figure 7 shows a linear relationship, statistically significant, between hole diameter and carapace length (LC) of individuals used (n° ind. = 17, $R^2 = 0.73$, d.f. = 15, F = 41.8, $P < 0.001$) in the absence of predator. Of the 16 in-

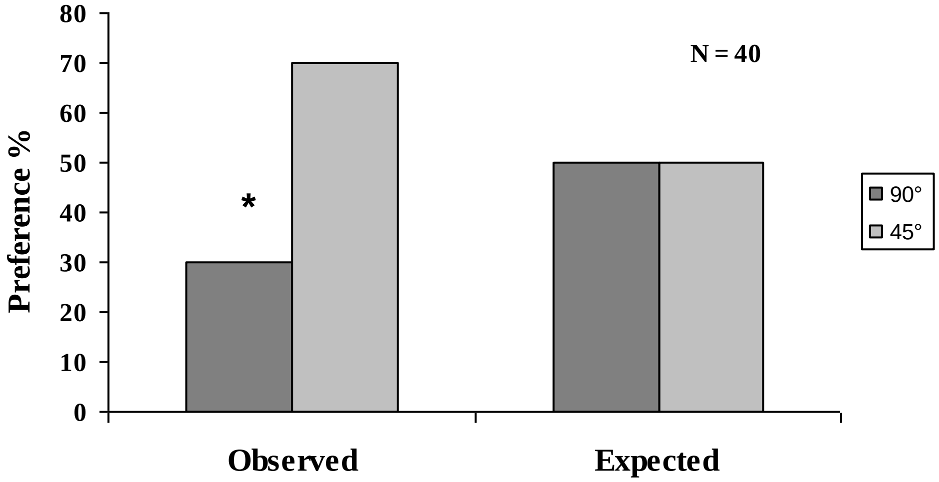

Figure 6: Number of individuals observed on the different substrate slopes. Differences were tested using a G-test, with a probability of substrate slope choice was 0.5; G = 6.481, (d.f. = 1), *P < 0.025.

dividuals used, 16 where found in circular shape holes at the end of the experiment, while 2 where found close to the artificial structure but not within holes. These two individuals were not included in the analysis. The introduction of the predator altered the relationship between lobster and refugium size. When the predator was present, individuals tended to choose refugia casually, mainly as a function of distance, probably trying to reduce the time of exposure to potential attacks. Figure 7 shows the relationship between hole diameter and carapace length (LC); however, there appear to be no significant difference (n° ind. = 16, $R^2 = 0.059$, d.f. = 14, F = 0.83, P > 0.1) .

3. Lobster vulnerability to octopus predation.

   This experiment allowed to test whether a potential predator (textitO. vulgaris) attacks more frequently *P. elephas* associated at different substrate slopes. Octopus shown a clear preference to attack *P. elephas* when on a horizontal rather than vertical surface. 83.3% of the time (15), the octopus attacked the individuals on the vertical slope, while only the 16.6% (3) of the individuals tethered on the horizontal surface were attacked.

   Considering 0.5 the probability that both individuals will be attacked, the G-test showed how the predator attacked significantly the individuals on the vertical surface (G = 5.69, d.f. = 1, P $<$ 0.025) (Figure 8).

6 Conclusions

Lobsters represent an important commercial resource for fisheries worldwide. As a consequence of their high commercial value and of their complex and vulnerable

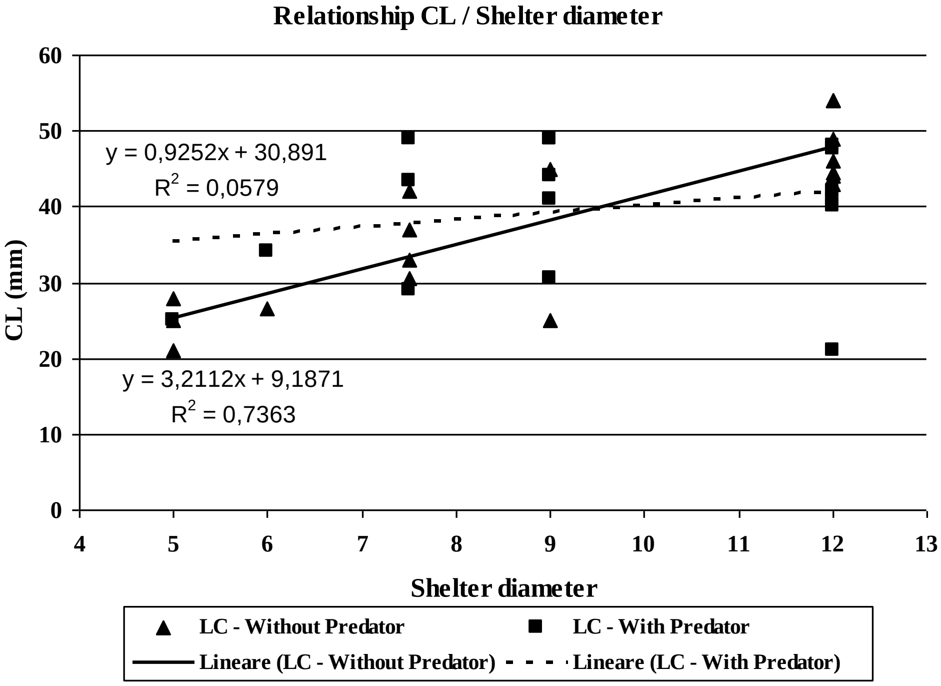

Figure 7: Relationship between LC and refuge diameter in the absence of predator (dot triangles) and in presence of predator (dot squares).

life cycle, lobster populations can be considered overexploited and declining. Several initiatives have been taken to encourage a sustainable management of this resource. However, apart from in a few countries (Australia and New Zeland), which have adopted severe restriction on lobster fishery, all other measures were proven not sufficient and showed poor results.

Management practice, such as minimum size catch, fishing ban for female bearing eggs and fishing ban during the reproductive period, do not seem to have a positive effect on the local populations. However, we believe that such measures have not shown positive effects for: a) the scarce enforcement by local authorities; b) the large amount of illegal fishing. This work, has proven that lobster fishery is largely illegal and that very few fishermen respect current regulations.

In the last decade however, despite local issues, effort has been focused onto improving *P. elephas* settlement as a management practice as the transition from the larval to the benthic stage represents an important step in determining size of lobster populations.

In the Mediterranean Sea, management initiatives have been scarce and few have evaluated the possibility to introduce practice such the one described above (catch, rear and release). However, the results contained in this study show that non inva-

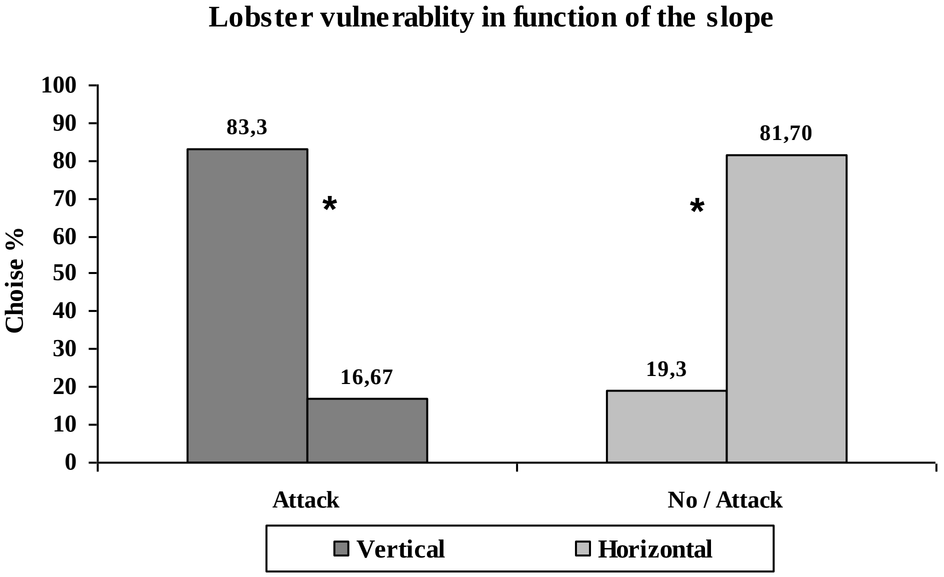

Figure 8: Vulnerability (the probability that a lobster is attacked and killed by textitOctopus vulgaris) of lobsters tethered on different substrate slopes and exposed to a potential predator (textitO. vulgaris). Differences were tested using a G-test, with a probability of substrate slope choice was 0.5; Vertical G = 5.92, (d.f. = 1), **P $<$ 0.005; Horizontal G = 5.45, (d.f. = 1), *P $<$ 0.025.

sive management practices can be adopted, such as the use of artificial structures, to increase lobster recruitment and settlement. Knowledge of location of nursery areas, habitat preference and substrate choice for settlement, recruitment season is essential to implement correct practice for a sustainable management of this resource.

The utilizations of artificial structures, assembled specifically for the aggregation of lobsters [9], is widely adopted in several tropical countries for the protection and aggregation of juveniles lobsters and it has shown encouraging results. In particular, positive results by Butler and Herrnkind [10], which built a series of artificial modules in Florida (USA) to aggregate juvenile lobsters, that reported a significant increase of juveniles and number of individuals recruited by fishing. Even if there are several differences (depth range, preferred habitat for recruitment) among the different genera of Palinuridae, we believe that some management practice proposed in other countries should still be considered an important reference point for the development of a non-invasive approach to lobster resource management in the Mediterranean region. The results presented in this work contributed to shed light on some biological features of *P. elephas* juveniles and allowed to investigate some behavioural aspects in a controlled environment.

Several other ecological and behavioral aspects are still unclear (size at which ontogenetic shift occurs, chemical stimuli exchange, MPA effects, etc.). However, a comparison between in situ and controlled experiments, can represent a fair compromise to approach ecological and behavioral aspects. The development of specific and targeted artificial modules, planned following the results presented here and the bibliography we included here, and coupled with other management practice (see next chapter), could represent an important tool for the recovery of *P. elephas* and to preserve and promote traditional and artisanal fishery, by guaranteeing a high and constant commercial yield.

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Fish Parasites in Polar Seas

M.R. Coscia, U. Oreste
Institute of Protein Biochemistry, CNR, Napoli, Italy
mr.coscia@ibp.cnr.it

Abstract

Parasitism is a very frequent event in the marine ecosystem. Nematodes (Nematoda) are the most common parasites in demersal and pelagic fishes throughout the world. This phenomenon has either human health or economic costs implications. In fact the ingestion of uncooked parasitized fishes causes the gastric *Anisakidosis* disease. Although the present knowledge remained incomplete, studies about parasite biology, phylogeny and ecology in Atlantic, Pacific, Mediterranean and polar areas achieved important results. The life-cycle of nematode parasites includes different vertebrate and invertebrate hosts, fish being only intermediate host, birds and mammals being the final one. The most common nematode larvae found in Arctic and Antarctic regions belong to the Anisakis, Contracaecum, and Pseudoterranova genera; in Antarctic and sub-Antarctic areas the nematode infection intensity is higher than in Arctic and sub-Arctic regions.

During three expeditions to Antarctica we verified a high incidence of parasitic infections in fish, particularly in the species of the families Channichthyidae and Bathydraconidae. We carried out some experiments to analyze the immune response of host to nematodes: we separated proteins of *Contracaecum osculatum* and Pseudoterranova decipiens larvae extracts and demonstrated the presence of nematode-specific antibodies in fish plasma and secretions. The antigenic proteins were identified and the antibody specificity in plasma and bile was compared.

1 Fish parasitism

Parasitism is a very frequent event in the marine ecosystem. Although the present knowledge remains incomplete, studies about parasite biology, phylogeny and ecology, but also taxonomy, and zoogeography in Atlantic, Pacific, Mediterranean and polar areas achieved important results. This phenomenon has either human health or economic costs implications. Nematodes (Nematoda) are the most common endoparasites in demersal and pelagic fishes throughout the world. The accidental ingestion of larval nematodes in raw or insufficiently pickled, salted, smoked, or cooked wild marine fish causes a gastric disease known as *Anisakidosis* [1]. Most of patients complain of acute symptoms such as epigastric pain, occlusion, diffuse abdominal pain, and appendicitis. Larvae are attached to the gastric mucosa eliciting an inflammatory response. The first human case of *Anisakidosis* was diagnozed in Holland and in 1960 van Thiel established the relationship between the human disease and eating infested raw herring. Infestation rate is high in fish: 80-90% in cod rock fish, herring, salmon, and mackerel.

There is a need to create a new classification system of these parasites; for this purpose, genetic differentiation and phylogenetic relationships of different groups based on detailed studies of individual species were revised and compared. Significant contributions to the study of parasite systematics, evolution and ecology have been provided by molecular methods, such as isozyme analysis, restriction fragment length polymorphisms, random amplified DNA analysis, mini and microsatellite DNA polymorphisms, mitochondrial markers, etc. However, a certain progress in the research of fish nematodes has been achieved during recent years, thanks to the use of some new methods, in particular Scanning Electron Micrographs and DNA studies.

Over the first two decades, the application of molecular systematics to the anisakid nematodes of the genera Anisakis, Pseudoterranova and Contracaecum, parasites of aquatic organisms, has advanced the understanding of their systematic, taxonomy, ecology and phylogeny. Different patterns of distribution of anisakid nematodes in various definitive and intermediate hosts have been proposed. Differences in the life history of related species can be due both to differential host-parasite coadaptation and coevolution, and/or to interspecific competition.

2 Fish immune response to parasite antigens

Interest in defence mechanisms of fish derives from the need to develop health management tools to support the aquaculture industry, and, at the same time, to clarify the evolution and origins of immunity in vertebrates.

The host-parasite relationship is based on fine balance between parasite survival and host defense. In this context, parasites often use the same or similar immune signaling molecules and molecular mimicry to escape host immunosurveillance. Both processes represent an adaptive strategy to ensure host immunocompatibility. In immunocompetent hosts, parasites evade the immune response mainly through two mechanisms: i) expression of appropriate antigens, either by antigen variation that is changing the expressed antigens fast enough to prevent any efficient immune response or by antigen mimicry that is expressing epitopes similar to those of host molecules; ii) modification of the host immune response either directly by its own molecules, or indirectly by disregulating the host effector cells. In vivo, antigen shedding in nematodes could be a parasite defence mechanism against antibody-mediated immune effector processes [2].

In an attempt to understand the molecular mechanisms of the host-parasite relationship, Politz and Philipp [3] conducted a biochemical study of the structure of the nematode cuticle. They defined three categories of protein components: 1) Surface associated proteins and glycoproteins, characterized by their ability to be solubilized from the cuticle by mild extraction procedures without reducing agents. They are expressed as the parasite colonizes a new host environment, and can elicit host immune response; 2) Collagens, hydrolyzed by collagenase, solubilized only with the aid of reducing agents; 3) The so-called cuticlins, cross linked by non-reducible bonds involving tyrosine residues [4].

Innate immunity plays greater role in resistance in fish compared to that in poikilotherm vertebrates [5]. The limited repertoire of adaptive immune mechanisms in poikilotherms has conferred a selective advantage on individuals or species possessing natural mechanisms of disease resistance. Models of innate immunity against parasites in teleosts are particularly interesting because of the life history, biochemical and genetic complexity of the eukaryotic parasites compared with viral or bacterial pathogens and because of the potential for greater diversity in anti-parasitic mechanisms [6]. A clearer understanding of the mechanisms of resistance may provide a novel perspective of host-parasite coevolution. Similarly, the survival of parasites in the host environment suggests that adaptation by parasite is a distinct feature.

Recent studies have begun to clarify the role of innate and adaptive immunity against parasitic infestation in teleosts. The skin and gills are common sites of parasite infestation despite the barrier functions associated with mucosal epithelia of fish. Parasites inhabiting the skin and gills are frequent causes of mortality, particularly among fish reared in aquaculture. The physico-chemical characteristics of skin mucus, the presence of haemolysins, lysozyme, C-reactive protein, complement, and lectins and the epidermal migration of inflammatory cells and their secretions may affect the proliferation of ectoparasites.

Many studies have noted that the level and nature of the immune response of fish is determined by a range of parameters including the duration of infection and environmental conditions such as temperature [7] and pollution [8].

The role of humoral factors such as complement and antibody in the immune response of fish to parasites has not been completely clarified. Although there have been several investigations on the association between complement and viral and bacterial pathogens in fish, there has been a limited number of studies on the antiparasitic activity of teleost complement. In contrast, there have been several studies that have also highlighted the involvement of antibodies in the host-parasite relationship in fish.

3 Antarctic fish parasitism

The study of Antarctic fish parasitism by nematodes is particularly interesting because a natural immune response (not elicited by immunization) has been found in fish living at an extremely low constant temperature (-1.8 °C). Many authors have investigated the effects of environmental temperature on fish antibody production. Le Morvan et al. [9] suggested that the decreased antibody production observed in carp maintained at low water temperature was a direct effect of temperature on cellular immune function modulated by membrane fluidity. Collazos et al. [10] found the proliferative response of lymphocytes of *Tinca tinca* to be lower at 12 °C than at 22 °C. Bly and Clem [11] attributed the low temperature-mediated immunosuppression of *Ictalurus punctatus* to the rigid nature of the T cell membranes; furthermore, they supposed that losses associated with the ‘winter kill’ syndrome may be attributable to a low temperature-induced immuno-deficient state [12].

Among the environmental antigenic stimuli of the fish immune system, parasite molecules play a major role. Parasites can be distinguished into ectoparasite and endoparasites. There have been very few research projects focusing on ectoparasites

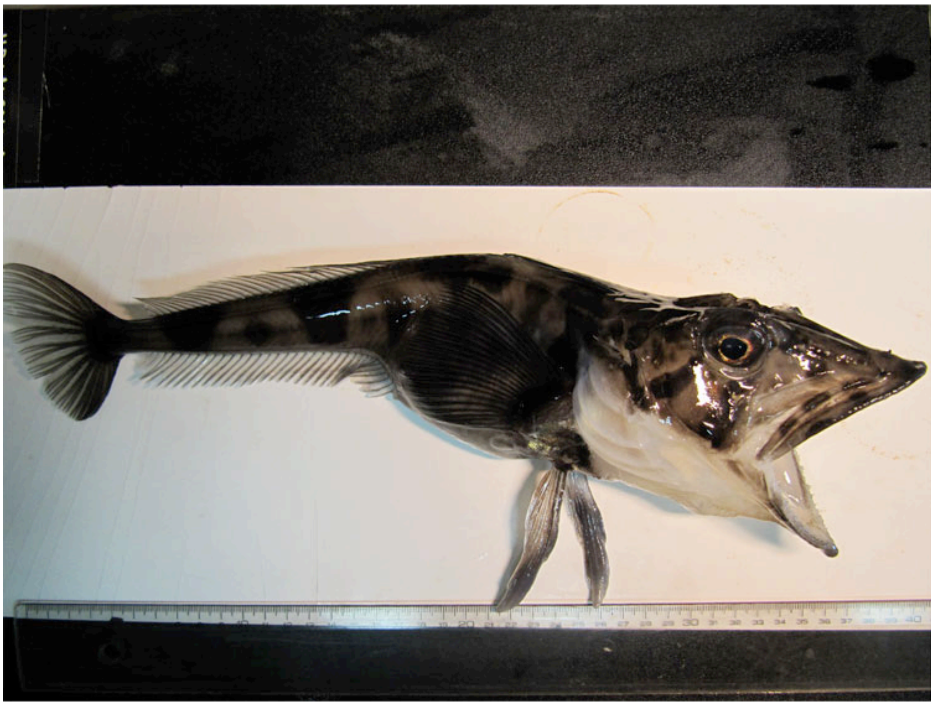

Figure 1: A *Chionodraco hamatus* specimen.

of Antarctic fish. However the prevalence and the intensity of leeches (Hirudinidae: Piscicolidae) infecting fish have been well documented. Among the ectoparasites, the copepod *Eubrachiella antarctica* and the leech *Notobdella nototheniae* are the most conspicuous and probably most ubiquitous species [13]. Endoparasites are much more numerous in terms of species and individuals than are ectoparasites. Like ectoparasites, most endoparasites show a low degree of host specificity. Fish feeding predominantly on krill have a reduced diversity of endoparasites; benthos-feeding and piscivorous species show a wide range of parasites [14].

Antarctic fish fauna is represented by sharks, skates (Chondrichthyes), and bony fishes (Teleostei). Sharks are uncommon in Antarctic waters and only 3 species from 2 families, Aqualidae and Lamnidae, were recorded off South Georgia and in the sub-Antarctic. All sharks in the Southern Ocean have been caught around islands and no data exist about the presence of pelagic species or the extension of the distribution of sharks along the Antarctic coast. All Antarctic skates belong to the family Rajidae and they represent about 10 species from 2 genera: Raja (Linnaeus, 1758), and Bathyraja (Ishiyama, 1958). Also, about 274 species of bony fish from 49 families occur in the Antarctic and sub-Antarctic waters and over 120 species belong to the suborder Notothenioidei [15].

Investigations of parasites of Antarctic fish

	Positive %	Negative %
C. aceratus	100	
C. hamatus	83	17
N. gibberifrons	50	50
N. coriiceps	100	
P. georgianus		100
T. bernacchii	20	80

Table 1: Summary of positive and negative results obtained by double immunodiffusion assay in six Antarctic fish species

have been mostly systemic studies. Inventories of parasites now exist for a number of notothenioids, primarily nototheniids and channichthyids, despite the taxonomy of some groups of parasites is still indeterminate. Infestation rates and infestation intensities of most fish species, as well as the life cycles of most parasites and relationships with their hosts, still await elucidation. Teleosts play an important role in the completion of life cycles of many parasites species. They serve as either definitive or intermediate hosts. Chondrichthyes are definitive hosts only. Trematodes, monogeneans, nematodes, and cestodes, which are known to represent a major component of the parasite fauna in fish living in temperate water [16, 17], are also found infesting Antarctic fish.

Seventy-three helminth species occur as the adult stage in fishes: Digea (45), Cestoda (14), Nematoda (6), Acantocephala (8). Also, 11 larval stages of Cestoda and Nematoda are known. One digenean species, Otodistomum cestoides, matures in skates. Antarctic Chondrichthyes are not infested with nematodes and acanthocephalans. Larval nematodes represent Anisakidae, parasites of fish, birds and mammals. All acanthocephalans, almost all digeneans, the majority of cestodes, and some nematodes occur mainly or exclusively in benthic fishes. Among parasites using fishes as definitive hosts, all Cestoda, most Digenea and Nematoda, and almost all Acanthocephala have a range of hosts restricted to one order or even to 1-2 host species [18].

In Antarctic fish, the parasitic fauna appears to be impoverished in terms of species. Infestation rates and infestation intensities varied considerably between species, being 100% in some species such as *Pseudochaenichthys georgianus* and Notothenia rossii at South Georgia.

Acanthocephalans show pronounced site fidelity in that each species settles in a definitive section of the digestive tract of the host. Digean trematodes are found predominantly in the esophagus and in the stomach.

The first report on the occurrence of adult cestodes in the Antarctic fishes was given by Linstow (1907) who described a new species, *Phyllobothrium dentatum*, from unidentified shark which was found on the coast of South Georgia. Cestodes found are primarily larvae and hence unidentifiable to genus or species level. Cestode larvae probably use planktonic copepods as first hosts whereas fish are the second intermediate host. Adults have been found in Bathyraja species and only occasionally in nototheniids, but seals are considered as prime candidates for potential definitive host [19]. Infestation rates in some nototheniids and channichthyids in the Scotia Arc region and the Weddell Sea ranged from 20% to 100%.

Nematode parasites of Antarctic fish are primarily third-stage larvae of the anisakid genera Contracaecum [20] and Pseudoterranova [21]. Fish are the paratenic hosts of the larvae, which mature after being ingested by the final host, seals or seabirds. A positive relationship between sea eutrophication and fish parasitism by nematodes has also been suggested, the parasite fauna being the result of the feeding habitat and the trophic level of the host [22]. Moreover, the infection levels of anisakid nematodes from various areas of the Boreal and Austral regions have been used as biological indicators of fish stocks and food-web integrity in areas at high versus low levels of habitat disturbance (pollution, overfishing, by-catch) [23].

Nematode larvae may live in fish for a couple of years. The main target sites of *Contracaecum larvae* in the fish host include liver, mesenteries and stomach wall [24]. The parasitic load of the liver of some species of Antarctic fish is often very high. Infestation intensities are up to 60-150 nematode larvae in piscivorous species, such as *Chaenocephalus aceratus* and *Chionodraco rastrospinosus* in the Antarctic Peninsula region and *Chionodraco myersi* and *Chionodraco antarcticus* in the Weddell Sea [25]. Their parasitic load is positively correlated with the size of the fish. This is likely to be due to the accumulation of larvae with age of the host, and to size-specific change of its diet. Acanthocephalans and larvae of the nematode Pseudoterranova are primarily found in fish feeding on benthic crustaceans. Larvae of Contracaecum species use both planktonic and benthic transmission pathways. This close relationship of feeding to the extent of parasitic infestation is also evident in some channichthyids, which shift their diet considerably when changing from pelagic to demersal life. As a result, infection rates with *Contracaecum larvae* increase sharply from 0-20% to 90-100% [25]. Piscivorous fish, such as channichthyids, play an important role as accumulators of acanthocephalan larvae [26] and *Contracaecum larvae* [20].

The genus Pseudoterranova consists of three species: *P. kogiae*, *P. ceticola* and*P. decipiens*. The life cycle of the latter, the only cosmopolitan species, has been described by McClelland [27]: the third larval stage occurs in benthic fish and the main sites of infestation are the liver, mesenteries and stomach walls.*P. decipiens* larvae have been recorded also in the Antarctic notothenioid species by many authors and the Weddell seal (Leptonychotes weddellii) is known to be an important final host [21]. *P. decipiens* was first found in *T. bernacchii* by Johnston [28]. Other fish species infested by*P. decipiens* are *Notothenia coriiceps*, *Champsocephalus gunnari*, *Chionodraco kathleenae*, *Notothenia gibberifrons*, *Pseudochaenichthys georgianus* and *Notothenia neglecta* living in the Antarctic Ocean. Also, in the North Atlantic Ocean, commercially important species, including Atlantic cod (*Gadus morhua*), are infested by*P. decipiens*. Based on the results of multilocus electrophoresis,*P. decipiens* larvae recovered from North Atlantic fish have been separated into three sibling, morphologically indistinguishable species, A, B and C [29].

4 Genetic diversity of anisakid nematodes

In order to test for a correlation existing between the genetic diversity of anisakid nematodes and habitat disturbance, the genetic variability, estimated by nuclear markers (19 allozyme loci), was evaluated among several anisakid populations from fish and marine mammals in various areas of the Boreal and Austral regions. Antarctic and sub-Antarctic populations showed significantly higher levels of genetic diversity than those from the Arctic and sub-Arctic species. Analysis between the degree of genetic variability and the levels of parasitic infections within their hosts revealed higher intensities in anisakid infections in Antarctic and sub-Antarctic hosts, presumably resulting from a lower degree of habitat disturbance in less stressed areas. The absence of disturbance presumably allowed anisakid species to reach a larger population size, with a reduced probability of genetic drift in their gene pools. This suggests that anisakid nematodes, and their levels of genetic diversity may be suitable indicators of the integrity of marine food webs and of the general biodiversity of a marine ecosystem [30].

5 Antibody response to *C. osculatum* antigens

To investigate the presence of antibodies to *Contracaecum osculatum* antigens in the plasma of several Antarctic fish, first the immunoglobulin fraction was purified from a plasma pool of *Chionodraco hamatus* specimens (Figure 1): 25.9% of the plasma proteins were bound by the thiophilic adsorbent [31]. The bound fraction was resolved into three peaks by size-exclusion FPLC; the relative molecular mass of the most representative peak (81%) was estimated to be 780 kDa. The serum concentration of this fraction was calculated to be 12.6 mg/ml. It was analyzed by SDS-PAGE under reducing conditions. Two bands were obtained: the higher (75 kDa) was suggested to be the IgH chain, the lower (27 kDa) the IgL chain. To investigate the specificity of *C. hamatus* antibody, we tested for the presence of anti-nematode antibodies in the purified Ig fraction. *C. osculatum* somatic proteins (Co SP) were subjected to gel electrophoresis. At least 14 separated bands of relative molecular mass ranging between 21 and 200 kDa were identified. The most representative bands were those with relative molecular mass of about 200, 91, 47, and 36 kDa. To identify the antigenic components of Co SP, SDS-PAGE separated bands were electroblotted onto nitrocellulose sheet and then incubated in sequence with Ig purified from plasma of *C. hamatus* or from *Boops salpa*, as uninfested fish, and 125I rabbit IgG anti-*C. hamatus* plasma. Only some protein components were identified as anti-

Figure 2: Pseudoterranova decipiens larvae.

gens by autoradiography of the sheet incubated with *C. hamatus* Ig. In contrast, no antigen was identified with B. boops Ig, although the labelled reagent recognized B. boops IgH and L chain in immunoblotting. The 91-kDa-band component was found to be the major antigen bound by *C. hamatus* antibodies; in addition two other antigens were identified (47 and 63 kDa, respectively).

A further confirmation of the specificity of the antigen-antibody binding was obtained by affinity chromatography. Co SP was immobilised on a HiTrap NSH-activated column, and the *C. hamatus* plasma pool was loaded onto the column. The bound fraction was demonstrated to correspond to Ig as only two bands with the same electrophoretic mobility as the H and L chains were revealed by SDS-PAGE under reducing conditions.

Plasma of individual fishes of six Antarctic species was tested for the presence of anti-*C. osculatum* antibodies in double immunodiffusion assay. Immunoprecipitates were observed with several plasma samples from *Trematomus bernacchii*, *Chaenocephalus aceratus*, *Notothenia coriiceps*, *Notothenia gibberifrons* and *C. hamatus* species.

None of the tested *Pseudochaenichthys georgianus* plasma samples gave immunoprecipitation. Table 1 summarizes all the results obtained by double immunodiffusion assay.

To assess the potential of using antibodies as an indicator of infestation in Antarctic fish, the presence of antibodies specific for nematodes usually infesting fish was investigated. The 91-kDa Co SP major antigen showed a high specific binding activity to *C. hamatus* antibodies, as a weakly stained band gave a very high signal in autoradiography. Testing Co SP with antibodies purified from plasma of other fish species, may confirm the antigenicity of the 91-kDa component. Neither the physiological role

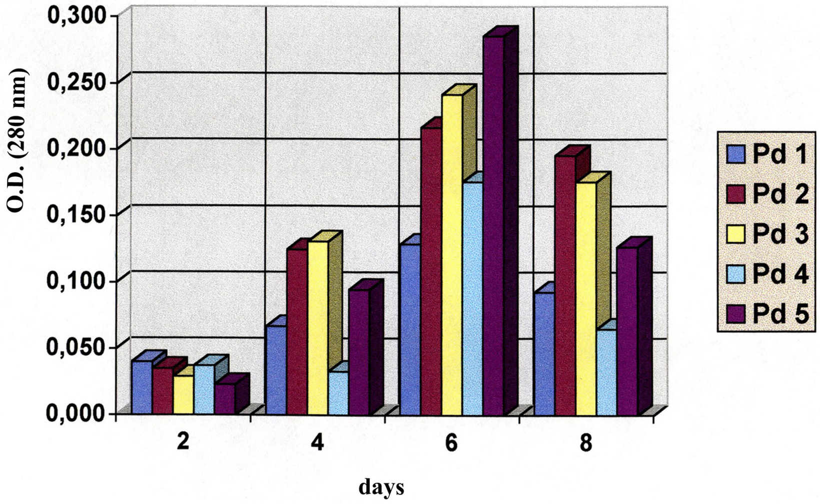

Figure 3: Proteins released by five*P. decipiens* larvae maintained in individual vessels at 18 °C for eight days.

of this molecule in the parasite nor the significance of the antibody response in protection is presently known.

6 Antibody response to*P. decipiens* antigens

We have examined the parasitism of *T. bernacchii* by*P. decipiens* using three approaches:

1. looking for *T. bernacchii* antibodies specific for different*P. decipiens* protein preparations (excreted/secreted, surface associated and cuticle soluble proteins);
2. comparing the antigen-binding activity of plasma and bile antibodies;
3. identifying the most relevant antigens [32].

The nematodes were collected from a *T. bernacchii* liver (Figure 2). The nematode proteins were recovered as follows: live nematode larvae were maintained in individual vessels at 18 °C for eight days and a thermal shock at 37° C was given twice a week. The larvae released similar amounts of protein in the medium (Figure 3). Subsequently, all the recovered samples were pooled, concentrated by ultrafiltration and dialyzed. The total amount of protein was referred to as excreted/secreted protein (ESP). To obtain the surface associated protein (SAP), the dead larvae were incubated for 16 h, at 37 °C in PBS pH 7.2 containing protease inhibitors. After cen-

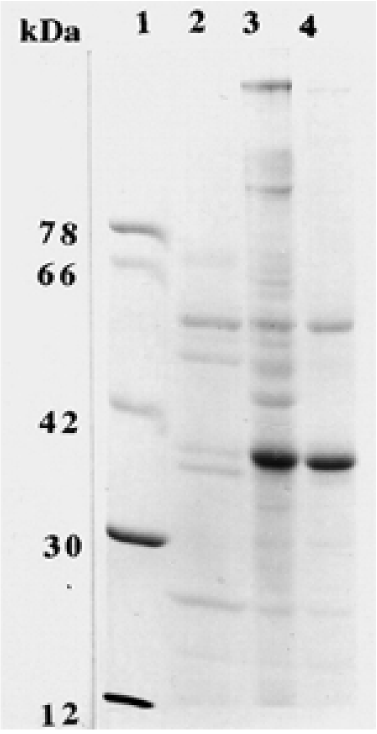

Figure 4: Coomassie staining of 8% SDS-PAGE gel run under reducing conditions. Lane 1: molecular weight markers. Lane 2: ESP. Lane 3: CSP. Lane 4: SAP.

trifugation, SAP was recovered. To collect the soluble fraction of the cuticle (CSP), the precipitate of the previous centrifugation was boiled in PBS, pH 7.2 containing 2% SDS and 10% β mercaptoethanol; the mixture was then incubated for 16 h at 37 °C and centrifuged, the dialyzed supernatant contained the CSP fraction.

Different methods were used to characterize the protein components of the three preparations ESP, SAP, and CSP. SDS-PAGE, run under reducing conditions, showed that the number of protein components differed among the three preparations (Figure 4): 26 in CSP ranging between 200 and 21 kDa; 12 in SAP and only 6 in ESP. Four bands of CSP (58, 54, 40 and 25 kDa) were found also in ESP and SAP; 40 kDa was the main component of CSP and SAP. To assess the biological activity of the 40-kDa protein, the band was blotted onto an Immobilon sheet and its N-terminal amino acid sequence was determined. The database search for identity of the sequence of the first 20 amino acid residues gave a high percent identity with the oxygen transporter molecule of*P. decipiens*, extracellular globin precursor (Pd glb ps); minor differences could be attributed to subspecies of*P. decipiens*. The amino acid sequences are as follows:

- PdSP 40 kDa SKXKTREL-

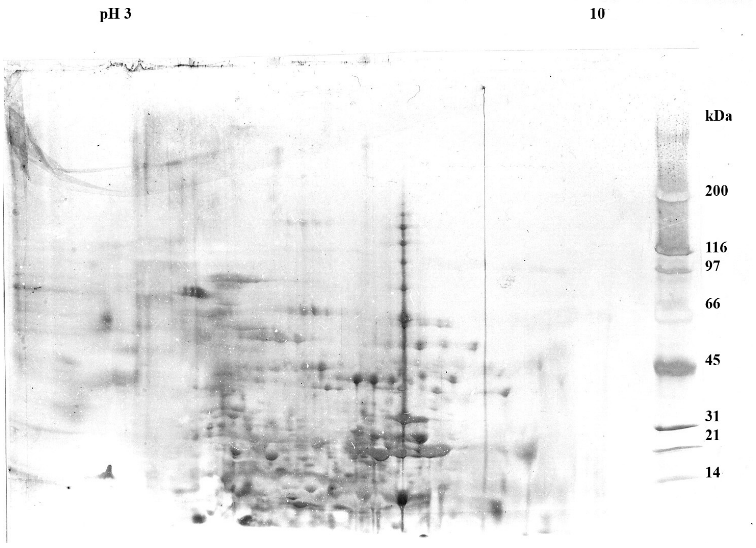

Figure 5: Two-dimensional electrophoresis of the CSP preparation. The first dimension was run using an immobilized linear pH 3-10 gradient. The second dimension was run on a SDS-PAGE gel (gradient 8-18) under reducing conditions. Molecular weight markers are reported on the right.

GMKELEKAKVGT,

- Pd glb ps MHSSIVLAIVLFVA-IASASKTRELCMKSLEHAKVGT.

The extracellular globin from*P. decipiens* has been cloned [33] and is thought to be antigenic in grey seals.

We used two-dimensional electrophoresis to analyze the highly heterogeneous CSP preparation (Figure 5). The first dimension was carried out on Immobiline DryStrip gel in the pH range 3-10, the second on precast ExcelGel SDS, gradient 8-18.

The specific antibody binding activity of CSP and SAP was tested by ELISA using the same protein amount of antigen coated in the wells and different dilutions of purified *T. bernacchii* plasma Igs (Figure 6). SAP had a specific antibody binding 1.3 times higher than CSP. As control of unspecific binding, Igs purified from plasma of a specimen of *B. salpa* were used at the same dilutions. The unspecific binding of control Igs was about 7.7 times lower than that of *T. bernacchii* Igs. Because of the scarce amount of ESP, it was not included in this experiment.

We examined nematode specific antibodies in the bile and compared their ability to recognize ASP to that of plasma Igs. The same dilution of *T. bernacchii* plasma or bile Igs was tested by dot blot immunoassay against ASP, which was demonstrated, by ELISA, to be highly reactive; the unspecific binding was tested against bovine serum albumin. The bile Ig-generated signal was clearly more pronounced than the plasma signal, suggesting the presence of an immune response against parasite at both systemic and secretory level. This implies that the spe-

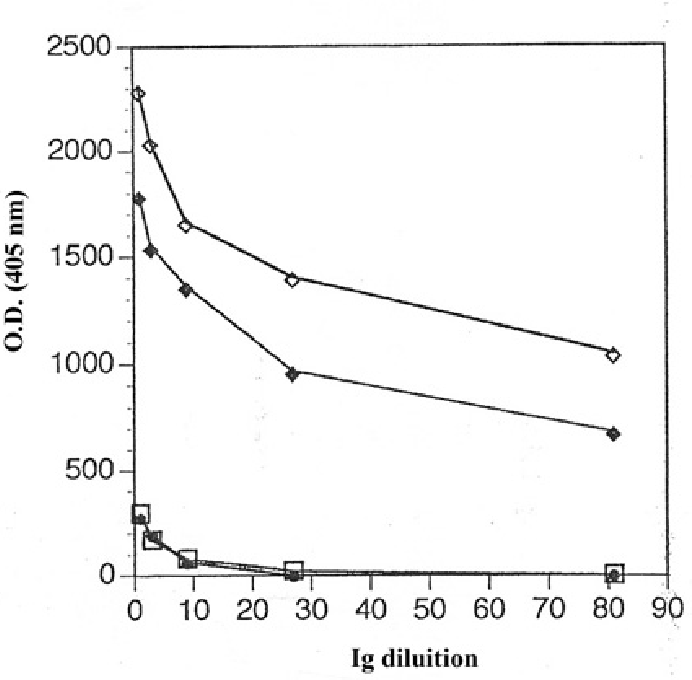

Figure 6: ELISA of specific antibody binding activity of CSP (♦−♦) and SAP (◊−◊), using the same protein amount of antigen coated in the wells and different dilutions of purified *T. bernacchii* plasma Igs. As control of unspecific binding, Igs purified from plasma of a *B. salpa* specimen were used at the same dilutions with CSP (•−•) and SAP (□−□).

cific antibody concentration is higher in the bile than in plasma, confirming the dot-blot results, and with a larger spectrum of specificities. It is noteworthy that specific antibodies are present in the bile of parasitized fish. The high titers of bile antibodies detected, and the fact that they recognized an antigenic repertoire larger than that recognized by plasma antibodies could be attributed to the greater topic immune response at intestinal level. In this context, the occurrence of hepato-biliary transport of immunoglobulin in *T. bernacchii* was demonstrated [34]. In fact, by immunohistochemistry IgH- and IgL-reactivity was detected in perisinusoidal cells, bile canaliculi and pre-ductules. In the anterior intestine, the intraluminal mucus retained a significant Ig-immunoreactivity, while the mucosa housed a limited density of Ig producing cells. These findings strongly indicate that Ig could be transported across the hepatocytes to be secreted into the bile and protect the intestinal epithelium. In addition, the presence in the liver of IgM and µ chain-specific mRNA was demonstrated.

We used immunoblotting to identify the pattern of antigenic molecules of the nematode preparations. Seven SAP and only 12/26 CSP protein components were identified as antigens. SAP was highly immunogenic, which is in line with antigen shedding as a parasite immune escape mechanism. CSP was a very complex mixture, less antigenic perhaps because of the strong conditions used, and certainly including proteins not exposed on the body surface.

The major CSP antigen had a relative molecular mass of 90 kDa; the corresponding SAP antigen was slightly heavier. The 81, 65, 58, 54, and 49 kDa SAP bands corresponded to additional antigens as well as the 40-kDa band, which gave a weak autoradiographical signal despite occurring at high concentration.

The immunoblotting analysis was repeated for SAP using *T. bernacchii* bile Igs: the number of antigenic components was higher, the relative intensity was different, the 49 kDa giving the strongest signal. The major*P. decipiens* antigen (90 kDa) seems to be the antigen recognized by *C. hamatus* antibodies in *C. osculatum* protein preparations [31] also the 47-kDa antigen could be shared by the two anisakid parasites.

We presented evidence that a humoral immune response occurs in parasitized Antarctic fish. In fact, the concentration of Ig concentration was higher than that generally detected in fish plasma. This finding is relevant because *T. bernacchii* lives at a temperature of -1.8 °C and the immune response induced by the helminth parasite is absent at low temperatures.

In conclusion, the role of these nematode antigens is obscure. Studies conducted so far concern antigen recognition by antibodies purified from a pool of samples from different *T. bernacchii* specimens. An analysis of antibody titers from individual fish of a documented level of parasitism might indicate that the host's immune response to parasite nematode exerts a protective effect.

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Benthic Communities as Indicators of Habitat Saprobity

D. Tagliapietra[1], M. Sigovini[2,3], P. Magni[1,3]
1, Institute of Marine Sciences, CNR, Venezia, Italy
2, Department of Environmental Sciences, University “Ca’ Foscari”, Venezia, Italy
3, Institute for Coastal Marine Environment, CNR, Oristano, Italy

davide.tagliapietra@ve.ismar.cnr.it

Abstract

We delineate the concept of saprobity as a state of an ecosystem resulting from organic matter (OM) metabolism processes. An overview is given on conceptual models describing the succession of benthic communities along a gradient of organic enrichment. Similarities between different models, e.g. between the Pearson-Rosenberg [1] and the Guelorget-Perthuisot [2] models, are highlighted. Based on a critical analysis of existing models and indices, we propose a general framework where the processes of OM metabolism are a major structuring factor the benthic communities in microtidal lagoons, and saprobity is used as a state descriptor of these process. We assume that saprobity cannot be quantified by considering only the amount of OM. In fact, saprobity is the result of both input of OM and other processes, such as mineralization, sinking, dilution and export of OM. The same organic input can, therefore, generate different degrees of saprobity in different systems/areas. Saprobity acts on benthic communities together with other components of the transitional gradient, such as salinity but, in coastal lagoons, the effect of the latter is primarily in oligohaline and hyperhaline areas. Due to difficulties to quantify saprobity itself, we are developing a numerical index of saprobity which takes into account the memberships of benthic species to different saprobic groups. Main characteristics of this index are described in this paper and a zonal approach is also foreseen.

1 The concept of Saprobity

Saprobity (from the Greek: *σαπρός*, Sapros = rotten, putrid, putrefaction, decay and *βίοσ*, Bios = life) is the expression of the metabolism of organic matter subjected to microbial degradation and of its ecosystemic balance. It was first developed for rivers in relation to the occurrence of organic waste discharges [3, 4, 5].

A definition of saprobity was accepted at the Symposium CMEA on “Questions of Saprobity” (Prague, 26-28 April 1966): ”Within the bioactivity of a body of water, saprobity is the sum total of all those metabolic processes, which are the antithesis of primary production. It is therefore the sum total of all those processes which are accompanied by a loss of potential energy. In combination with the biogenic and physical oxygen component, it determines the saprobity level of a body of water. This level can be ascertained both by metabolic-dynamic measurements and by analysis of the living communities" [6, 7]. This definition points out that saprobity is not the result of the mere presence of organic matter but involves ecosystemic processes. The saprobity is therefore the state an ecosystem assumes as a consequence of the decomposition of organic matter. In general terms, saprobity is given by the balance between an input of catabolites generated by the metabolism of the organic matter, and their removal from the medium. The removal of catabolites from the medium depends mainly on their utilization by microorganisms (remineralization or self-depuration), on the dilution into the water column and on the export from the system. The sum of all removal processes can be regarded as the "clearance" of the ecosystem. As result of a metabolic processes, saprobity is strictly dependent on oxygen availability and temperature.

Saprobic processes in sedimentary environments, such as coastal transitional ecosystems (CTE), occur mostly close to the sediment surface. Benthic microbial metabolism generates oxygen demand (Sediment Oxygen Demand) subtracting the gas from the overlying water. Often just few centimeters of sediment and water are affected by oxygen deficiency but this is sufficient to shape the benthic biota [8]. The texture of sediments and bioturbation affect the oxygen penetration in the substrate (as can be evidenced by the depth of the Redox discontinuity layer RPD) and the flux of reduced and toxic products. Hydrology and physical mechanisms plays a key role in the clearance process. For instance, tides act both on the dilution of catabolites bringing new seawater and to the physical export toward the sea. The marine water supply usually conveys dissolved oxygen to the system. Re-mineralization processes can be enhanced by water mixing through oxygenation and increased contact of catabolites with various kind of surfaces such as the bottom, suspended matter and other physical and biological supports (e.g. shells, cobbles, plant stems). The different components of the clearance (i.e. self-depuration, sinking, dilution, export) have different influence in different zones of the same lagoon depending on the hydrodynamics. In outer areas, close to the inlets, (lotic areas) the export play a major role in the clearance process, whereas in the innermost areas (lentic areas) self-depuration and burrowing can have a dominant role. Similarly, different types of lagoons naturally express different saprobity a shallow lagoon with reduced hydrodynamics, little oxygenated waters and fine sediments is likely to have a higher saprobity than a sandy leaky lagoon. The same organic input can, therefore, generate different degree of saprobity in different habitats.

2 Effects of saprobity on benthic communities

The main effect of saprobity on macroscopic biocoenosis is that the number of species that can cope with it decreases progressively as the saprobity increases, leading to a reduction of species richness and diversity. Species change along a saprobity gradient gives rise to a succession/seriation according to species-specific tolerance levels to saprobity. Along this gradient, the density and biomass of an individual species, as well as the total density and biomass of the community, show characteristic trends. As an example, organic enrichment associated to low saprobity of the system can promote the vitality of the biocoenosis. Generally, along a gradient of organic enrichment, trophic groups move from suspension/filter feeding towards sediment feeders. The degree of saprobity of an aquatic system is often measured on the basis of the capacity of its biological community to cope with phenomena which are consequential to the degradation of organic matter present in the system, mostly with oxygen depletion and reducing conditions of the medium with release of toxic substances [9]. Several conceptual models have qualitatively described how species or group of organisms reduce their abundance in a given order as environmental stress increases and how the number of taxonomic groups is reduced as stress increases. In most cases these methods are based on macroinvertebrate since this biological component includes a wide range of taxa, providing sufficient presence of sensitive species, and organisms have limited spatial and temporal mobility and integrate environmental conditions over longs periods of time.

3 Models of response to organic enrichment

Conceptual models are an important framework for developing benthic indices, which are used to evaluate various types of environmental stress. This path was first recognized in freshwater and only in a second time in marine and transitional ecosystems. Conceptual models for rivers were developed at the beginning of the 20th century [3, 4, 5] and were later developed into biotic indices [10, 11, 12]. Fundamental contributions for marine and coastal transitional ecosystems were brought by [13, 14] and [15]. These contributions were addressed toward the use of marine benthic invertebrates as indicators of organic pollution. They drove the attention on the effect of organic pollution both on single species and communities. Four zones were generally identified: a) ”dead” zone, b) ”polluted” zone, c) ”subnormal” zone, and d) ”pure water” ”normal” zone [14, 15]. Few years later, a conceptual models was developed in the Baltic area, describing the seriation of benthic invertebrates along a gradient of organic enrichment. In 1975, Leppäkoski [16] published his research on the relationships between magnitude of disturbance and spatial or temporal changes of benthic assemblages.

In 1978, Pearson and Rosenberg published the well-known “Pearson and Rosenberg model” (P-R model), refining the work of Leppäkoski [17, 16]. This conceptual model illustrates the qualitative relationships between magnitude of disturbance and spatial or temporal changes of the main macro-descriptors of benthic assemblages such as (total) Species, Abundance, Biomass by means of the well-known SAB curves. The authors reviewed the response to organic enrichment for about a hundred of soft-sediment benthic macro-invertebrates. The model foresees a seriation of species from “opportunistic species” [18], characterized by a “r” reproductive strategy [19] that dominate in organic over-enriched conditions, toward sensitive species characterized by a “K” reproductive strategy. This change is accompanied by a progressive increase in the number of species. Opportunistic species have short life cycle, little body size, fast growth, often polivoltine reproduction, the dominant feeding group is the detritivores, particularly polychaets. Similarly to Leppäkoski [17, 16] who categorized the species into initial colonizers, first and second order progressive species, Pearson and Rosenberg [1] divided the species into groups: the "First-order opportunistic species" are the first colonizer of defaunated substrates or, the last group of species that could be found as the organic load increases. This group is followed by the "Second-order opportunistic species" and by the "Enrichment opportunists" which are "initial colonizers only in organically rich areas". According to this classification, [1] identified four "zones" in the changes in fauna and sediment structure along a gradient of enrichment, i.e. Normal, Transitory, Polluted and Grossly Polluted.

The "normal zone" is characterized by marine organisms and a RPD of more than 2 cm deep, the "polluted zone" is characterized by genera such as Capitella and Scolelepis with a RPD between 1 cm and the surface, and a "transitory zone" is a transition between the two. The "grossly polluted zone" zone have no macrofauna. In the conclusion of the paper, the authors defined five successional stages occurring both in space and in time two endpoints constituted by "Afaunal" and "Normal" (i.e. marine) situation and three intermediate stages, i.e. the "peak of opportunists" with few species in great number, the "Ecotone point" with low abundance and high diversity, and the "Transition zone". [20] and [21] synthesized the Pearson and Rosenberg zones into four stages, respectively Stage 3 (Normal zone/ Normal stage), Stage 2 (Transitory zone/ Transitory stage), Stage 1 (Polluted zone/peak of opportunists), Stage 0 (Grossly polluted zone/Afaunal stage), the "Ecotone point" dividing Stage 1 from Stage 2. The P-R model relates the succession to organic enrichment with a strong dependence to the redox conditions of the sediments [22], nevertheless the authors referred the seriation to "organic enrichment" and, in case of heavy input to "pollution", that is to the load of organic matter, not to saprobity. Although this model remains descriptive and qualitative [23], it opened the conceptual way to quantitative approaches. Recently some attempts have been made to relate more quantitatively the sedimentary organic matter to the main features of benthic communities [24, 25].

4 Models of response to "confinement"

In the late 1970's, the meeting between a biologist (O. Guelorget) and a geologist (J.-P. Perthuisot) drove the attention towards the biological organization of lagoons at the landscape scale. These authors draw the attention toward a common feature of all lagoons: the existence of a biological seriation with substitution of species along an environmental gradient [2, 26, 27]. In the years of "organic pollution", Guelorget and Perthuisot did not try to use fauna to evidence any kind of pollution. On the contrary, they evidenced the existence of a spatial biological seriation in all lagoon whatever their state of naturalness, including lagoons with different degree of anthropic impact. The authors stated that their zonal model is a common feature of coastal transitional ecosystems (CTE) and also of some seas but their scheme is particularly relevant for micro-mesotidal lagoons (see criticism of [28]).

The factor (the authors used the term "parameter") that mainly controls the distribution of organisms and the features of living populations was defined as "the time of renewal of the elements of marine origin at any given point" and called it "confinement" since it is strictly related with the degree of separation (seclusion) from the sea and the distance from the sea-inlets. They recognized the "confinement" as a common, emergent feature of all sedimentary environments "with relation to the sea" and called the whole class "paralic domain" (for a discussion of the terms "paralic" and "paralic domain" see [29]). The authors stated that the confinement reduces the availability for the biota of the "life-giving elements" such as mineral salts, trace elements etc. that come from the sea, hence biological communities organize themselves according to this rarefaction. The authors did not support with evidences the rarefaction of these elements, nevertheless defining the "confinement" as a "time of renewal". They drew attention towards those processes that are driven by the hydromorphology of the lagoonal systems.

Guelorget and Perthuisot [2, 26] affirmed that, by definition, "a confined environment is not synonymous with a reducing environment" since it would be the supply of "vital element" the limiting factor for community, not the saprobity, although they admitted that "in the paralic domain, confinement often leads to the reducing character of the milieu, notably in the region of the bed" ([2], [30], Annex III). The authors listed a series of characteristics of the confined environments that favor the instauration of a reducing medium such as low hydrodynamics, tendency to oxygen depletion, high biological production and the presence of saprobic microorganisms which contribute to the oxygen consumption and to the production of reduced compounds. They also recognized that reducing environments are more frequent in the innermost parts of the lagoon, the so-called "far paralic" indirectly asserting the presence of a reductive gradient due to organic matter decomposition (i.e. a saprobic gradient) related to the "confinement gradient". They also recognized that "organic pollution" can induce local variations in the species succession.

A confinement scale was proposed consisting in six discrete spatial zones. This scale was conceived as valid for the Mediterranean lagoons. The succession, according to the authors, is recognizable both in hypohaline and hyperhaline environments. The zones were identified mostly using four main groups of indicator species: "strictly thalassic species", "thalassic species", "mixed species" and "paralic species" called also "strictly paralic species". "Strictly thalassic species" belong to the biocoenosis of Sables Fins Bien Calibrés (SFBC WCFS Well Calibrated Fine Sands biocoenosis by [31], that are the more "stenohaline" species. "Thalassic species" are marine species that colonize the lagoonal areas which are in close contact with the sea. "Mixed species" are present both in the marine environment and in the lagoonal environment, they decrease in density as the confinement increases but have a high biomass inside the lagoon. "Strictly paralic species" are typical of lagoons, their density generally increase moving from the sea along the confinement gradient.

A short list of species divided among these zones was given. It is remarkable that Guelorget and Perthuisot did not mention a number of little polychaetes such as Spionids and Capitellids. As matter of fact, although giving a wide series of examples, Guelorget and Perthuisot did not give a systematic and quantitative description of the distribution of species along the ”paralic” seriation, leaving room for many criticisms. [28], criticized the possibility of application of the ”confinement” species to estuaries and tidal-flat habitats of the northern macrotidal Europe, characterized by a strong hydrodynamics and water renewal. This author also recognized the non direct dependence of the distribution of ”brackish fauna” on salinity. He pointed out that the majority of the species listed by Guelorget and Perthuisot can be considered as euryhaline, or particularly euryhaline, marine species that penetrated also estuaries. However, most importantly, he stressed the existence of ”lagoonal species”, i.e. species that lives in nanotidal or microtidal lagoons but are usually absent from macrotidal estuaries and tidal-flat habitats, that are coincident with the ”strictly paralic species” of Guelorget and Perthuisot. These ”lagoonal species” are adapted to lentic, little flushed CTE, the environments that are more subjected to develop saprobic conditions. The G-P model was not followed by indices that quantify the “confinement”, with the exception of a biotic index proposed by [32, 33] conceived to assess environmental quality in Mediterranean lagoons.

5 Convergence of the P-R and G-P models

Applying the P-R model to lagoons, we notice that there is an evident parallelism with the biological succession foreseen by the G-P model. In fact, both the P-R and G-P models foresee a shift in trophic habits from a dominance of filter feeders and suspension feeders towards an almost complete dominance of deposit feeders, first surface deposit feeders and then subsurface deposit feeders. This roughly corresponds, in terms of systematic groups, to a passage from a bivalve-dominated assemblage toward a polychaetes-dominated one. Also, it seems there is a strong parallelism between the “ecological groups” as defined by the P-R model (and the subsequent derived indices) and the G-P groups of species. The seriation starts from the sea with ”sensitive species” the “thalassic species” of G-P model, continue with ”mixed species” defined also ”coastal/estuarine species” by [28], ”tolerant species” or “strictly paralic” or “lagoonal” in Barnes. These evidences suggest that the P-R and G-P models describe substantially the same phenomenon: the succession of macrobenthic species in relation to a gradient of saprobity. However community would not be structured by a general input of ”vital elements of marine origin”, but by processes linked to the metabolism of organic mater as for the P-R model. Therefore both models describe the effects accumulation, metabolism and clearance of organic matter catabolites and the related subtraction of oxygen from the system. In this regard, the two models acknowledge the fundamental importance of hydrodynamics both in the genesis of the organic enrichment gradient and in clearance processes. Descriptions differ because of the habitat investigated, coastal marine, estuarine or lagoonal characterized by different hydrodynamics and sedimentology and because of the nature of the organic input, anthropogenic or more natural. The application of the G-P conceptual model, however, suffered from the same drawbacks as the P-R model, that is poor quantification and formalization.

Moreover, the reduced supply of "vital elements", invoked by the "confinement" parameter, lacked of the evidences that substantiated the "organic enrichment" approach.

The lack of reference to the majority of opportunistic species, especially sedentary polychaets such as Capitellids and Spionids, is another major drawbacks in comparing the biological seriation in the two models. Anyway, these taxa, as well as many other opportunistic species are currently found in lagoons with a distribution consistent with the G-P model [34, 35, 36, 37]. Moreover the distribution of a wide assortment of species is considered in agreement with the "confinement" in many studies conducted in Mediterranean lagoons [38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49].

6 From models to indices

Expanding upon the P-R model [1] and the work of previous authors [50, 14, 15], a group of French scientists [51, 52, 53, 54, 55] developed a model based on the categorization of benthic invertebrates in five "ecological groups", according to their dominance along a gradient of organic enrichment and oxygen depletion. Species were grouped as follows: Species very sensitive (Group I), Species indifferent to enrichment always present in low densities (Group II), Species tolerant of excess organic enrichment (Group III), Second order opportunistic species (Group IV), First order opportunistic species (Group V); a list of those species was given in [55]. The method consists in the individuation of seven "Biotic indices" (BI) each one characterized by the dominance of one ecological groups (e.g. BI 0 is dominated by Group I) which were used to define different stages of community degradation. This approach is in between a conceptual model and biotic index since the computation of each BI was not clearly defined.

In Europe, the Water Framework Directive (2000/60/CE) gave a new impulse to the development of "quality indices". [56] (2000, AZTI Marine Biotic Index, AMBI) improved the method described above, giving a formal structure with formula, permitting the derivation of a series of continuous values (called in the paper "Biotic Coefficient"), and assigning a "sensitivity coefficient" to each group (i.e. 0 GI, 1.5 GII, 2 GIII, 4.5 GIV, 6 GV). The Biotic Coefficient is subjected to a subdivision into 7 classes of quality (called in the paper Biotic Index, BI), in agreement with the 7 "Biotic indices" given by [55]. [56] used the same groups of [55] updating regularly the list of species and their attribution to the ecological groups.

[57] (2002, Bentix) produced a version of the index by dividing the species into three ecological groups (Group I. Sensitive species, Group II. Species tolerant to disturbance or stress and second-order opportunistic species, Group III. first order opportunistic species, pioneers, colonizers, or species tolerant to hypoxia). The index is based mostly on the discrimination among sensitive and no sensitive species since in the formula it sums together the frequency of group II and III. The "sensitivity coefficient" used in the formula for group I is 2 and for GII and III is 1. Subsequently, [58], produced a more formal and quantitative version of their own model as well (Indice d'Evaluation de l'Endofaune Côtière, I2EC). More recently, among others, [59] (2008, BITS) proposed the application of an analogous method for coastal lagoons based on the principle of taxo-

nomic sufficiency, listing sensitivity groups using the taxonomic level of family instead of species.

[60] argued that the attribution of different taxa to "ecological groups" were made in a subjective way on the basis of the experience of the authors and, therefore, vary between scientists and geographical areas. The authors suggested a more objective method to obtain "tolerance values" for different species, based on the axiom that "tolerant species", associated to disturbed situations, mainly occur in areas with low diversity and, in contrast, "sensitive species" occur in areas with high diversity. In this way "tolerance values" are structurally correlated with diversity (ES50).

7 Towards a benthic index of lagoon saprobity

Based on the above analysis of existing models and indices, we propose a general conceptual framework where the processes of organic matter (OM) metabolism are a major structuring factor the benthic communities in microtidal lagoons, and saprobity is used as a state descriptor of these process. In fact, we believe that saprobity has a fundamental ecological role which can be used in the definition of lagoon typologies. We are aware that saprobity cannot be quantified by considering only the amount of OM *per se*, but should take into account also the processes generally described as "clearance". In fact, saprobity is the result of both input of OM and other processes, such as mineralization, sinking, dilution and export of OM. The same organic input can, therefore, generate different degrees of saprobity in different systems and in different part of the same system. Saprobity acts on benthic communities together with other components of the transitional gradient, such as salinity but, in coastal lagoons, the effect of the latter is primarily in oligohaline and hyperhaline areas. Due to difficulties to quantify saprobity itself, we are developing a numerical index of saprobity which takes into account the memberships of benthic species to different saprobic groups. It is not intended to be a quality index, nor an index of organic pollution. Instead, it should be a structural descriptor of CTE taking into account both local benthic communities and the physiographic characteristics of specific habitats within CTE. In particular, we assess the saprobity of a system based on the benthic species as indicators of a certain level a saprobity. We will thus assign to each species a numerical value which takes into account their sensitiveness to saprobity, and derive main ecological groups. This value combined to numerical abundance of each group (biomass may also be considered subsequently) will be the basis for the index. Only the dominant species, accounting e.g. for 90% of total abundance and/or biomass at an individual station, will be used. This because these species are representative of the whole community, and also because their ecology and, therefore, their saprobic values is more likely to be known, thus less subjected to subjectivity in the definition of their ecological position. A zonal approach will be used by dividing the index values in ranges that will identify different zones. It is important to highlight that these ranges and zone will be not a quality identify. This index will serve to describe both spatial variability and temporal changes. We will attempt to model the saprobity of lagoons in order to identify reference values for each lagoon typology. The index should thus be used to identify saprobic zones inside CTE landscapes.

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Benthic Diversity in Oligotrophic Waters: the Case Study of Is Arenas (Western Sardinia, Italy)

G.A. Fenzi[1], P. Magni[1,2]
1, Institute for Coastal Marine Environment, CNR, Oristano, Italy
2, Institute of Marine Sciences, CNR, Venezia, Italy
paolo.magni@cnr.it

Abstract

We report the distribution of benthic assemblages in Is Arenas Bay, a coastal marine area of western Sardinia characterized by oligotrophic waters and the presence of different habitats, including the seagrass *Posidonia oceanica* and unvegetated sediments. Gammarids tended to dominate in proximity of the seagrass meadow, while nematods, polychaetes (Lumbrineridae) and bivalves (Tellinidae) increased in well-sorted fine-sand sediments. Among these benthic communities, a wide range of taxonomic groups included as the polychaetes Phyllodocidae, Nuculanidae and Gliceridae, the crustaceans Ostracoda, Caprellidae, Processidae, Isopoda and Portunidae, the mollusks Nuculanidae, Glycymeridae, Naticidae, Eulimidae, and the echinoderms Astropectinidae, Ophiuroidea, Holoturoidea. Overall, the benthic communities of Is Arenas were dominated by taxa sensitive to anthropogenic impact, indicating a high biodiverse and healthy coastal marine ecosystem.

1 Introduction

Coastal marine ecosystems play a key role on the whole marine environment, providing a variety of ecological functions [1]. As an example, physical [2], chemical [3, 4] and biological processes take part in the gas regulation and nutrient cycling at different spatial and temporal scales [5]. These environments are also essential for many organisms, providing essential habitats as a nursery ground for juveniles of many marine, estuarine and freshwater fishes [6]. Moreover, coastal areas have direct or indirect junctions with different economical services and human values. These natural resources should be considered as renewable and preserved through the conservation of biodiversity and the mitigation of climate change effects [1] to maintain constant their productivity in the future.

The spatial and temporal distribution of macrozoobenthic assemblages and the main features of sediments provide valuable information of the ecological status of the system [7, 8]. Indeed, benthic dynamics are tightly related with processes occurring both in the water column and sediments. Distributional changes, altered abundance and reduced diversity in the benthos can be used as biological tools of past and present disturbances [9, 10]. Because of the sedentary lifestyle and the longevity (several months to a few years) of benthic organisms, they can be a direct evidence of environmental changes in the marine habitats [11, 12].

Several aspects of benthic communities can be described by their functional diversity. Tilman [13] defined it as “the number of different functional groups that exist within a given community or ecosystem" (see also [14, 15, 16, 13]). It is generally accepted that functional diversity plays a key role for the ecosystem processes such as productivity, stability, resilience or invadability [17, 18, 19, 20, 21]. At the same time, the functional diversity decreases with increasing environmental constraints or stress. In fact, when environmental constraints increase, coexisting species are more likely to be similar to one another because environmental conditions (i.e. abiotic properties of the habitat) act as a filter, allowing only a narrow spectrum of species to survive [22].

The Is Arenas Bay is an area renowned for its natural heritage, the local economy being mainly focused on the tourism and fishery activities. In the last decade, several companies have held interest on this area, considering its high potential for both tourism and business on alternative energies (wind, solar). A conscious exploitation of these resources should be provided to assure a sustainable productivity without impoverishment of this area in the future. Therefore, detailed monitoring studies are required to investigate the ecological processes related to the ecosystem functioning [13] and to assess the environmental quality of the area by detecting any possible natural and anthropogenic stresses [23]. At the Is Arenas Bay there are not previous ecological studies about the spatial distribution of macrozoobenthic communities. Therefore, detailed studies are required to asses which are the main environmental features of this important naturalistic site and how they may influence the distribution of the benthic fauna.

This study is a preliminary attempt to analyse the spatial variability of benthic assemblages in the soft bottom sediments of Is Arenas Bay. We tested the hypotheses that benthic fauna distribution may vary (1) at different depths and (2) at different distances from the *Posidonia oceanica* meadows.

2 Materials and Methods

2.1 Study Area

The Is Arenas Bay is located on the western coast of Sardinia (Italy) (Figure 1). There are two main habitats: (i) fine sand soft bottom and unvegetated sediments (8 km^2) investigated in this study and (ii) hard-bottom (10 km^2) colonized by *Posidonia oceanica* meadow. The depth varied between 20 to 30 m, and the distance from the shoreline is about 1.500 m. The main freshwater inputs are from the artificial channel of the Is Benas lagoon and the rainwater drained flux from the catchment basin (Figure 1). There are not industrial nor urban discharges, with low anthropogenic pressure. According to the ARPAS report, the Is Arenas Bay is an oligotropthic and stable habitat [24].

2.2 Sampling

Four different areas (A, B, C and D), thousands of meters apart, were sampled 1 and 2 August 2009 (Figure 1). Referring to the distance from the *Posidonia oceanica*, C and D areas are closer ($\sim$ 0.5 km) than A and B areas ($\sim$ 2.5 km). As for water depth, A and D areas ($\sim$ 30 m) are deeper thnn B and C areas ($\sim$ 20 m). Sediments are sandy in all areas, varying from Very Fine Sand to Medium-Fine (Table 1).

Within each area, two sites were chosen randomly (hundreds of meters apart). Four replicate samples (meters apart) were col-

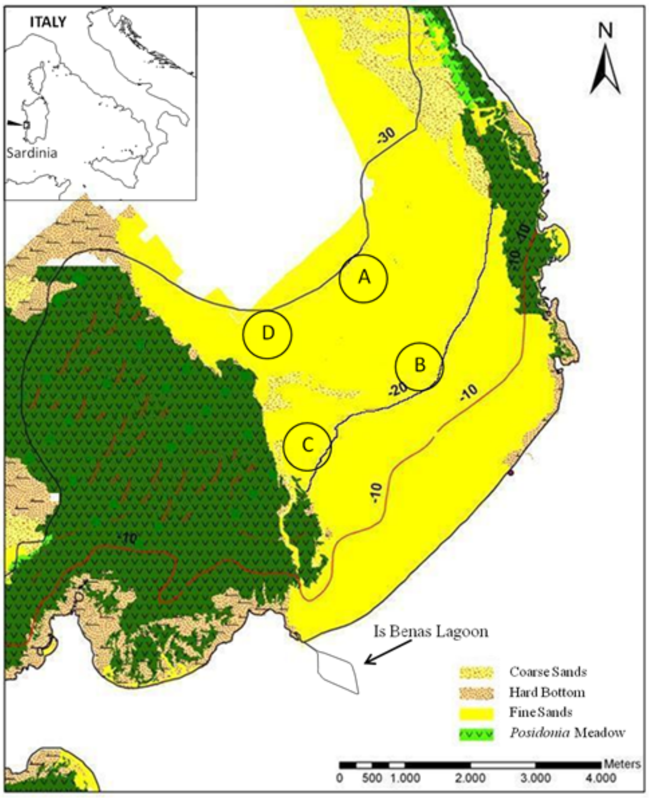
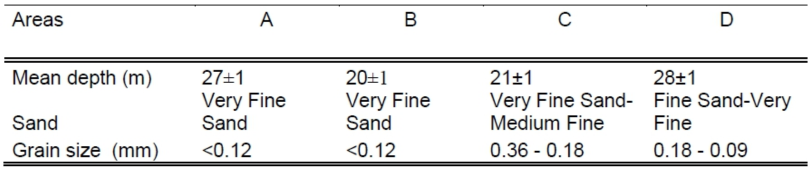


Figure 1: Location of Is Arenas Bay, western Sardinia (Italy). The four study areas (A, B, C and D) are circled.

lected per site for a total of 32 samples. Macrozoobenthic samples were collected by means of a van Veen grab with a sampling area of 360 cm^2. The samples were sieved through a 0.5 mm mesh and then preserved in 4% formaldehyde. In the laboratory, all organisms were identified to the family level.

2.3 Analysis

For each sample and each taxonomic level the number of taxa and the Shannon diversity index (H' $\log_2$ based, [25]) were calculated. Non-parametric multivariate analysis of community structure was performed using the software package PRIMER v.6 [26]. The abundance data (no transformation) were used to detect the differences between sampling areas, using test ANOSIM (Analysis of Similarities) based on the Bray-Curtis (dis)similarity measures with no transformation [27]. A non-metric multidimensional-scaling (nmMDS) was used to plot the differences between areas in the macrobenthic assemblage. The SIMPER procedure was used to identify species contribution to similarity between samples.



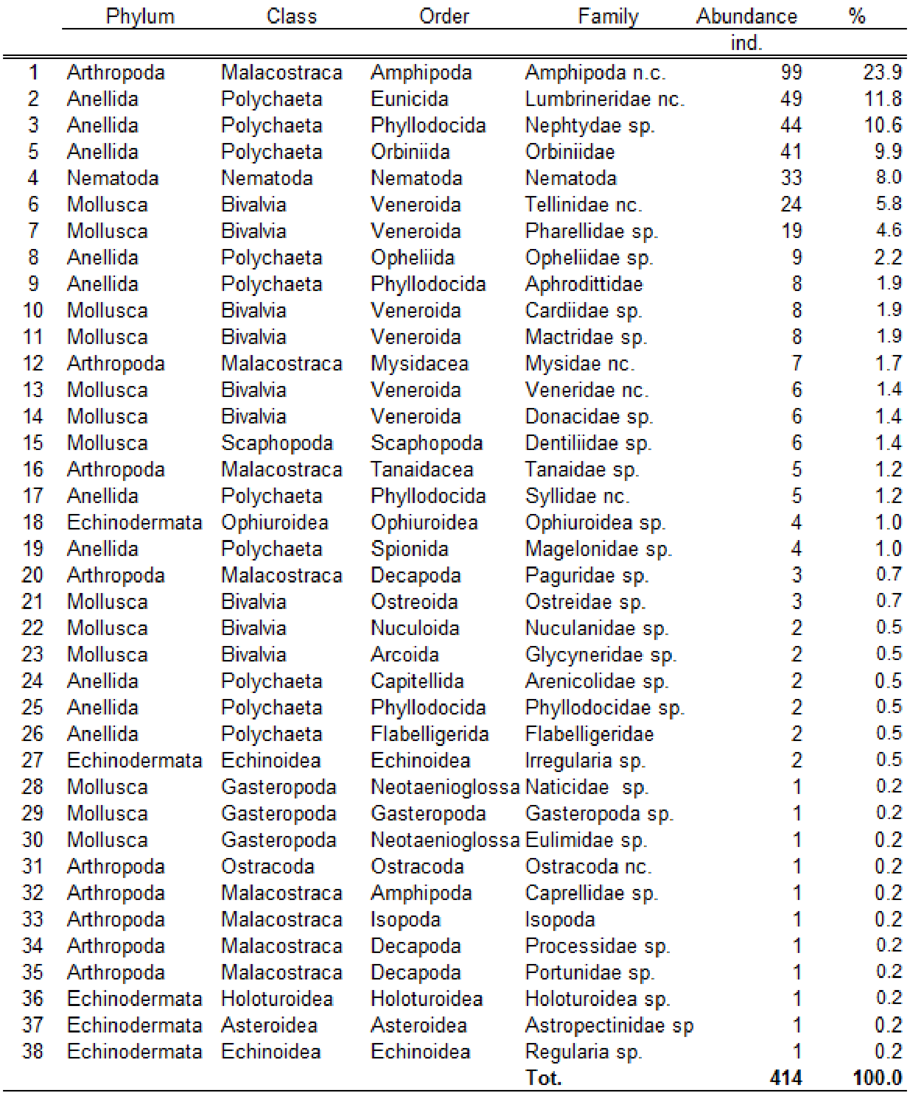
Areas	A	B	C	D
Mean depth (m)	27±1	20±1	21±1	28±1
Sand	Very Fine Sand	Very Fine Sand	Very Fine Sand-Medium Fine	Fine Sand-Very Fine
Grain size (mm)	<0.12	<0.12	0.36 - 0.18	0.18 - 0.09

Table 1: Water depth in the 4 areas (A, B, C and D) and main characteristics of sediments.

3 Results

A total of 414 individuals were collected, belonging to 38 taxa. Amphipods were the most abundance taxa, accounting for 23.9% of the total abundance, followed by polychaetes with the families of Lumbrineridae (11.8%), Nephtydae (10.6%) and Orbiniidae (9.9%). Nematods accounted for 8.0%, while bivalves included the families of Tellinidae (5.8%) and Pharellidae (4.6%). The rare taxa (<0.5%) were spread across a wide range of groupd including polychaetes (Phyllodocidae, Nuculanidae, and Arenicolidae), crustaceans (Ostracoda, Caprellidae, Processidae, Idopoda and Portunidae), Mollusks (Nuculanidae, Glycymeridae, Naticidae and Eulimidae), and echinoderms (Astropectinidae, Ophiuroidea and Holoturoidea) (Table 2). The highest number of taxa was found in area B, the lowest in D. In B and D abundance was higher than in A and C (Figure 2). The Shannon-Wiener index (H') decreased from A to D (Figure 2).

The ANOSIM test (1-way, Global R = 0.234, P<0.01) pointed out differences in taxa composition among assemblages. D differed significantly from all the others and B differed significantly from C. There were not differences between A and C, and at A and B the dissimilarity (p = 0.049) was low. The nmMDS plot provides a visual representation of the distances among the four areas sampled (Figure 3). The SIMPER (cut-off: 90%) highlighted the taxa which give the main contribution to the average dissimilarity among the areas (Table 3). Amphipods were dominant in deeper areas (i.e. D and A) and more abundant close to the *P. oceanica* meadow (Figure 4). On the contrary, Polychaetes were dominant in lower depth areas (i.e. B and C).



	Phylum	Class	Order	Family	Abundance ind.	%
1	Arthropoda	Malacostraca	Amphipoda	Amphipoda n.c.	99	23.9
2	Anellida	Polychaeta	Eunicida	Lumbrineridae nc.	49	11.8
3	Anellida	Polychaeta	Phyllodocida	Nephtydae sp.	44	10.6
5	Anellida	Polychaeta	Orbiniida	Orbiniidae	41	9.9
4	Nematoda	Nematoda	Nematoda	Nematoda	33	8.0
6	Mollusca	Bivalvia	Veneroida	Tellinidae nc.	24	5.8
7	Mollusca	Bivalvia	Veneroida	Pharellidae sp.	19	4.6
8	Anellida	Polychaeta	Opheliida	Opheliidae sp.	9	2.2
9	Anellida	Polychaeta	Phyllodocida	Aphrodittidae	8	1.9
10	Mollusca	Bivalvia	Veneroida	Cardiidae sp.	8	1.9
11	Mollusca	Bivalvia	Veneroida	Mactridae sp.	8	1.9
12	Arthropoda	Malacostraca	Mysidacea	Mysidae nc.	7	1.7
13	Mollusca	Bivalvia	Veneroida	Veneridae nc.	6	1.4
14	Mollusca	Bivalvia	Veneroida	Donacidae sp.	6	1.4
15	Mollusca	Scaphopoda	Scaphopoda	Dentiliidae sp.	6	1.4
16	Arthropoda	Malacostraca	Tanaidacea	Tanaidae sp.	5	1.2
17	Anellida	Polychaeta	Phyllodocida	Syllidae nc.	5	1.2
18	Echinodermata	Ophiuroidea	Ophiuroidea	Ophiuroidea sp.	4	1.0
19	Anellida	Polychaeta	Spionida	Magelonidae sp.	4	1.0
20	Arthropoda	Malacostraca	Decapoda	Paguridae sp.	3	0.7
21	Mollusca	Bivalvia	Ostreoida	Ostreidae sp.	3	0.7
22	Mollusca	Bivalvia	Nuculoida	Nuculanidae sp.	2	0.5
23	Mollusca	Bivalvia	Arcoida	Glycyneridae sp.	2	0.5
24	Anellida	Polychaeta	Capitellida	Arenicolidae sp.	2	0.5
25	Anellida	Polychaeta	Phyllodocida	Phyllodocidae sp.	2	0.5
26	Anellida	Polychaeta	Flabelligerida	Flabelligeridae	2	0.5
27	Echinodermata	Echinoidea	Echinoidea	Irregularia sp.	2	0.5
28	Mollusca	Gasteropoda	Neotaenioglossa	Naticidae sp.	1	0.2
29	Mollusca	Gasteropoda	Gasteropoda	Gasteropoda sp.	1	0.2
30	Mollusca	Gasteropoda	Neotaenioglossa	Eulimidae sp.	1	0.2
31	Arthropoda	Ostracoda	Ostracoda	Ostracoda nc.	1	0.2
32	Arthropoda	Malacostraca	Amphipoda	Caprellidae sp.	1	0.2
33	Arthropoda	Malacostraca	Isopoda	Isopoda	1	0.2
34	Arthropoda	Malacostraca	Decapoda	Processidae sp.	1	0.2
35	Arthropoda	Malacostraca	Decapoda	Portunidae sp.	1	0.2
36	Echinodermata	Holoturoidea	Holoturoidea	Holoturoidea sp.	1	0.2
37	Echinodermata	Asteroidea	Asteroidea	Astropectinidae sp	1	0.2
38	Echinodermata	Echinoidea	Echinoidea	Regularia sp.	1	0.2
				Tot.	**414**	**100.0**

Table 2: List of taxa identified in the study area sorted by total abundance and percentile composition.

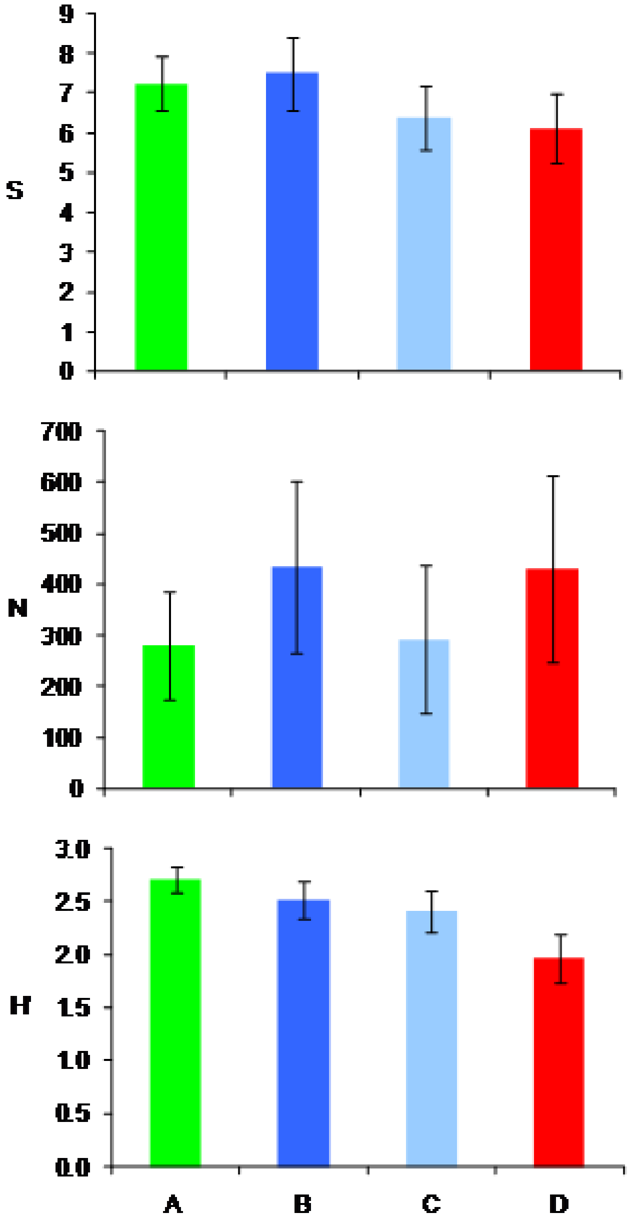


Figure 2: Distribution of species richness (S), density (N, ind. m^{-2}) and Shannon diversity (H') in the four sampled areas (A, B, C and D).

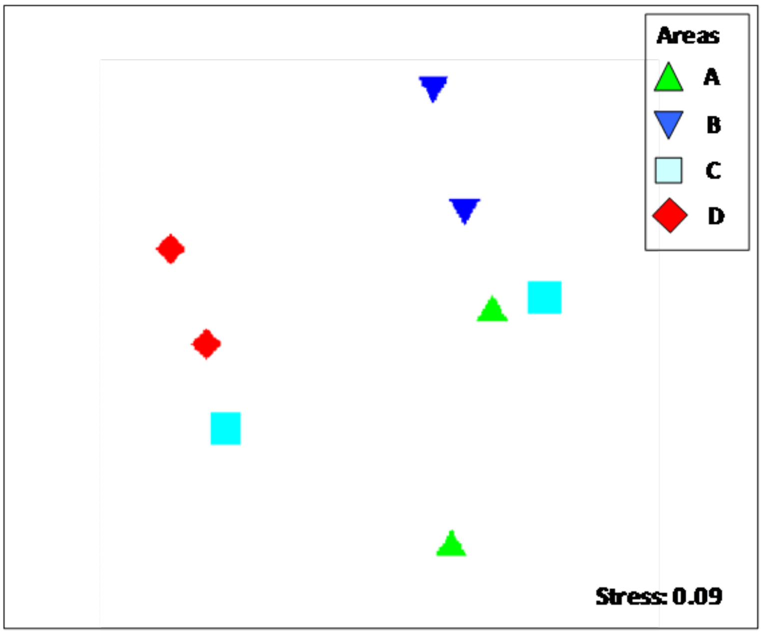


Figure 3: nmMDS plot of benthic abundance in the four sampled areas (A, B, C and D). Each symbol represent the mean of four replicates per site within an area.

	Areas						
Taxa	A		B		C		D
Gammaridae spp.	1.13	<	1.88	>	1.75	<	7.63
Nephtydae sp.	063	<	0.75	<	2.5	>	1.25
Nematoda spp.	0.88	<	1.25	>	0.88	<	1.13
Lumbrineridae spp.	0.88	<	4.13	<	0.88	>	0.25
Tellinidae spp.	0.63	<	1.00	>	0.63	<	0.75
Orbiniidae sp.	-		2.63	>	-	>	0.25

Table 3: Mean density (ind.m^{-2}) of taxa contributing most to the average dissimilarity among areas.

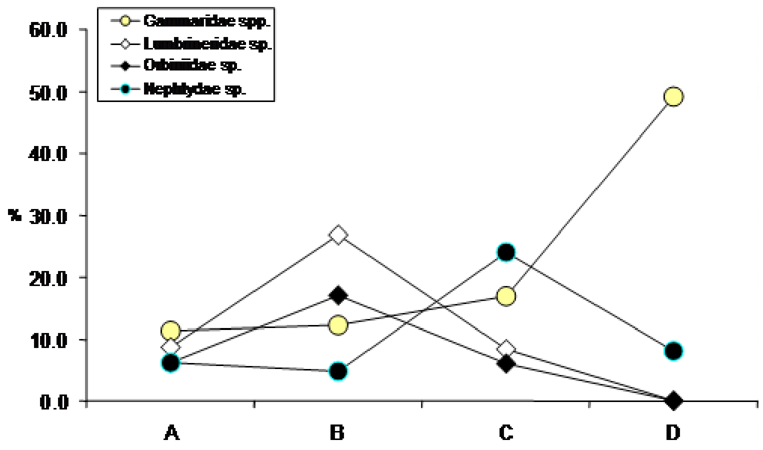


Figure 4: Abundance (%) of dominant taxa in the four sampled areas (A, B, C and D).

4 Discussion

Soft bottom benthic communities can be considered as valuable bioindicators of ecosystem health (or stress) due to their tight relation with the environmental features of water and sediments [28]. In our study area, taking into account the homogeneous composition of sediment grain size, the distribution of macrozoobenthos appeared to be mainly conditioned by different depths and distances from the *P. oceanica* meadow. This was indicated by the dominance of gammarids in deeper areas (A and D), and the dominance of polychaetes in lower depth areas (B and C). Moreover, the distribution of gammarids highlighted a close relation between the *P. oceanica* meadow and the soft bottom habitats; in fact the abundance decreased gradually from the area closer to the *P. oceanica* meadow (D) to that more distant (A) (Figure 4). This trend suggests possible migrations of organisms between the two habitats, considering that some amphipods, common in *P. oceanica* meadows [29] are vagile [30, 31, 32, 33] and able to colonize nearby soft bottom sediments [34].

As for benthic diversity, it is known that high Shannon index (H') values are found in *P. oceanica* habitats [29, 35, 36, 37]. Thus, we would have expected higher H' values in D than what we actually observed (Figure 2). This fact can be explained taking into account that in this study organisms were classified to the family or a higher taxonomic level, possibly precluding a finer definition of the diversity index [38, 39]. Nevertheless, the univariate measures considered here, including the diversity index, the number of taxa (S) and the total abundance (N), indicated that the Is Arenas area is in a good ecological status [40, 41]. In fact, benthic communities in polluted sediments and poor ecological status would be characterized by a much lower number of taxa (S) and a higher abundance (N). The reason is that few tolerant and opportunistic species can adapt to stressed conditions, reaching a considerable number of organisms to the detriment of the rare and sensitive species. The good ecological status of Is Arenas area can also be inferred by the dominance of amphipods which are sensitive species to organic pollution [42, 43, 30, 38].

Finally, taking into account the oligotrophic status of the water, the grain size composition of sediments and the benthic communities typical of environments with low levels of organic matter, the monitored area can be referred as to a biocenosis of Fine, Well-Sorted Sand according to the definition of Pérès and Picard [44]. In conclusion, this preliminary study provides basic information about the main benthic features of Is Arenas Bay. A detailed monitoring program is required to investigate the spatial and temporal distribution of the macrozoobenthic assemblages in order to provide further insights on the benthic dynamic and ecological processes of Arenas Bay.

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Benthic Foraminifera in the Lagoon and Gulf of Venice: a Proxy to Monitor Coastal Environments

S. Donnici[1], R. Serandrei-Barbero[1], A.D. Albani[2]
1, Institute of Marine Sciences, CNR, Venezia, Italy
2, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sidney, Australia
sandra.donnici@ve.ismar.cnr.it

Abstract

The recent benthic foraminiferal distribution in the Lagoon and Gulf of Venice has been described through the quantitative analysis of about 1000 bottom samples collected in the ‘80s and ‘90s. The benthic foraminiferal assemblage shows a high level of uniformity and stability within areas characterised by similar physical and chemical parameters (biotopes). The foraminiferal biotopes have a response time to environmental changes of the order of a few years, so the temporal comparison between biotopes before and after anthropic interventions is particularly useful for applicative approaches. To assess the degree of recent environmental change, the quantitative data have been used for a comparison using the Kolmogorov-Smirnov non-parametric test in two area with great anthropic influence, in the central lagoon and at the Lido Inlet. Between 1980-1990 and 2001 little or no biotope change occurred over more than 50% of the lagoon. On the Lido Inlet a comparison was obtained between samples collected in 1983 and 2006: the high similarity in the foraminiferal assemblages of the two periods strongly suggests that the physical and chemical characteristics, within the sampled area, have not greatly changed during the intervening 23 years. The knowledge of the biotopes distribution gives an overall picture of the characteristics of the Lagoon of Venice and Northern Adriatic Sea for monitoring the effects of the engineering interventions ongoing at the lagoon inlets.

1 Introduction

Being small organisms with a calcareous test that is well preserved in soft sediment, benthic foraminifera (class Foraminifera, according to the suprageneric classification of [1]) enable an understanding of the evolution of the coastal environments to be obtained through a reconstruction of the palaeoenvironments [2, 3, 4, 5, 6]. In transitional environment under anthropic pressure, the benthic foraminifera are particularly effective as biological indicators since the distribution of benthos is closely related to environmental parameters [7].

The large number of species in each sample (assemblage), combined with the large quantity of individuals of each species (population) even in restricted environments, provides a statistically valid methodology in monitoring the coastal and transitional environments. These characteristics make possible to recognize any slight changes in the total assemblage as well as in the various populations, and thus to detect any persistent alteration in the water qualities.

In the biological investigations a species is considered an environmental indicator when its presence-absence-abundance relates to biotic processes that often covary with natural and anthropogenic environmental gradients, in particular in a lagoon environment given the great variability of the physical and chemical parameters. However, the species as indicator is less efficient and reliable than the whole assemblage [8] as the totality of the species present reflects the physical-chemical parameters prevailing.

The foraminifera of the Lagoon of Venice were described for the first time by Silvestri [9] and later in the earlier studies on the lagoon [10, 11, 12, 13, 14]. Their sensitivity to environmental changes makes them a proxy to identify future changes.

2 The recent foraminifera of the Venice coastal area

In the '80s a systematic sampling of the Lagoon of Venice and Northern Adriatic seabed was initiated and over 700 sediment samples were collected. The total foraminiferal fauna was identified and counted in each sample and the abundance and distribution of 80 benthic species within the lagoon and the Gulf of Venice has been established [15, 16].

Numerical analyses were based on the total assemblage of both living and dead individuals as that gives an integrated view of the assemblage [17]. The short-term variations of the living assemblage, being mainly due to the availability of phytoplankton and biotic interactions, result more irregular with respect to the total association [18].

The average assemblage of benthic foraminifera in the lagoon is composed of 11 species; the assemblages reach 37 species in the vicinity of the marine inlets and reduce to 5-6 species in the more confined areas. *Ammonia beccarii, Haynesina paucilocula, Aubignyna perlucida* and *Cribrononion granosum* are distributed over 90% of the area and appear to be the most tolerant species; other 57 species are present in sediment covering less than 10% of the lagoon. The foraminiferal assemblages show a high level of uniformity and stability within an area (biotope) that is characterised by similar physical and chemical parameters, hence allowing biotopes to be identified by Q-mode cluster analysis [19, 20]. A percent matrix using the Pearson correlation coefficient [19] is obtained from the percentage data set and cluster analysis is performed on such correlation matrix. The output of the cluster is generally presented in the form of a dendrogram. Cluster analysis is a metod for linking stations according to their similarity in faunal content, the closer the foraminiferal assemblages are, the higher is the similarity between stations.

The foraminiferal biotopes [21, 22, 23, 24] reflect the characteristics of the lagoonal and coastal environment during the years 1980 - 1990. Each biotope is characterised by the association of a number of key species that are more tolerant to the extremes conditions of the different areas (Table 1). The distribution of benthic foraminifera in the Lagoon of Venice today appear to be largely controlled by residence time, pollution, fresh water inputs and presence of intertidal morphologies (Figure 1), parameter well tolerated respectively by *Ammonia beccarii, Haynesina*

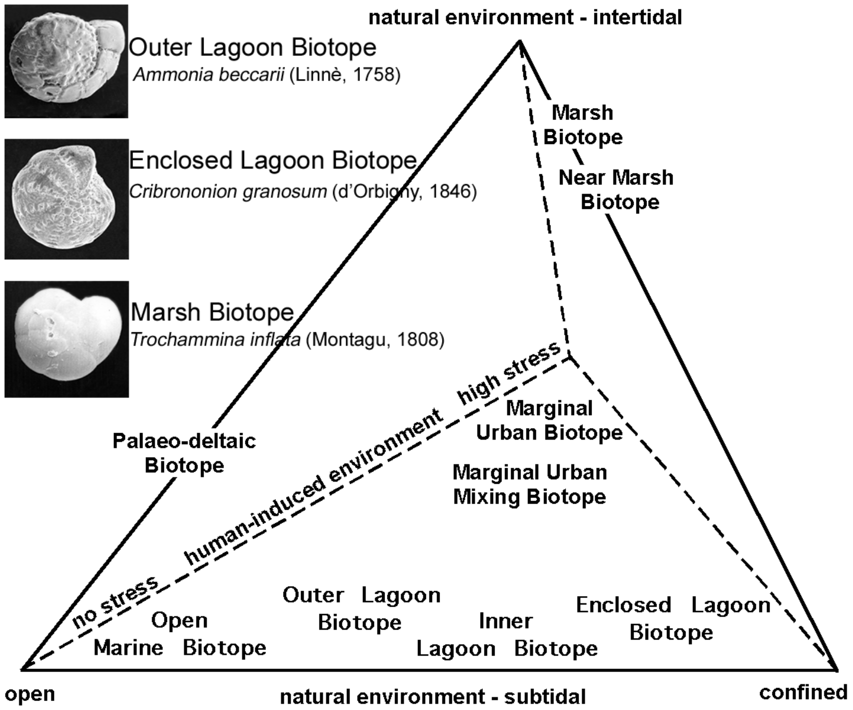


Figure 1: Lagoon biotopes terminology and their relationship with intertidal morphology and environment. The main control factors are the degree of confinement or residence time (from open to confined), inversely proportional to the water exchange time with the sea; the human-induced stress, which is covariant with residence time; the elevation with respect to mean sea level, affecting the intertidal morphologies. As an example, the dominant taxon in the main lagoonal biotopes are illustrated.

paucilocula, Cribrononion granosum and *Trochammina inflata*. In the northern Adriatic continental shelf, three different foraminiferal associations discriminate the shallow, nutrient rich area, with *Ammonia beccarii* dominant; the deep, nutrient rich area of clay-rich sediment where inputs from the Po River are concentrated, dominated by *Nonionella opima* and the nutrient-poor area, dominated by *Textularia agglutinans*.

The detailed knowledge of the distribution of the different recent biotopes (biotopes of the lagoon northern basin are shown in Figure 2), has given an overall picture of the characteristics of the lagoon environment at the onset of important and far-reaching engineering interventions. Their effects can be verified in the future through the answer of the populations and assemblages.

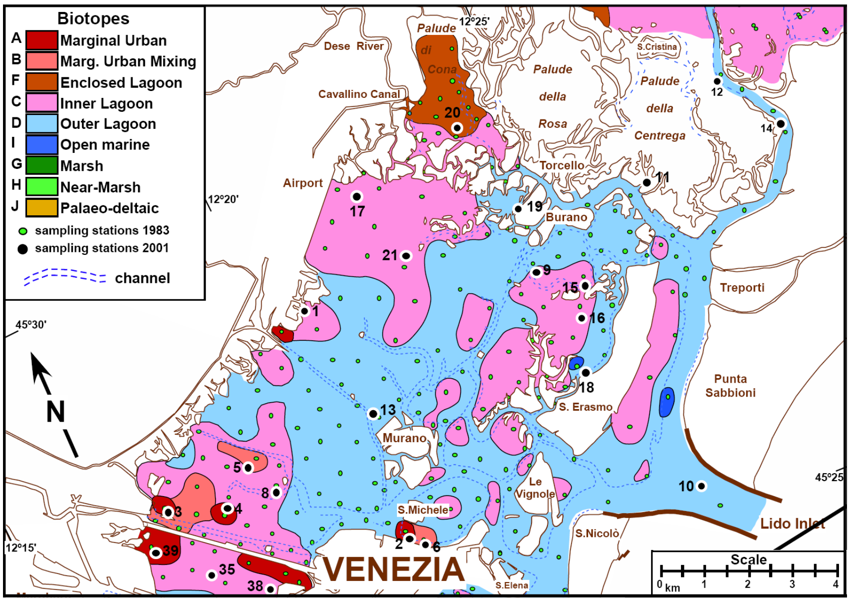


Figure 2: Northern sector of the Lagoon of Venice with the 1983 biotopes distribution and the location of the 2001 samples an cores.

3 The monitoring of the present-day lagoon

Recent laboratory studies have dealt with trying to establish a linear relationship between benthic foraminifera and natural or anthropogenic events [25, 26, 27]. However, this relationship is not clear because the foraminiferal biofacies are the result of mutual interactions of the different variables, both biological and non-biological, whose total effect influences the field data. The pollution deals with the diversity and with the abundance of the assemblage more than with the single population, while, in the single specimen, pollution may cause test deformation or changes in the size and age distribution [28, 29, 25]. However, differential adaptations to the complex conditions characterizing estuarine environments make it difficult to separate natural properties from pollution effects.

The foraminifera have a response time to environmental changes of the order of a few years, so the comparison of biotopes obtained from samples collected in the Venice Lagoon in 1980-1990 compared to those from samples collected in 2001 is particularly revealing of these applicative aspects. To assess the degree of environmental change, the quantitative data have been used for a comparative study using the Kolmogorov-Smirnov non-parametric test [30]. This test compares the cumulative frequency values of each pair of samples and records the value of the difference. The combination of the Kolmogorov-Smirnov test, which determines the level of similarity between each pair and the cluster links, determines not only the degree, but also the

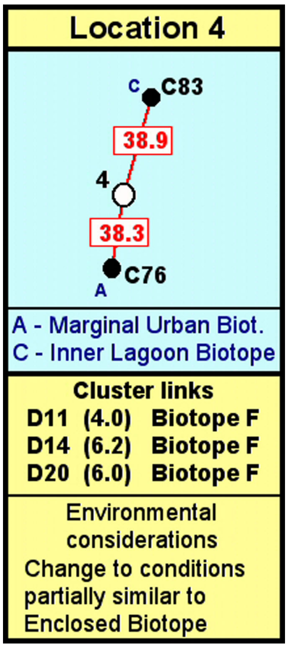


Figure 3: Relationship between the 2001 foraminiferal fauna in sample station 4 (open dot) and the 1983 faunas (solid dots, corresponding to 1983 sampling stations). The Kolmogorov-Smirnov index for each pair of stations is shown in the small rectangle. The link with Enclosed Biotope (Biotope F) derives from the combined cluster analysis of the 1983 and 2001 foraminiferal faunas.

direction of the change, if any.

Between 1980-1990 and 2001, the results indicate that little or no biotope change occurred over more than 50% of the area sampled [31]. In some of the areas with great anthropic influence a reduction of the environmental stress has been found (samples 3, 4, 5 and 39 in Figure 2). Along the ”canale dei petroli”, in the central part of the lagoon, the collapse of some saltmarshes appears to be related to the increased tidal hydrodynamics caused by channel dredging. In the southern basin of the lagoon a mudflat shows a foraminiferal fauna pertaining to the Marsh Biotope that is due to the rapid lowering of the floor of the southern lagoon since 1983.

Figure 3 refers to the sampling stations 4 in Figure 2 and illustrates the comparison of the samples of 1983 vs. the sample of 2001. The main environmental changes that have occurred between the two surveys appear to be the improvement of the water quality in the area near Marghera. Since 1986, the wastewater treatment plant of the Consorzio Comunale per gli Impianti di Depurazione (CCID) has led to a progressive decrease of the phosphorous load [32] that is reflected in the foraminiferal fauna through its control on the algal bloom. In the Lagoon of Venice, the presence of phytoplankton appears to be the main cause for the productivity of benthic foraminifera also with a mechanism of interspecific competition [3]. Benthic foraminifera, in fact, are consumers of phytoplankton.

The foraminiferal fauna is therefore capable to identify and distinguish the environmental changes due to both the natural and the human-induced processes.

4 The monitoring of the coastal environment

In the Gulf of Venice, where the conditions are oligotrophic, the occasional availability of phytoplankton is the main factor influencing the productivity of benthic foraminifera and fluctuations in dominant and subdominant taxa are linked to biotic interactions [18].

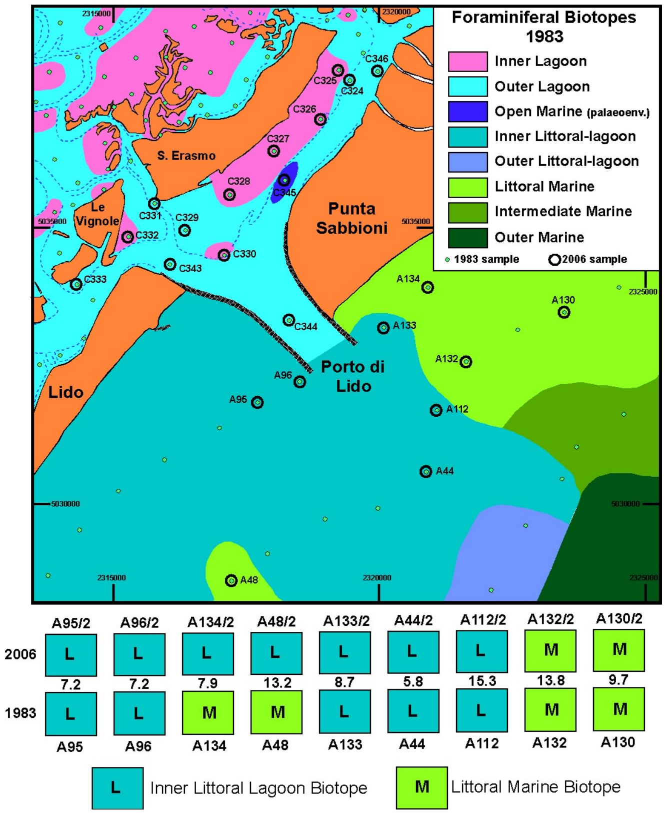
The good correspondence with the foraminiferal distribution described in the sixties [33], indicates that, in spite of the occurrence of anoxic and dysoxic events, the environmental conditions in the Northern Adriatic may be considered stable [34]. At the Lido Inlet, in 2006 sampling was repeated at 23 of the sites sampled in 1983 using the same methodology and identical laboratory processing: the foraminiferal assemblages of the two sampling periods are very similar, strongly suggesting that the physical and chemical characteristics of the water masses have not greatly changed during the intervening 23 years [35]. Figure 4 shows the map of the benthic foraminifera biotopes based on the 1983 survey and the comparison diagrams between 1983 and 2006 biotopes for the Adriatic Sea stations. No major biotope changes are noted in the Adriatic portion of the area, there being only a small broadening of the extent of the Inner Littoral Lagoon Biotope (stations A48 and A134), indicating an increase in the flow energy.

5 Conclusions

The detailed knowledge of the foraminiferal biotopes gives a picture of the distribution of the environmental parameters in the Lagoon and Gulf of Venice and draws the boundaries of the areas under different influences with the present coastal regime.

The distribution of the various biotopes reflect the lagoonal hydrodynamics of the last decades, which have been substantially unchanged since the late ‘60s. Therefore, because these organisms are preserved within the unconsolidated bottom sediments, benthic foraminifera represent highly suitable and readily mapped biological indicators as they synthetically reflect a great variety of physical and chemical parameters of the water masses.

The foraminiferal assemblages react to the environmental changes with a response time of a few years and for this reason they act as proxies of environmental trends; the comparison between biotopes before and after the engineering interventions of defence from the high waters will make possible to monitor changes caused by the mobile barriers ongoing at the lagoon inlets.



	A95/2	A96/2	A134/2	A48/2	A133/2	A44/2	A112/2	A132/2	A130/2
2006	L	L	L	L	L	L	L	M	M
	7.2	7.2	7.9	13.2	8.7	5.8	15.3	13.8	9.7
1983	L	L	M	M	L	L	L	M	M
	A95	A96	A134	A48	A133	A44	A112	A132	A130

L Inner Littoral Lagoon Biotope

M Littoral Marine Biotope

Figure 4: Coastal foraminiferal biotopes based on the 1983 survey and comparison between 1983 and 2006 biotopes for the Adriatic Sea stations. The squares 1983 and 2006 show the respective biotope: the Kolmogorov-Smirnov value (smaller the value, smaller is the difference between samples) is given between the biotopes squares.

Biotope	A- Marginal Urban			B-Marginal Urban Mixing		
Major foraminiferal species	average	median	range	average	median	range
Trochammina inflata (Montagu)	0,8	0,6	0,2 - 1,6	1,9	1,1	0,2 - 6,9
Eggerella scabra (Williamson)						
Quinqueloculina seminulum (Linné)	2,9	1,9	0,4 - 12,3	2,8	1,6	0,2 - 12,2
Aubignyana perlucida (Heron-Allen & Earland)	3,9	2,4	0,5 - 17,4	4,3	3,1	0,8 - 13,7
Haynesina paucilocula (Cushman)	47,3	46,9	30,9 - 66,0	24,9	25,6	16,3 - 37,1
Nonion politum (d'Orbigny)	0,2	0,2	0,2 - 0,2	6,2	6,2	4,0 - 8,3
Ammonia beccarii (Linné)	38,6	38,3	27,7 - 51,0	54,2	53,8	43,0 - 62,6
Elphidium granosum (d'Orbigny)	6,8	5,2	1,1 - 20,6	9,8	10,1	1,7 - 16,2
stations	12 = 2.01%			17 = 2.85%		
species	18 = 24.32%			25 = 33.78%		
Biotope	C- Inner Lagoon			D-Outer Lagoon		
Major foraminiferal species	average	median	range	average	median	range
Trochammina inflata (Montagu)	1,7	0,5	0,2 - 13,4	0,5	0,3	0,2 - 5,3
Eggerella scabra (Williamson)	3,4	0,6	0,2 - 17,2	1,9	0,7	0,2 - 8,7
Quinqueloculina seminulum (Linné)	3,2	2,3	0,1 - 23,7	1,9	1,3	0,2 - 14,8
Aubignyana perlucida (Heron-Allen & Earland)	6,6	6,2	0,3 - 17,7	6,6	6,3	0,3 - 16,2
Haynesina paucilocula (Cushman)	11,5	9,7	1,3 - 38,6	6,7	6,3	0,3 - 18,1
Nonion politum (d'Orbigny)	1,8	1,0	0,2 - 7,7	1,8	1,3	0,2 - 7,7
Ammonia beccarii (Linné)	62,0	62,6	28,0 - 81,7	71,7	72,4	43,0 - 93,0
Elphidium granosum (d'Orbigny)	11,7	10,2	0,3 - 43,0	7,7	6,7	0,3 - 25,2
stations	186 = 31.16%			317 = 53.10%		
species	68 = 91.89%			72 = 97.30%		
Biotope	I-Open Marine			J-Palaeo-deltaic		
Major foraminiferal species	average	median	range	average	median	range
Trochammina inflata (Montagu)				0,3	0,3	0,3 - 0,3
Eggerella scabra (Williamson)				12,3	12,3	7,5 - 17,0
Quinqueloculina seminulum (Linné)	15,3	15,4	6,0 - 23,3	0,8	0,3	0,3 - 2,3
Aubignyana perlucida (Heron-Allen & Earland)	1,7	1,7	1,2 - 2,5	2,8	3,2	0,8 - 4,5
Haynesina paucilocula (Cushman)	3,4	3,7	0,7 - 5,3	12,8	13,7	0,7 - 20,3
Nonion politum (d'Orbigny)	0,7	0,7	0,4 - 1,0	8,8	11,7	0,3 - 15,7
Ammonia beccarii (Linné)	46,9	50,2	35,0 - 58,7	69,3	70,6	57,1 - 78,0
Elphidium granosum (d'Orbigny)	13,6	10,3	5,4 - 34,2	2,8	1,6	0,8 - 6,0
stations	6 = 1.01%			8 = 1.34%		
species	41 = 55.41%			16 = 21.62%		
Biotope	F-Enclosed Lagoon			G-Marsh		
Major foraminiferal species	average	median	range	average	median	range
Trochammina inflata (Montagu)	0,3	0,2	0,1 - 0,5	26,4	24,1	17,7 - 39,8
Eggerella scabra (Williamson)				0,5	0,5	0,3 - 0,7
Quinqueloculina seminulum (Linné)	2,9	1,2	0,2 - 13,3	0,3	0,3	0,3 - 0,3
Aubignyana perlucida (Heron-Allen & Earland)	5,7	5,9	2,8 - 7,4	4,9	4,8	3,3 - 6,7
Haynesina paucilocula (Cushman)	17,1	19,7	6,3 - 27,3	5,7	5,5	4,0 - 7,7
Nonion politum (d'Orbigny)				6,7	6,7	5,3 - 8,0
Ammonia beccarii (Linné)	33,2	33,7	20,8 - 43,8	54,1	53,3	49,4 - 60,3
Elphidium granosum (d'Orbigny)	39,2	40,6	18,7 - 56,1	1,8	1,8	0,7 - 2,9
stations	11 = 1.84%			4 = 0.67%		
species	14 = 18.92%			19 = 25.68%		
Biotope	H-Near Marsh					
Major foraminiferal species	average	median	range			
Trochammina inflata (Montagu)	8,3	8,8	1,4 - 13,4			
Eggerella scabra (Williamson)						
Quinqueloculina seminulum (Linné)	2,7	1,5	0,3 - 11,4			
Aubignyana perlucida (Heron-Allen & Earland)	4,1	3,8	0,3 - 8,2			
Haynesina paucilocula (Cushman)	11,8	9,4	5,2 - 21,0			
Nonion politum (d'Orbigny)	1,5	0,6	0,3 - 3,7			
Ammonia beccarii (Linné)	67,7	68,1	64,1 - 71,7			
Elphidium granosum (d'Orbigny)	3,4	3,2	0,7 - 8,1			
stations	7 = 1.17%					
species	25 = 33.78%					
The % values are calculated based on 74 total species and 559 stations						

Table 1: Biotopes faunal composition, as relative abundance of each species in the total assemblages of about 300 individuals, based on the 1983 survey. Only significative species are represented with their range of relative abundance, average, median value and occurrence expressed as number of stations and percentages of total stations (areas).

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Study of the Biomass and Spatial Distribution of Deep Pelagic Fish Larvae of the Sicilian Channel in Relationships to the Physical and Chemical Parameters of the Water Column

A. Cuttitta, L. Caruana, B. Patti, A. Bonanno, C. Patti, B. De Luca, S. Bonomo, E. Quinci, G. Tranchida, M. Musco, T. Masullo, M. Borghi, G. Buscaino
Institute for Coastal Marine Environment, CNR, Capo Granitola (TP), Italy
angela.cuttitta@iamc.cnr.it

Abstract

Mesopelagic fish represent one of the most important links in the food chain of the marine ecosystem. Their abundance in the Mediterranean sea makes them the most important members of the deep water fauna and a potential resource for future fisheries. The aim of this work is the study of the spatial distribution, composition and biomass of mesopelagic fish larvae species in relationship with the oceanographic parameters of the Sicilian Channel. This area is characterized by different water masses associated with the typical hydro graphic structures of the Channel (Atlantic Ionian Stream-AIS), influencing the presence and distribution of fish species larval stages. Samples were collected during oceanographic surveys, on board of URANIA O/V in years 2005, 2006 and 2007. Distribution maps of the hauls, grouped in clusters, showed similar patterns during all analyzed years. The most common spots we found were located under Pozzallo, Cape Passero and Siracusa, though there were small spots along the central shelf of the western coast. These results would be influenced by the existence of different water masses on the surface, associated with the AIS path and with the hydrographic structures. The occurrence of these species in shelf areas would be associated with the coastal upwelling characterizing the northern part of the study area. In addition, the accumulation of larvae on the south-eastern edge could be related to the strong salinity front off Cape Passero.

1 Introduction

Spatiotemporal patterns of ichthyoplankton communities, with respect to abundance, distribution and species composition, have been traditionally a basic topic of scientific research in fisheries oceanography [1]. Ichthyoplankton surveys provide useful data for the assessment of important parameters of commercially important fish populations (i.e. spawning stock biomass, recruitment), but can also improve our knowledge on the agents structuring larval assemblages [2]. A large number of biological and physical factors contribute to the formation, maintenance and disruption of larval fish assemblages as it has been pointed out for several coastal

Families	**Species**	**2005**	**2006**	**2007**
Gonostomatidae	*Cyclothone braueri*	100%	100%	100%
Sternoptychidae	*Maurolicus muelleri*	100%	-	100%
Phosichthyidae	*Vinciguerria attenuata*	22%	12%	19%
	Vinciguerria nimbaria	3%	8%	5%
	Vinciguerria poweriae	39%	37%	29%
	Vinciguerria spp	36%	43%	47%
Paralepididae	*Lestidiops jayakari*	7%	10%	18%
	Pseudosphyraenoides sp			
	Paralepis affinis	45%	38%	42%
	Paralepis coregonoides	19%	12%	12%
	Arctonozenus risso	2%	-	-
	Sudis hyalina	-	-	3%
	Paralepide spp	27%	50%	25%
Myctophidae	*Ceratoscopelus maderensis*	22%	25%	16%
	Lobianchia spp	4%	5%	4%
	Diaphus holti	-	-	0,4%
	Electrona risso	8%	4%	12%
	Hygophum spp	15%	2%	22%
	Lampanyctus crocodilus	11%	19%	25%
	Lampanyctus pusillus	2%	-	0,6%
	Myctophum punctatum	4%	5%	11%
	Myctophum humbolditi	-	-	-
	Myctophum spp	34%	40%	9%
Stomiatidae	*Stomias boa*	100%	-	100%

Table 1: List of mesopelagic species larvae detected during July 2005, 2006, 2007 and the percentage of each species among the belonging families.

systems around the world's oceans since the early 90's [3, 4].

Regarding the Mediterranean waters, research on the structure of larval fish communities has been progressively developed in the last two decades. A large amount of information has been published concerning the western part of the Mediterranean Sea (Catalan Sea, Balearic Archipelago) [5]. Latitudinal and vertical distributions of fish larvae in relation to short-term mesoscale variability have been also examined in the western Alboran Sea [6]. In the central and eastern Mediterranean information on ichthyoplankton assemblage structure and distribution is scant (straits of Sicily: [7]; northern Aegean Sea: [8]; [9]).

Mesopelagic fish are scotophil species living from 100 m to 1000 m of depth. They have typical larval stages of photic zone.

Their abundance in the Mediterranean sea [10, 9] makes them the best represented members of the deep water fauna and they are very likely to be preys of other fish species. They represent one of the most important link in the food chain of the marine ecosystem [11]; in fact, mesopelagic fish are highly relevant in the diet of economically important species and can be considered as a link in the energy transfer between the deeper environment and the higher layers of the water column. Taking into account the important function of these species in the trophic marine chains, and

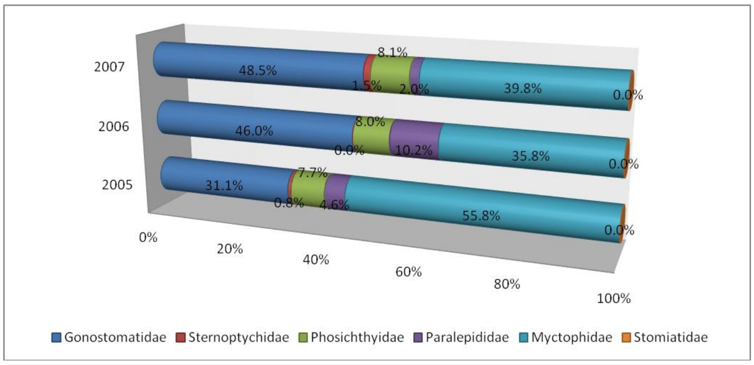


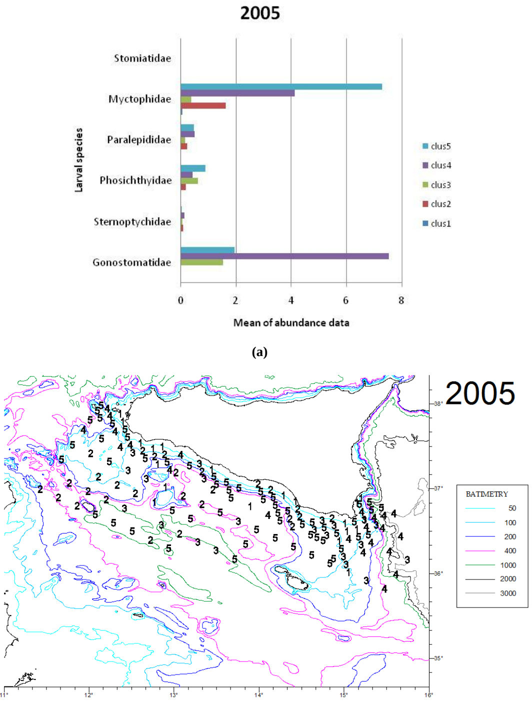
Figure 1: Percentage of abundance of each larval families in years 2005, 2006 and 2007.

their potential use as a resources for features fisheries, it is important to collect data on their distribution, abundance and life cycle. The aim of this study is focused on the improvement of mesopelagic species spatial distribution, on their composition and abundance through the knowledge of their larval stages in relation to Sicilian Channel's oceanographic parameters.

This area is characterized by different water masses associated with the typical hydro graphic structures of the Channel influencing the presence and distribution of fish species larval stages. Specifically, the surface circulation in this area is controlled by Atlantic-Ionian Stream (AIS, [12]) flowing from west to east in the surface layer of the Mediterranean basin. Below this layer corresponding to the mesopelagic environment, the Levantine Intermediate Water (LIW) flows in opposite direction: it is a colder and more saline water compared to the surface layers. The AIS has a cyclonic pattern: it enters the Channel by its west boundary and follows a large cyclonic meander, which embraces the Adventure Bank. Then, it moves close to the shore, in the middle of the southern coast of Sicily. In fact, the AIS pattern makes a cyclonic meander out of Cape Passero, separating again when it encounters the shelf of Malta. This surface circulation pattern supports the upwelling of water derived by LIW and thus determines the enrichment of surface layers.

2 Materials and Methods

Biological data were collected in the south of Strait of Sicily during the BANSIC hydrographic and icthyoplanktonic surveys carried in July 2005, 2006 and 2007 years on board of O/V URANIA. The sampling was made on a station grid of 4×4 nautical miles in sea zones closer to the coasts; a grid of 12×12 nautical miles was adopted for the off-shore areas. In each station ichthyoplankton samples have been collected by means of a Bongo40 net, which is composed by two coupled nets with the inlet mouth diameter of 40 cm and mesh size 200 µm. The plankton oblique tows were carried out to a depth of 100 m, wherever possible, with a constant speed of 2 knots. The filtered water volume of each mouth



(a)

(b)

Figure 2: (a) Mean of abundance of larval species belonging each family among hauls, grouped through cluster analysis in year 2005. (b) Distribution of hauls of BANSIC 05, grouped through cluster.

was measured by a calibrated flow-meters (type G.O. 2030).

The zooplanktonic samples were stored in alcohol at 70% and then observed in the laboratory by stereomicroscopy for the taxonomic analysis of the ichthyoplanktonic species. In order to identify the distribution of different species in relation of AIS trajectory the hauls were grouped through the cluster analysis, based on $\log_{10}$ (X+1) transformed species abundance data. Bray-Curtis distance [13] was used and Ward's method[14], as grouping method.

3 Results

155 stations were sampled during the year 2005. Among mesopelagic larval species the most abundance ones were Gonostomatidae and Myctophidae, which represented 31.13% and 55.82% respectively. Other families as Sternoptychidae, Phosichthyidae and Paralepididae were detected in very low proportions (Figure 1). Among species belonging to Gonomastidae family only one species was founded (Cyclothone braueri) [15], while among species belonging to Mycthophidae family the more abundant founded species

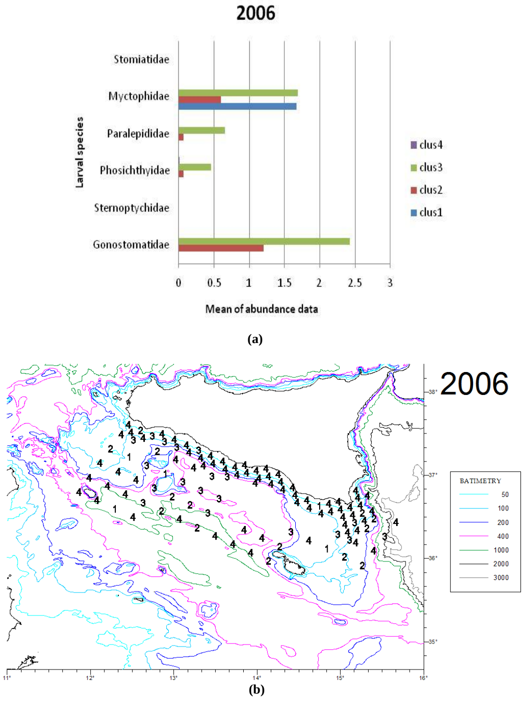


Figure 3: (a) Mean of abundance of larval species belonging each family among hauls, grouped through cluster analysis in year 2006. (b) Distribution of hauls of BANSIC 06, grouped through cluster.

were *Myctophum* spp and *Ceratoscopelus maderensis*[16], with 34% and 22% of the total respectively (Table 1).

123 stations were sampled during the BANSIC 2006 and the 13% of the total larval fish were mesopelagic species. The most abundance mesopelagic larval species were Myctophidae and Gonostomatidae, which respectively represented 46.02% and 35.80% of the total. Other families as Phosichthyidae and Paralepididae were detected in very low proportions (Figure 1). In the Myctophidae family 7 species were detected, *Myctophum* spp and *Ceratoscopelus maderensis*[16] were the most abundant species, accounting for 40% and 25% of the Myctophidae. Among species belonging Gonostomatidae family, *Cyclothone braueri* [15] was the only species we found (Table 1). This year, the weight of the zooplankton samples has been 315.95 g.

161 stations were sampled during the BANSIC 2007 and the 24% of the total larval fish were mesopelagic species. The most abundance mesopelagic larval families were Gonostomatidae and Myctophidae, which represented 48.48% and 39.85% of the total respectively. Other families as Sternoptychidae, Phosichthyi-

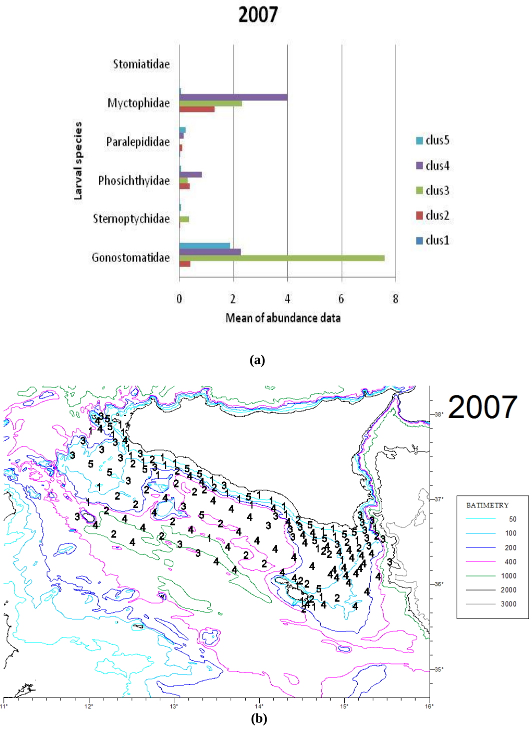


Figure 4: (a) Mean of abundance of larval species belonging each family among hauls, grouped through cluster analysis in year 2007. (b) Distribution of hauls of BANSIC 07, grouped through cluster.

dae, Paralepididae and Stomiatidae were detected in very low proportions (Figure 1). Among Gonostomatidae family, *Cyclothone braueri* [15] was the only species we detected, while among Myctophidae family 9 species were detected, *Lampanyctus crocodilus* and *Hygophum spp* were the most abundant species, accounting for 25% and 22% (Table 1). In 2007, the weight of the zooplankton samples was 795.61 g.

In year 2005, the hauls with absence of larvae (cluster 1) are located mostly inshore. Those of cluster 2 and 3, that have a minimal presence of Sternoptychidae and Myctophidae are in the north-west of the study area (Adventure Bank), while the hauls with major abundance of Gonostomatidae (cluster 4) are located in the east side (under Cape Passero) and the hauls of cluster 5 with major abundance of Myctophidae are located in the south-east (Figure 2).

In year 2006, the hauls with absence of larvae (cluster 1) and with minimal presence of Phosichthyidae (cluster 4) are mostly inshore. Hauls of cluster 2 have a minimum percentage of Gonostomatidae and Myctophidae, the same species but with a higher percentage characterize the cluster

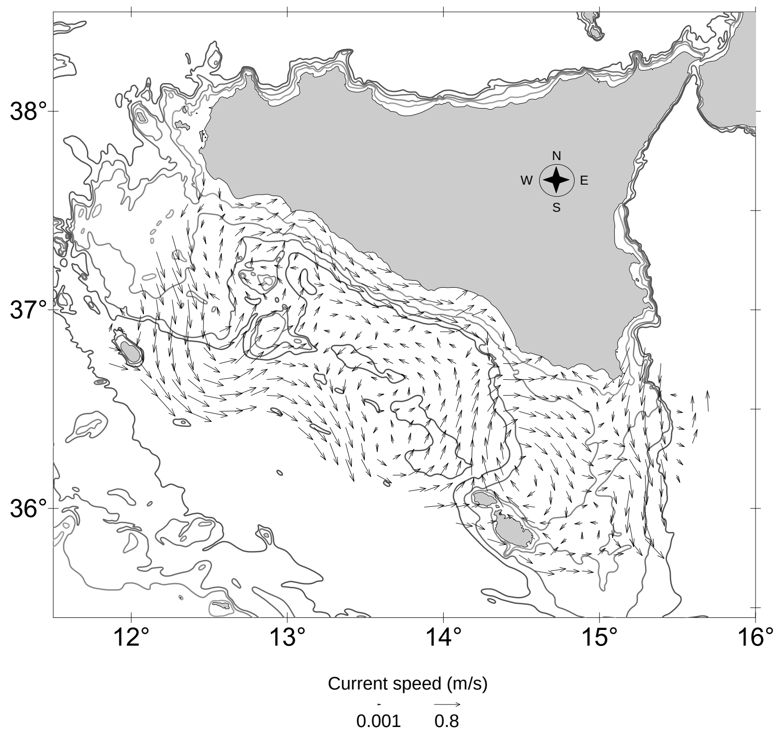


Figure 5: Surface geostrophic current pattern during July 2005.

3. Both clusters are offshore (Figure 3). In year 2007, the hauls with absence of larvae (cluster 1) are inshore. The hauls of cluster 2 have a low percentage of Phosichthyidae and Myctophidae and are located in the western part of the study (Adventure Bank) and in the Maltese Bank. The hauls of cluster 3 and 5 show a low percentage of Gonostomatidae and are located mostly in the north-east, those of cluster 4 have a minimum presence of Myctophidae and are located in the south-east side (under Licata and Capo Passero) (Figure 4). Figures 5-7 show the path of the geostrophic currents estimated of CTD data collected in July 2005, 2006 and 2007. From such pattern it is possible to evaluate the AIS pattern in the study area.

In 2005, the AIS goes away from the Egadi Archipelago, moving southward.

Once it arrives at Pantelleria Island, it moves eastward, shifting into two arms: the first one goes to the cost and the second one flows parallel to the coast up to Malta, where it heads to the coast and then goes southward (Figure 5). In 2006, the AIS skims the coast of Marsala during its move to southward. After going over Pantelleria, the AIS moves eastward, flowing in the north of Malta and offshore Pozzallo (Figure 6).

In 2007, the AIS moves from Marsala to the east of Pantelleria, doing immediately a reverse close to the coast from Gela to

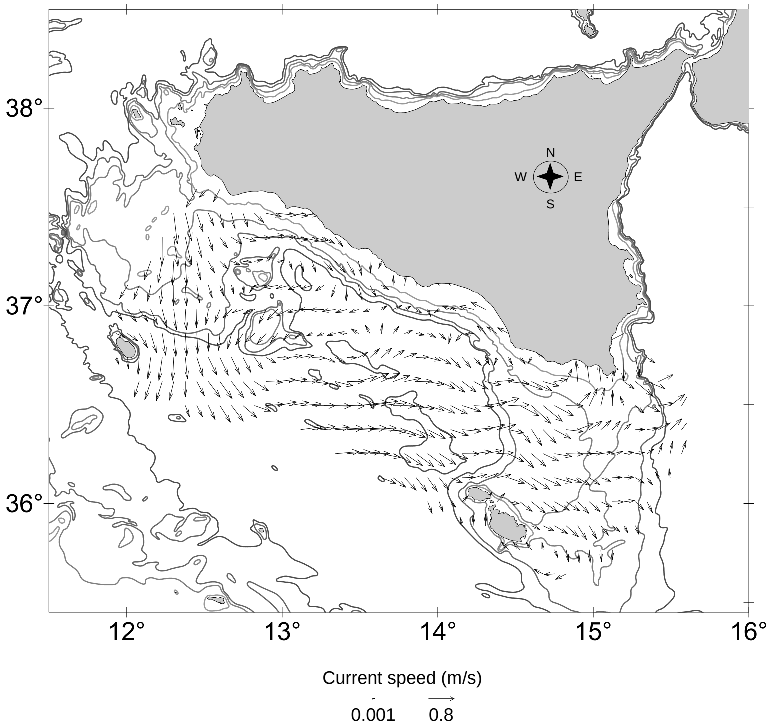


Figure 6: Surface geostrophic current pattern during July 2006.

Pozzallo, then heading southward (Figure 7).

4 Discussion

Distribution maps of the hauls, grouped in clusters showed similar patterns during all analyzed years. In fact, the most common spots we found were located under Pozzallo, Cape Passero and Siracusa, though there were small spots along the central shelf of the western coast. Distribution maps for the cluster with major abundance of Gonomastodae basically showed the presence of spots along the Egadi Islands and in the same sites where we found the Myctophidae. The distribution of cluster with presence of Phosichthyidae was along the central shelf of the western coast (from Porto Empedocle to Pozzallo) and under Cape Passero. During the sampling (July), the AIS skims along the Sicilian coasts only in the neighbours of Egadi Islands and Cape Passero, while there is a shift of the tide in the central part of the south-western coast. The trajectory of the identified AIS core suggests that the AIS was flowing offshore and only approached the shore near the edge of Sicily, over the Maltese Shelf. Path irregularities in the southern part of the Sicilian Channel might be caused by the very small differences in

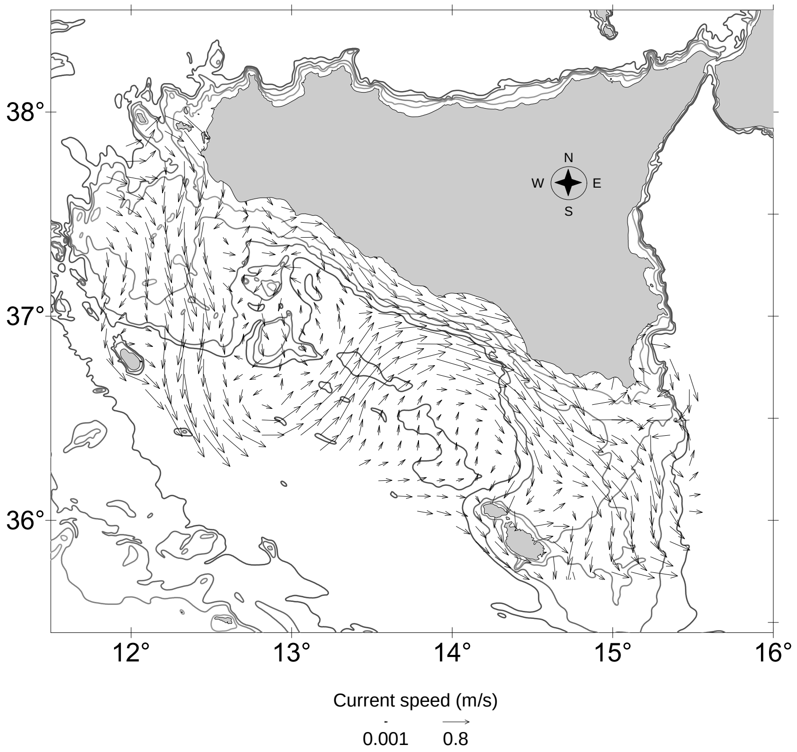


Figure 7: Surface geostrophic current pattern during July 2007.

the numerical value of the minimum salinity found in the different offshore legs, but also by interference with other small-scale oceanographic features that may be generated in the neighbourhood with a strong salinity front of Cape Passero. The higher salinity values along the south-eastern Sicilian coast reveal the presence of a well-developed coastal upwelling event. This is also confirmed by the temperature distribution, which exhibits a very large spatial variability.

5 Conclusions

Results showed the existence of different water masses on the surface, associated with the AIS path and with the hydrographic structures. AIS path presents year-to-year variability that has consequences on the predominant hydrological phenomena occurring in the region, such as the extent of upwelling and the formation of frontal structures [17].

These appear in turn to affect the distribution of mesopelagic species larvae observed. In fact, the presence of these families offshore would testify the bathypelagic environment of the adult fraction in their respective populations, whereas the occurrence of these species in shelf areas would be associated with the coastal upwelling characterizing the northern part of the study area.

In addition, the accumulation of larvae on the south-eastern edge could be related to the strong salinity front of Cape Passero.

The higher larval concentration of Cape Passero raises the question of whether there are physical reasons for defining it as a retention area. There, the presence of a surface thermohaline front linked to the Ionian slope fronts (ISFs) [18] would be able to provide a suitable scenario of enrichment, concentration and retention (the fundamental triad; [19, 20]), thus forming a favourable nursery habitat for larvae [21].

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Marine Hydrocarbonoclastic Bacteria: a New Perspective for Biotechnological Applications

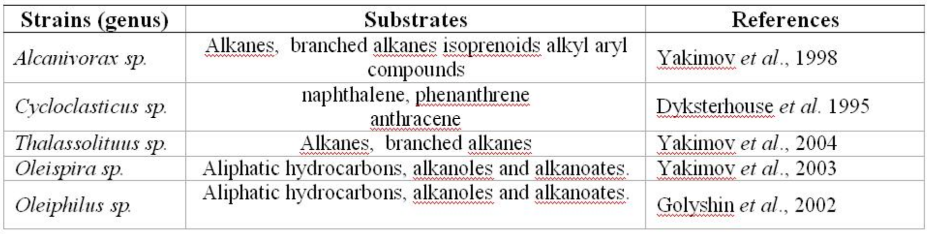
R. Denaro, S. Cappello, M. Genovese, L. Genovese, L. Giuliano,
M.M. Yakimov
Institute for Coastal Marine Environment, CNR, Messina, Italy
renata.denaro@iamc.cnr.it

Abstract

Over the past few years, a new and ecophysiologically unusual group of marine hydrocarbon-degrading bacteria, the obligate hydrocarbonoclastic bacteria (OHCB), has been recognized and shown to play a significant role in the biological removal of petroleum hydrocarbons from polluted marine waters. The introduction of oil or oil constituents into seawater leads to successive blooms of a relatively limited number of indigenous marine bacterial genera: *Alcanivorax, Marinobacter, Thalassolituus, Cycloclasticus, Oleispira* and a few others (the OHCB) which are present at low or undetectable levels before the polluting event. The types of OHCB that bloom depend on the latitude/temperature, salinity, redox and other prevailing physical-chemical factors. These blooms result in the rapid degradation of many oil constituents, a process that can be accelerated further by supplementation with limiting nutrients. Genome sequencing and functional genomic analysis of *Alcanivorax borkumensis*, the paradigm of OHCB, has provided significant insights into the genomic basis of the efficiency and versatility of its hydrocarbon utilization, the metabolic routes underlying its special hydrocarbon diet, and its ecological success. These and other studies have revealed the potential of OHCB for multiple biotechnological applications that include not only oil pollution mitigation, but also biopolymer production and biocatalysis.

1 Obligate marine hydrocabonoclastic bacteria

Marine DOC hydrocarbons-degrading bacteria are definitely rare, only 19 genera among Eubacteria and two strains belonging to Firmicutes and Bacteroidetes phyla have been identified. Among these, a group of obligate hydrocarbonoclastic marine bacteria (OHCB) show a very restricted range of substrates profile when assayed with BIOLOG® system, namely on Tween 40 and Tween 80, that contain long-chain alkyl moieties. This highly specialized substrate specificity obligate hydrocarbon utilization seems to be more characteristic for marine hydrocarbon-degrading bacteria than soil bacteria thus far, only three paraffin-degrading bacteria possessing similar narrow substrate profiles have been isolated from terrestrial environments [1, 2]. Since the description of the first OHCB, *Alcanivorax borkumensis* [3, 4], four further genera of OHCB, represented by seven validly published species, have been reported. The genera *Alcanivorax* and *Cycloclasticus* include both OHCB, like *A. borkumensis, A. jadensis*, [4, 5] *A.*



Strains (genus)	Substrates	References
Alcanivorax sp.	Alkanes, branched alkanes isoprenoids alkyl aryl compounds	Yakimov *et al.*, 1998
Cycloclasticus sp.	naphthalene, phenanthrene anthracene	Dyksterhouse *et al.* 1995
Thalassolituus sp.	Alkanes, branched alkanes	Yakimov *et al.*, 2004
Oleispira sp.	Aliphatic hydrocarbons, alkanoles and alkanoates.	Yakimov *et al.*, 2003
Oleiphilus sp.	Aliphatic hydrocarbons, alkanoles and alkanoates.	Golyshin *et al.*, 2002

Table 1: OHCB show one of the higher affinity towards hydrocarbons. The table show strains and substrates they consume.

dieselolei [6], *C. pugetii* [7] and *C. oligotrophus*, [8] and more nutritionally versatile species with less restricted substrate profiles, like *A. venustensis* and *C. spirillensus* [5, 9]. The three other genera of the OHCB group, despite being represented by numerous members, are monophyletic and represented by the three type species *Oleiphilus messinensis* [10] , *Oleispira antarctica* [1] and *Thalassolituus oleivorans* [11]. Moreover other two interesting genera, *Marinobacter* [12, 13] and *Neptunomonas* [14], consume limited carbon sources but prefer hydrocarbons. Table 1 show specific substrates for each OHCB strains.

1.1 Biogeography of OHCB

A recent study on the monitoring of microbial communities associated to marine hydrocarbons polluted areas revealed that OHCB have a global distribution (Figure 1) with the exception of *Oleispira sp.* which was detected in cold environment [15].
Alcanivorax-affiliated bacteria have been isolated or detected in surface water, shallow and deep sea water bodies, sediments [16, 17], hydrothermal vents and mud volcanoes, ridge flank crustal fluids and grey whale carcass [18, 19], in corals, sponges and aquaculture-poisoning dinoflagellates [20, 21].
Alcanivorax like organisms have also been detected in a few terrestrial environments that share relevant properties (salinity, presence of hydrocarbons) with marine ecosystems for example, a selenium-contaminated hypersaline evaporation pond in California [22], saline subsurface waters in Africa and Australia [23], hydrocarbon-polluted saline soil and various geothermal areas in Italy and USA (Yellowstone). This ubiquity of *A. borkumensis* presumably results from its versatility to use many saturated petroleum fraction and on biogenic hydrocarbons. Two other OHCB, *Talassolituus oleivorans* and *Cycloclasticus spp.*, are also widely distributed, although so far they have mostly been found in the Northern hemisphere (Figure 1), possibly due to sampling bias. The GenBank and RDP (Ribosomal Databse Project) databases currently contain 16S rRNA gene sequences of 59 *Thalassolituus*-like bacteria originating from microbial communities inhabiting both marine (Baltic, Barents, Mediterranean, North, Okhotsk and South China seas, and the Atlantic, Pacific and Polar oceans) and terrestrial environments (subsurface caves and ground waters) [24, 25] All of the 38

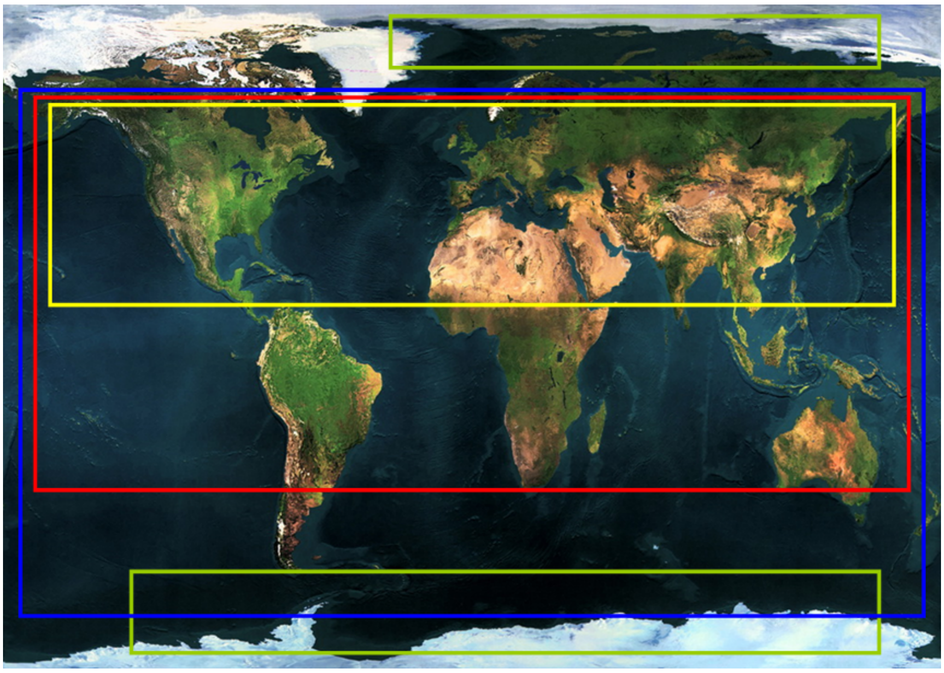


Figure 1: The marine areas where OHCB have been detected is represented by rectangles, namely, yellow sector is referred to *Cycloclasticus sp.* Red to *Alcanivorax sp.* Blue *Thalassolituus sp.* In Artic and Antarctica regions was retrieved *Oleispira antarctica* (green sectors).

sequences in the databases assigned to the genus *Cycloclasticus* were retrieved exclusively from marine microbial communities, or PAH-supplemented enrichments [26, 27]. In contrast, the distribution of the psychrophilic OHCB *Oleispira antarctica* (55 sequences to date) is thus far limited to the colder waters found at high latitudes [28, 29]: the least cold sites at which *O. antarctica* has been found are Cape Cod (MA, USA GenBank AM117931), and South Tasmania [30].

The most enigmatic marine OHCB, *Oleiphilus messinensis* [10], initially isolated from harbor sediments (Messina, Italy) seems to thrive elsewhere as a sponge symbiont, as *Oleiphilus*-like 16S rRNA gene fragments have been recovered from bryozoan and dictyoceratid sponges sampled in North Atlantic and equatorial Pacific oceans.[31, 32].

1.2 Genomic basis of OHCB ecophysiology

Marine OHCB occupy a special trophic niche among marine heterotrophic bacteria participating in the global carbon cycle, as they mediate degradation of chemically stable saturated and aromatic hydrocarbon species that are not substrates for most bacteria. However, a few studies have revealed unusual and interesting features of the marine OHCB lifestyle. Organisms analysed so far exhibit features typical of oligotrophic bacteria. The most detailed studies, carried out with *C. oligotrophus*, have shown that the outer cellular membrane is enriched for a wide range of transport systems for the capture of nutrients and diverse oligo-elements from the generally nutrient-poor marine environment [33, 34]. The affinity of *C. oligotrophus* cells for toluene is the highest microbial cell/ substrate affinity reported so far and is sufficient for bacterial growth in seawater containing hydrocarbons at exceptionally low concentrations [33]. Genomic analysis of the *A. borkumensis* strain SK2 has revealed a large repertoire of genetic determinants for the uptake of mineral nutrients that are limiting in marine environments, particularly following a sudden input of oil, which leads to severe imbalances in carbon/nitrogen and carbon/phosphorus ratios. This battery of genetic determinants for scavenging functions enables *A. borkumensis* SK2 to efficiently exploit its alkane catabolic functions in response to a sudden appearance of hydrocarbons and to adapt to carbon: nutrient imbalances that occur (e.g. after an oil spill). These functions could explain the competitive advantage *Alcanivorax* enjoys in such circumstances. The SK2 genome specifies multiple systems for hydrocarbon catabolism, namely two alkane hydroxylase systems AlkB1and AlkB2 and three P450 cytochromes [34, 35, 36, 37, 38]. A similarly ubiquitous genetic organization also exists for determinants specifying degradation of aromatic hydrocarbons. Analysis of the organization of gene clusters in *Cycloclasticus sp.* for the degradation of (poly)aromatic hydrocarbons revealed a cluster of six open reading frames xylXMKGC1C2 that specifies utilization of all three forms of xylene [39]. The cluster also exhibits similar organization to the catabolic determinants of the PAH degrading in freshwater. Sequence analysis of a 10.5 kb DNA fragment from texitCycloclasticus sp. A5 revealed a cluster of phn genes specifying degradation of naphthalene, methylnaphthalene, phenanthrene and dibenzothiophene.

The broad PAH substrate range characteristic of Cycloclasticus might reflect the existence of multiple dioxygenase determinants in the genome of this bacterium, analogous to the situation for alkane monooxygenases found in *Alcanivorax.* Interestingly, a third putative dioxygenase, homologous to the initial enzyme of the pathway for the degradation of 2,4-dichlorophenoxyacetic acid, has been detected in *C. oligotrophus.* This indicates that *Cycloclasticus spp.* might degrade not only diverse (poly)aromatic hydrocarbons, but also chlorinated derivatives thereof consistent with their ubiquity in marine environments.

2 Potential biotechnological applications

The exploiting of natural resources, with special attention to microorganisms, requires accurate knowledge on the physiology, genetic and ecology. Among OHCB, Alcanivorax spp. has been deeply studied because of the de-codification of its entire genome. For this reason next sections on biotechnological application will be mostly referred to *Alcanivorax spp.* Marine microrganisms which exhibit special properties receive research interest for their biotechnological potential. Especially for bacteria inhabiting extreme environment (for natural parameters or accidental stress conditions). In particular OHCB promise attractive perspectives for their exceptional physiological and genetic specialization.

2.1 Bioremediation

Bioremediation is a natural process carried out by microrganisms which result in the reduction or disappearance of pollutant. As an example an oil input in marine environments induces a response of microbial community which changes its composition towards a new structure dominated by hydrocarbons-degraders bacteria.

Environmental conditions play a crucial role on the promotion of biodegration, for instance, a typical limiting factor is the bioavailability of nutrients such as nitrogen and phosphorus.

Marine oil degraders are universally found in inshore waters, shelf seas, deep oceans including the abyss, beaches and bottom sediments. Together with OHCB (very specialised in the hydrocarbons consuming), there are bacteria belonging to a wide range of genera including *Vibrio, Pseudomonas, Acinetobacter, Achromobacter* and *Corynebacteria* which not consume hydrocarbons preferentially but seems to degrade it "accidentally" as cometabolism. Microcosm experiments have shown that an influx of oil in a seawater sample causes, after 10 days, population densities of OHCB to transiently increase up to 90% of the total microbial community in presence of nutrients.

Aliphatic hydrocarbon-degraders, in particular *Alcanivorax*, are the first to bloom and are succeeded by microbes, particularly *Cycloclasticus spp.*, specialized for the remaining compounds that are more difficult to degrade [40, 41, 16]. Yakimov and co-authors [42] studied community shifts in microcosms of superficial sediments from a chronically polluted area of the Milazzo Harbour oil refinery, Sicily. The samples were experimentally spiked with oil, tetradecane or naphthalene, with or without supplementation with mineral nutrients. Figure 2 shows the strong effect, operated by substrates, on the microbial community diversity and overall on the dominance of specific signatures which were undetectable in the natural sample. The clone library of the same samples showed that the initial community, was composed mostly of vibrios, low-GC Gram-positive bacteria, *Arcobacter spp.* and a few crenarchaea, became dominated by HCB after addition of hydrocarbons: whereas *Alcanivorax spp.* dominated the OHCB bloom in crude oil-spiked microcosms, *Thalassolituus spp.* dominated tetradecane-spiked microcosms and Neptunomonas-like microbes dominated naphthalene spiked microcosms. Similar results indicating the central role of OHCB, and *Alcanivorax spp.* in particular, were obtained in a mesocosm study involving a 14000 L bioreactor [43] Mckew et al.[24] observed microcosms of Thames salt marsh water from a site close to an oil refinery that were experimentally spiked with crude oil or oil constituents showed blooms of *Thalassolituus spp.* and *Roseobacter spp.*, *Alcanivorax spp.* was observed when samples were spiked with the branched aliphatic hydrocarbon pristine, and *Cycloclasticus spp.*, when spiked with PAHs. In the same occasion nutrients supplemented microcosms were bioaugmented with *Thalassolituus spp.* and *Alcanivorax spp.* enhancing the degradation rates up to 80% . Chemical analyses showed a clear correspondence between the reduction of hydrocarbons amount and the increase of OHCB spe-

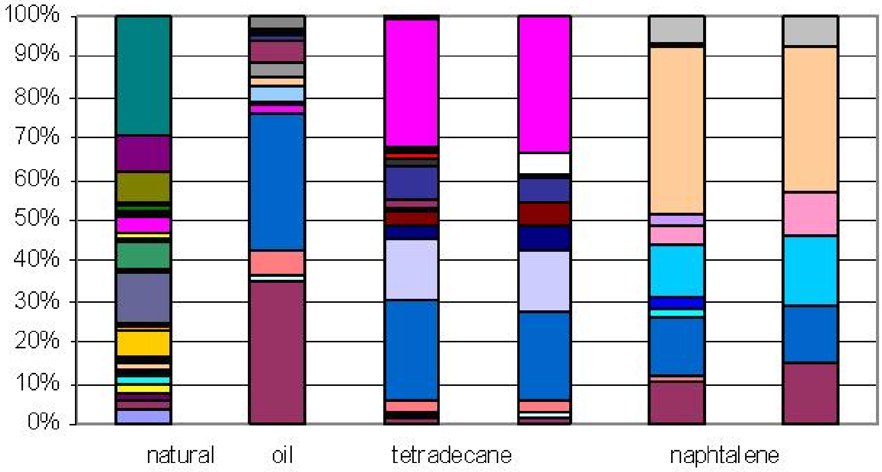


Figure 2: Microbial community diversity analysed by Terminal Restriction Fragment Polymorphisms (T-RFLP). In this case it is clear the selective effect of the substrates on the microbial diversity. The first column is the natural sample collected in a hydrocarbons polluted site near a refinery, the following columns are enrichments of the natural sample with hydrocarbons and nutrients (two column for tetradecane and two for naphthalene 1/100 - 1/1000 dilution).

cific population [24].

These and other studies in micro/mesoscale [43, 44] let hypotheses on the determinant role of OHCB in natural attenuation of hydrocarbons pollution and their potential application for the recovery of marine oil polluted areas, but it is still questionable whether results of small-scale laboratory experiments can be extrapolated to the field.

Field studies have shown that many of the features observed in laboratory microcosms are also observed under field conditions [45]. In beach sediments collected in the field, bacterial communities have limited diversity and are homogeneous in composition in space and time. The overall response of the bacterial community in the field to oiling and bioremediation treatments is variable, similar to the response in laboratory microcosms. In common with laboratory experiments, specific hydrocarbon degrading organisms (for example, *Alcanivorax spp.*) were selected in the field under conditions in which nutrient addition resulted in an increase in nutrient concentrations in interstitial waters [45].

Interesting, some broad similarity is observed in the dynamics of microbial communities in response to spilled oil and bioremediation, in particular, the selection for *Alcanivorax* and *Cycloclasticus spp.* seems to be a recurrent response.

Phylogenetic-based community dynamics tools associated with analysis of catabolic genes expression suggests that alkane-hydroxylase gene expression could be associated to Alcanivorax spp., activity. In fact there is some indication that different genotypes of *Alcanivorax* can become prevalent under conditions of high and low nutrient input [16]. Two distinct *Alcanivorax* genotypes were identified in laboratory and field oil-spill bioremediation experiments, and the patterns of the relative abundance of 16S-rRNA sequences in clone libraries from sediments that were treated with different amounts of nutrients indicate that the different genotypes are adapted to higher or lower concentrations of nutrients. Different *Alcanivorax spp.* have different physiological properties [3, 6], and the selection of different *Alcanivorax* genotypes during bioremediation might affect the kinetics and patterns of biodegradation that occur.

Although the pivotal role of OHCB in bioremediation in marine environment is confirmed by several studies, in nature, biodegradation is the results of cooperation and interaction within microbial community acting in concert or sequence, rather than as single strains. For example, Tagger et al.[46] point out that of 9 oil-degrading isolates from marine sediments, only 2 strains were able to grow on napthalene as sole carbon source, but the remaining 7 used metabolic intermediates. The importance of such trophic interactions and succession have to be interpreted not only for environmental recovery but also as a method for organic carbon balance and recycling. On this bases it is noticeable the effect of hydrocarbon-degrading predators. Studies in mesocosms revealed the presence of nanoflagelates which, not only tolerate the presence of oil, but seemed to feed in *Alcanivorax spp.* dominated microbial community.

Predators indirectly affect contaminant biodegradation, owing to their ability to selectively graze on, and control the biomass of bacteria. The influence of protozoa on biodegradation is often negative, because it decreases the number of degrading bacteria in comparison to the situation in the absence of predators [47]). However, predation has been shown to stimulate bacterial degradation of toluene and benzene considerably on a per-cell basis [48, 49]. In addition, predation creates a nutritional loop, because predators can re-mineralize nutrients, which in turn increases bacterial growth.

The knowledge on the use of hydrocarbons-degraders as an alternative intervention to the chemical one lacks of theoretical basis to underpin and predict the behaviour and interactions of hydrocarbon-degrading bacteria in situ. Further studies are required to elucidate the biochemistry, genetic basis and regulation of hydrocarbon-degrading pathways. Nevertheless experiments carried out in micro-meso/scales simulate the natural responses of the marine microbial community to an oil spill event, many questions remain about the dynamic of hydrocarbons-degraders and their predators, the succession of degradation pathways, how bacteria compete for substrate utilization (special capturing and transport systems, toxic metabolic intermediates, antibiotic production).

The needs on bioremediation studies are related to field application of OHCB in consortium with other hydrocarbons degraders and the optimization of intervention strategy in terms of quantity of biomass, nutrients, surfactant monitoring environmental parameters also.

2.2 Bioproducts: Biosurfactant applications to increase bioavailability

Biosurfactants are surface-active microbial products that have numerous industrial applications.

The production of biosurfactants facilitates emulsification of hydrophobic organic substrates, enhances their bioavailability and increases the degradation. Many microorganisms, especially bacteria, produce biosurfactants when grown on water-immiscible substrates (*Rhodococcus, Acinetobacter, P. aeruginosa*). Most common biosurfactants are glycolipids in which carbohydrates are attached to a long-chain aliphatic acid, while others, like lipopeptides, lipoproteins and heteropolysaccharides, are more complex. The most promising applications of biosurfactants are in the cleaning of oil-contaminated tankers, oil-spill management, transportation of heavy crude, enhanced oil recovery, recovery of oil from sludge and bioremediation of sites contaminated with hydrocarbons, heavy metals and other pollutants [50, 51]. *Rhodococcus sp.* 094 can oxidize alkanes in crude oil emulsified by nonionic chemical and biological surfactants Iwabuchi et al. [52] observed that in oil-polluted sea water supplemented with nitrogen, phosphorus and iron, significant biodegradation of an aromatic fraction of crude oil occurred only when extracellular polysaccharides from Rhodococcus rhodochrous were present. The extracellular polysaccharides resulted in emulsification of oil, followed by changes in the composition of the bacterial community, with *Cycloclasticus spp.* becoming dominant [53]. *A. borkumensis* forms stable emulsions of hydrocarbon in water and produces biosurfactants [54]. These biosurfactants are anionic glucolipids carrying four fatty acids of varying chain lengths. The genetic organization of the glucolipid biosynthesis remains unclear in *A. borkumensis* SK2, but genome annotation revealed candidate genes potentially involved in biosurfactant production. ABO_1783 and ABO_2215 encode glycosyltransferases, exhibiting significant homology with RhlB from *Pseudomonas aeruginosa* and glycosyltransferase protein family, respectively. These gene products possibly provide the sugar moiety of the glucolipids, yielding glucose lipid surfactants. *A. borkumensis* SK2 also expresses an OprF/OmpA protein encoded by ABO_0822, which is upregulated when grown on alkane

2.3 Bioproducts: Polyhydroxyalkanoic acids (PHAs)

One of the currently overcome of modern society is the production of environmentally friendly materials. This is particularly thru for the plastic objects which have a deleterious environmental impact. Polyhydroxyalkanoic acids (PHAs), are natural polymers whose production/degradation cycle reduces undesirable wastes and emissions.

PHAs are extremely versatile polymers as their crystalline aspect can be manipulated to provide a broad range of mechanical and barrier properties, in some cases matching the performance of engineered thermoplastics. PHA biodegradable polymers are still largely at the development stage of market development, although there a few commercial applications available.

These polymers are synthesised as storage substances by bacteria in specific conditions of nutrients and carbon source. For carbon-limited microbes, an increase in carbon allows an increase in growth rate until another growth limitation is reached.

The first survey of PHA was observed on *Bacillus megaterium* which produced an intracellular polymer of hydroxybutyrate monomers. In the following years biopolyesters with longer side chains (medium-chain-lengthPHAs [mcl-PHAs]) were also described in *Pseudomonas putida* GPo1 growing on alkanes [55]. Moreover has been detected in some archaea and a wide range of gram-positive and gram-negative bacteria in aerobic and anaerobic environments. Although there are many microorganisms that are able to produce PHA, mcl-PHA production had been repetitively restricted to Pseudomonas strains [55, 56, 57, 58, 59].

The appearance of alkanes in oligotrophic environments like most marine habitats allows *Alcanivorax* to "bloom" initially until nitrogen limitation is experienced. Under conditions of high C/N ratios, many microbes synthesize carbon storage materials, like polyhydroxyalkanoates (PHAs). ABO_1418, one of two *A. borkumensis* phaC PHA synthase genes, was solely expressed in alkane-grown cells, whereas another, ABO_2214, was not expressed at detectable levels in such cells. Since PHA is also produced at high C/N ratios in cells grown on non-alkane substrates (Sabirova et al., unpublished), it seems that *A. borkumensis* produces one of two distinct PhaC PHA synthases in response to different growth substrates, probably having different substrate specificities that reflect distinct metabolites produced from the different growth substrates.

A recent functional genomics study of *A. borkumensis* SK2 revealed that inactivation of a 'TesB'-like hydroxyacyl-coenzyme A-specific thioesterase leads to hyperproduction and extracellular localization of PHAs , even though the normal food storage compounds found in this strain and other OHCB (*Marinobacter* and *Thalassolituus*) are triacylglycerols and wax esters. The extracellular localization of PHA is an exceptional novelty both because of the extraction technique and the amount of the production.

Currently, other potential applications are being assessed, including environmental and medical applications, such as the use of PHAs in wastewater denitrification bioreactors, in which they serve as both the source of reducing equivalents for denitrification and asthe matrix for biofilm formation [60], and as biocompatible materials for implants, scaffolds for tissue engineering, and drug carriers [61, 62].

2.4 Bioproducts: Enzymes

Metabolic specialization and biotechnological potential *A. borkumensis* SK2, like other hydrocarbonoclastic bacteria, has a limited substrate range for its growth. The absence of a functional PEP-dependent sugar/phosphotransferase system or other types of sugar transporters, as well as the lack of several determinants for key enzymes of the glycolytic, pentose phosphate shunt and Entner-Doudoroff pathways, is consistent with the inability of *A. borkumensis* SK2 to utilize simple hexoses and other simple carbohydrates for growth. The *A. borkumensis* SK2 genome encodes a number of proteins putatively involved in metabolic reactions of potential biotechnological interest, including eight hydrolases of the haloacid dehydrogenase/epoxide family, two determinants for dienelactone hydrolases(ABO_1618 and ABO_1886), three for deacylases, 36 for variouscytochrome proteins and 30 for oxidoreductases. Various oxidoreductase genes are clustered together or in operon-like structures with determinants for monoxygenases, aldehyde dehydrogenases, decarboxylases, Fe-2S family proteins, transcription regulators and/or transporter-coding genes. The genome also includes 11 genes coding for different lipases/esterases of unknown specificity. Two of these esterases were purified and functionally characterized. They exhibit excellent enzymatic activity up to two orders of magnitude higher than typical esterases, have a wide substrate profile, remarkable enatioselectivity and chemical resistance, which underscores their potential for the resolution of chiral mixtures in biocatalysis (M.F. et al., unpublished data). Thus, overall, *A. borkumensis* SK2 exhibits a biotechnological potential that goes well beyond its application to marine oil degradation.

2.5 Genetic potential

Chaperonins are ubiquitous proteins in bacteria, archaea and eukaryotic mitochondria and chloroplasts, engage in important activities, including their ability to fold over 30% of cellular proteins under conditions in which spontaneous folding cannot occur, or assist proteins duringfolding. The cpn60 and cpn10 genes from psychrophilic bacterium, Oleispira antarctica RB8, showed a positive effect in *Escherichia coli* growth at low temperature, shifting its theoretical minimal growth temperature from +7.5°C to-13.7°C. It is noteworthy that, if the temperature range of growth of organisms generally prove to be modifiable by recruitment of heterologous chaperones, this could become a generic means of altering their biogeography and of making them more robust either for a wide range of environmental application that are subject to climate-related fluctuations in temperature (waste treatments, bioremediation, microbially mediated plant growth promotion and protection, retting, biomiming, etc) or for biotechnological processes at temperature that are stressful for the organisms used.

2.6 Biosensor

Traditionally, pollution risk assessment is based on the measurement of a pollutant's total concentration in a sample. The toxicity of a given pollutant in the environment, however, is tightly linked to its bioavailability, which may differ significantly from the total amount. Physico-chemical and biological parameters strongly influence pollutant fate in terms of leaching, sequestration and biodegradation. Bacterial sensor reporters, which consist of living microorganisms genetically engineered to produce specific output in response to target chemicals, offer an interesting alternative to monitoring approaches. Since the high specialization of OHCB on the use of hydrocarbons we expect to find their as a target in case of oil spill. Moreover since their activity is carried out if the pollutant is bioavailable and/or bioaccessible, the survey of OHCB presence and activity could be associated to a process of degradation in real time. The enzymatic system that could be useful for this application is the alkane hydroxylase (previously described) which is involved in the degradation of m-chain alkanes. Proteomic profiling suggested that both AlkB systems and all three cytochromes P450(a–c) participate in catabolism of saturated hydrocarbons. Quantitative real-time transcriptional analysis showed that the P450(b) and P450(c) genes, which encode identical polypeptide sequences, were expressed only in the presence of alkanes, whereas P450(a) was also expressed in cells growing on pyruvate. Similar expression profiles of the alkB1, alkB2 and cytochromes P450 (a, b, c) genes were observed in exponential cultures growing on n-tetradecane. Interestingly, in phytane-grown cells, strong induction of the cytochrome P450(a) gene was found, but no transcription of alkB1. Such a differential expression of these two genes could be useful in the application of *A. borkumensis* gene transcription profiling as a possible bio-indicator of oil pollution.

3 Conclusions

Advancements in the study of physiological and genetic potential of hydrocarbonoclastic bacteria can reveal other biotechnological applications or improve adaptation of mechanisms already known to the specific requirements. In particular the needs are related to the whole genome sequencing of other strains OHCB as *Cycloclasticus sp.* which, at the moment, is the unique known marine PAH-degrader whose aptitude cover a wide range of PAH, as naphthalene, ethylnaphthalene, phenanthrene, dibenzothiophene. The analysis of the structure and function of the genes and the enzymes, which they encode will be determinant for the clarification of the degradation process. In fact little is known about the set of functional genes involved and their regulation.

Moreover, the response to environmental restrictions (as substrates, nutrients, iron, temperature etc.) is very different among OHCB as they exhibit different capability to use quite a few substrates or nutrients in a competitive manner. To this end, the investigation in depth of molecular tools of OHCB in the perception of the presence of hydrocarbons, activation of degradation process, cell-to-cell communication (quorum sensing) are important issues for the management of practices in bio-monitoring (biosensors development) and in the exploiting of natural attenuation for the recovery of marine polluted areas.

A third point which is related to the behaviour of individual functional members of such networks will also need to be assessed, as nicely exemplified by a recent case study on the anaerobic biodegradation of organic matter by a complex microbial community. For such modelling, statistically robust data must be obtained from real-time measurements in large-scale simulations. Moreover, new knowledge is urgently needed on the critically important activities and roles of predators and grazers (e.g. viral, prokaryotic and eukaryotic) on the composition, population dynamics and ecophysiological functioning of marine oil-degrading communities, and the role of lysogenic phages in their functioning, adaptation and evolution.

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