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#### AQUATIC CONSERVATION: MARINE AND PRESHWATER ECOSYSTEMS

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### Mucilages and climatic changes in the Tyrrhenian Sea

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#### ABSTRACT

- 1. Pelagic mucilage aggregates are composed of phytoplankton and bacteria, whilst macro- and micro-phytobenthos and bacteria make up the benthic ones. During the summer of 1991 massive mucilage aggregates, both pelagic and benthic, appeared in the Tyrrhenian Sea, seriously damaging fisheries, tourism and the benthic biocoenoses. Only a few, loose benthic mucilages have persisted during the last decade although the pelagic ones have shown a great resurgence during the last autumn.
- 2. <sup>14</sup>C autoradiographies show that the mucilage is photosynthesized and excreted by the microand macroalgae it contains. The mucilage aggregates are a microecosystem with biological and chemical composition, and a functionality that is different from the surrounding waters. The mucilages cannot be the result of a gelification of the Dissolved Organic Matter (DOM) in the water trapping the free algae present in the water.
- 3. Since 1991 up to the present, variations of Dissolved Inorganic Nitrogen (DIN) and Dissolved Inorganic Phosphorous (DIP) and of their ratio have been observed in the surface waters. These variations could be assumed as the cause of mucilages, however, the mucilage occurrence cannot be directly related to the change of nutrient concentrations and ratios because: i) the higher N/P ratio was persistent also during years without mucilages; ii) nutrients and their ratios have a continuous spatial distribution while mucilages have a discontinuous spatial and temporal distribution. It is suggested that mucilage aggregates are the result of a hyperproduction as a pathological response of the algae to a microbial infection. This hypothesis is also supported by some laboratory experiments.
- 4. The observed trophic changes in the surface Tyrrhenian waters have been related to the Levantine Intermediate Waters (LIW) as they are the main source of nutrients for the surface ones. Data collected from several areas between 1982 and 1998 show that changes in the temperature, salinity and nutrient concentrations and ratios took place in the LIW, so changing the Tyrrhenian climate.

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KEY WORDS: bacterial infection; Levantine Intermediate Waters; mucilages: N/P ratio; nutrient deficiency; Tyrrhenian climate change

#### INTRODUCTION

Mucilages heavily affect marine ecosystems, mainly when the massive development of aggregates covers large areas of the rocky substrate, suffocating and destroying benthic biocoenoses. Furthermore, fisheries

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and tourism are seriously damaged. A wide literature of laboratory and field studies about mucilages is available, but little is still known about the factors triggering the phenomenon (review in Vollenweider and Rinaldi, 1995). There is laboratory evidence that many environmental stresses cause the hyperproduction of exudates (high irradiance, temperature, nutrient availability). Nutrient deficiency is one of the main causes thus far investigated, particularly when the N/P ratio shifts toward the phosphorus deficiency (Myklestad, 1977; Jensen, 1984; Fogg, 1990; Myklestad, 1995). Biological causes too have been hypothesized: viral or bacterial infections could lead, more or less directly, to the abnormal production of mucilages (Innamorati, 1992; Murray, 1995; Azam et al., 1999).

In the Tyrrhenian waters both benthic and pelagic mucilages occurred, although these differed not only in their location but also in their biological features (Innamorati, 1995). Massive mucilages excreted by planktonic and benthic marine algae in the Tyrrhenian Sea have been observed since 1991, but previous observations of scuba divers and fishermen oral tradition reveal their presence in earlier decades too (Innamorati, 1995). The Tyrrhenian phenomenon was readily recognized as the same that occurred in the Adriatic Sea, where it was well known since 1800 (Fonda Umani et al., 1989). From analyses of the relationships between the trophic status of the water inside and outside the mucilages, there was evidence that during 1991 the N/P ratio was higher (> 30) than in the previous years, both in the surface (0-100) m) and intermediate (> 100 m) waters (Innamorati et al., 1993, 1994, 1995b). The Northern Tyrrhenian waters were previously characterized by a general nitrogen deficiency, showing a quite different pattern from the eutrophic northwestern Adriatic waters (Innamorati and Giovanardi, 1992; Innamorati et al., 1995a). These results led us to re-examine all our physical and chemical data collected in the Northern Tyrrhenian from 1982 to 1998, to recognize if some trophic changes really occurred (Innamora et al., 1998). Furthermore, as the Levantine Intermediate Waters (LIW) constitute the main source of nutrients for surface Tyrrhenian waters; river and terrestrial runoff and the oligotrophic North Atlantic Waters (NAW) contribute very little, it was supposed that any trophic change would be caused by a more general change in the LIW. To verify this hypothesis, we focused our attention on the layer 200 1000 m (Melley et al., 2000), examining data from the last two decades and from different Mediterranean areas.

In this paper we summarize our results on Tyrrhenian mucilages production together with a synthesis of our researches on the temporal trend of trophic conditions in the Mediterranean waters.

#### MATERIAL AND METHODS

Benthic mucilages were collected by scuba divers in different sites of the Archipelago Toscano (Capo d'Uomo dell'Argentario, August 1991: Montecristo Island, August 1991, July August 1992, July 1993: Formiche di Grosseto, August 1991, July-August 1992, July 1993 and from May to October 1995). Pelagic mucilages have been collected at Procida Island (September 1991, 1992; October 2000) from nets (300 × 20 m) left in the sea overnight, by wringing them out and dripping them in a bucket. On each cruise Conductivity Temperature Depth (CTD) profiles were measured (Idronaut Ocean Seven). In the samples of both mucilages and water column, the latter collected with Niskin bottles, the analysis of nutrients (nitrites plus nitrates and orthophosphates), phytoplankton biomass (chlorophyll) and composition (inverted microscope counting), Coloured Dissolved Organic Matter (CDOM) concentrations and <sup>14</sup>C incubations for primary production measurement (Riemann and Möller Jensen, 1991) and autoradiography (Becciolini and Balzi, 1992), were performed following standards methods.

For the analysis of the thermohaline and trophic conditions in the Tyrrhenian Sea and in the LIW of different Mediterranean areas, data collected during 28 cruises have been examined (Table 1). At first we analysed temperature, salinity and nutrients in the layers 0 99 m and 100-400 m from 16 cruises (Table 1) carried out in the Tyrrhenian Sea from 1982 to 1995; subsequently, we took in account the layer 200-1000 m (LIW) from cruises until 1998 and from other data available from the Mediterranean Sea

Table 1. Sampling period and area of the cruises. The number of samples used in this paper are indicated

Cruises	Sampling period	Area	Samples used		
			0 400 m	200 1000 m	
Marsili 82ª	6/6 19/1982	Southern Tyrrhenian		87	
		Sicily Channel			
		Western Ionian			
Elbal	10/2 8/1982	Archipelago Toscano	74		
Elba2	7/5–12/1983	Corsica Channel	223	29	
		Archipelago Toscano			
ATT1	9/3 12/1985	Corsica Channel	154	21	
ATT2	11/21 28/1986	Corsica Channel	131	18	
		Archipelago Toscano			
ATT3	3/12-23/1987	Corsica Channel	216	30	
		Archipelago Toscano			
ATT4	4/6 18/1988	Corsica Channel	1 <b>7</b> I	27	
		Eastern Ligurian			
ATT5	7/5–11/1988	Eastern Ligurian	166	14	
ATT6	11/22-12/4/88	Corsica Channel	17 <b>1</b>	8	
		Eastern Ligurian			
ATT7	2/15 3/2/1989	Corsica Channel	250	15	
		Eastern Ligurian			
ATT8	7/25 8/3/1989	Corsica Channel	91	14	
Tempot <sup>b</sup>	9/22 10/9/1989	Northern Tyrrhenian		49	
Tempo2 <sup>b</sup>	2/16-23/1990	Northern Tyrrhenian		48	
Arcil	6/19-7/2/1990	Archipelago Toscano	92		
Arci2	5/7 17/1991	Archipelago Toscano	100	4	
		Corsica Channel			
Arei3	9/3-22/1991	Corsica Channel	69	10	
		Northern Tyrrhenian			
POEMBC 091°	10/1991	Western Ionian		99	
MUCINUTRI*	12/28/91 7/28/92	Archipelago Toscano	30		
MARET	5/26-6/1/1993	Northern Tyrrhenian	63	23	
EOCUMM94	7/7-12/1994	Southern Tyrrhenian		26	
EOCUMM95	7/22 28/1995	Southern Tyrrhenian		34	
ARCIPICO*	3/16 -12/6/1995	Archipelago Toscano	77		
SYMPLEX96	4/15-5/15/1996	Sicily Channel		76	
SYMPLEX97	7/20-8/10/1997	Sicily Channel		27	
SALE2	8/14 25/1997	Southern Tyrrhenian		79	
	•	Sicily Channel			
		Western Ionian			
SYMPLEX98	3/27-4/20/1998	Sicily Channel		23	
NUGLOB*	6 9/1998	Archipelago Toscano	20	14	
Fronti 98	7/14 26/1998	Western Mediterranean		7	

<sup>&</sup>quot; See Faranda (1985).

(Table 1). Vertical profiles of temperature and salinity were performed with multiparametric probes (Idronaut Oc. Seven, KMS Meerestechnick, Neil Brown). The samples for analysis (Strickland and Parsons, 1972) of dissolved nitrogen (nitrites plus nitrates) and phosphorus (orthophosphates) were collected with Niskin bottles at fixed depths (0, 10, 25, 50, 75, 100 m and every 100 m until the bottom).

<sup>&</sup>lt;sup>b</sup> Cruise carried out by ISDGM (CNR) and CRAM (ENEA), La Spezia (Astraldi et al., 1995).

Cruise carried out by CNR, Trieste (Civitarese et al., 1996).

<sup>\*</sup> Two stations monthly sampled.

For the cruises carried out by other research groups, see Faranda (1985), Astraldi *et al.* (1995), Civitarese *et al.* (1996). The identification of the LIW was made from CTD vertical profiles, T-S diagrams and nutrient concentrations. From the total of stations sampled ( $\sim 2000$ ) those with a bottom depth of < 100 m and affected by rivers input were discarded, the arithmetic means of nutrient concentrations and N/P ratios are for the samples listed in Table 1.

#### RESULTS

The first signs of Tyrrhenian benthic mucilages were visible in spring, with the development of isolated and discontinuous metaphytic aggregates (Round, 1981), dominated by the filamentous algae Nematochrysopsis marina (= Tribonema marinum J. Feldmann) and/or Acinetospora crinita (Carmichael) Kornmann, and benthic pennate diatoms (Sartoni and Sonni, 1992; Innamorati et al., 1995b). Afterwards the mucilage spread out over the rocky bottom, reaching a maximum expansion and covering the erectile organisms at the height of summer. In the same period (July-August) mucilages showed the peak of chlorophyll, orthophosphates, total phosphorus, CDOM concentration and cell density (Melley et al., 1998). After the largest development, the mucilages buried the benthic biocoenoses under a layer of degrading material, which afterwards changed into a fine light-grey powdery layer covering the bottom with dead benthos remnants (Cincili, 1992).

Even assuming that the mucilage incorporates debris and organisms from surrounding waters, the autoradiography revealed (Plate 1) that the phytocoenose inside is the mucilage producer. Radioactivity both inside and outside of the cells showed a dense and insoluble mucopolysaccharides matrix (confirmed with alcyan blue PAS reaction) spreading out from the cell towards the surrounding medium, synthesized after the <sup>14</sup>C assimilation and then excreted.

The biological and trophic characteristics inside the mucilages (Table 2) are quite different from the surrounding waters (Innamorati *et al.*, 1995b; Melley *et al.*, 1998). The biomass, the carbon production and the cell density were higher, while the carbon assimilation per biomass unit (C/Chl or C/cell) was lower. Nutrient concentrations were higher too, mostly phosphorus, while the N/P ratio was lower. The ratio was lower than the Redfield's one (N/P = 16) while, in the pelagic environment during 1991, a higher N/P ratio ( $\gg 16$ ) was noted, if compared to the previous data obtained until 1990 in the Northern Tyrrhenian waters (Innamorati *et al.*, 1992, 1995b), when the ratio was  $\ll 16$ . The variation of the mean values of nutrients and N/P ratio from 1982 to 1995 (Figure 1), showed the evident decrease of phosphorus, the increase of nitrogen and the consequent increase of N/P from 1991, not only in the surface but also in the deeper layers. The trend of N/P ratio showed a nitrogen deficiency (N/P = 6.1) in the layer 0–99 m and a higher value (N/P = 19.9) in the deeper waters (100–400 m) until 1990. In the following years the data were distributed towards higher mean values (N/P = 19.6 in the layer 0–99 m and N/P = 36.0 in the layer 100–400 m), revealing the shift to phosphorus deficiency.

The summer physical structure of the water column before and after 1990 (Figure 2) was different with higher salinity in the surface layers after 1990 and the increase both in temperature (0.6°C) and salinity (0.15‰) of the LIW (Figure 2(b)). Furthermore, examining the nutrient concentrations from 1982 to 1995 vs. temperature in the LIW (Figure 3) there was evidence that the phosphorus decreased and the nitrogen increased, following the temperature increment and causing consequently the increase of the N/P ratio.

In Figure 4 the means of nutrients and N/P ratio obtained from the samples of the LIW of various Mediterranean areas (Table 1), vs. time are shown. Dissolved nitrogen (Figure 4(a)) showed an initial increase at the end of the 1980s followed by a decrease, similar to the phosphorus (Figure 4(b)). The effect of these fluctuations on the variation of N/P ratio (Figure 4(c)) results in an increase until the mid-1980s before stabilizing at a relatively high range.



Plate 1. Autoradiographies of a pennate diatom (a) and of Nematochrysopsis marina (= Tribonema marimum J. Feldmann) (b).

Table 2. Biological and	I trophic characteristics of th	e Tyrrhenian mucilages (N	4) compared with the	surrounding water (W)"

Characteristics		Montecristo		Procida		Formiche		Formiche 1995*	
		w	M	W	M	W	М	W	М
Chlorophyll Cell density	mg m <sup>-3</sup> cell litre <sup>-1</sup>	$0.047$ $8 \times 10^{4}$	11.09 8×10 <sup>6</sup>	0.182 $2 \times 10^{5}$	510 6×10 <sup>8</sup>	0.075 1 × 10 <sup>5</sup>	$33.87$ $3 \times 10^7$	$0.263$ $6.5 \times 10^4$	24.65 8 × 10 <sup>6</sup>
Chl./cell	pg cell <sup>1</sup>	0.59	1.42	0.96	0.79	0.72	1.30	4.03	5.50
Phaeop./Chl	9/6	$\sim 0$	18	$\sim 0$	32	~0	56	40	46
C production	mg m <sup>3</sup> h	0.13	14.30	0.79	173.00	0.46	48.30		
C/Chl	h T	2.82	1.29	4.30	0.34	6.13	1.43		
C/cell	pg cell h	1.66	1.80	4.20	0.27	4.40	1.86		
$NO_2 + NO_3$	μM	0.55	5.13			0.46	1.03	0.545	0.854
PO <sub>4</sub>	μ <b>M</b>	0.02	12,84			0.02	0.07	0.039	0.481
N/P	-	33.14	0.63			29.50	1.41	15.43	1,78

<sup>&</sup>lt;sup>a</sup> Chl = chlorophyll. Phaeop, = phaeopigments.

#### DISCUSSION

The results show that the producers of mucilage are the same algae, that are contained inside them (Plate 1; Table 2). The unicellular phytoplankters, during excretion, aggregate among them, or with filamentous algae, to form respectively the pelagic or the benthic mucilages. The latter must be considered as the metaphyton defined by Behere (Round, 1981) in order to distinguish the assemblage of cells kept around a macroalga by mucilage from the epiphytic cells firmly attached to a macrophyta. Both in the benthic and in the pelagic form the individuals (micro and macrophyta) and their relationships inside mucilage, constitute a true phytocoenose and can be named metaphytocoenose (or mucophytocoenose). They are a quite different system from the surrounding water, as the biological composition, the physical and chemical properties and the activities of production, decomposition together with the presence of massive numbers of bacteria indicate.

The abnormal mucilages hyperproduction, the high bacterial activity inside the aggregates and their patchy distribution, emphasizes the hypothesis of a biological cause, a microbial infection, as the triggering factor inducing the pathological response of the enhanced mucilages excretion (Innamorati, 1992, 1995). Some preliminary experiments (Innamorati *et al.*, 1996) on axenic phytoplankton cultures show that the majority of the cultures inoculated with filtrates from mucilages produced mucopolysaccharides. Therefore the role of bacteria for mucilage production must be clarified by further investigations.

The increase of the N/P ratio in the Tyrrhenian surface waters with the abnormal mucilage formation may suggest the relationship between the two phenomena. But, while the distribution of dissolved nutrients is continuous, mucilages have spatially and temporally discontinuous distributions. Furthermore, it is known that in the Adriatic Sea the mucilages are a sporadic recurrent phenomenon also scientifically well documented since the latest 1880 and during 1900. In the last 30 years the Adriatic waters have been always characterized by phosphorus deficiency as have the Tyrrhenian waters from 1991 up to the present. Therefore, since in the sea the mucilages have always been found with high N/P ratio — but more often, high N/P ratio can be found without mucilages — it seems that in nature the P deficiency, can be considered as a necessary condition for the appearance of mucilages, but not the only factor.

<sup>\*</sup> Means of monthly sampling from May to October 1995.

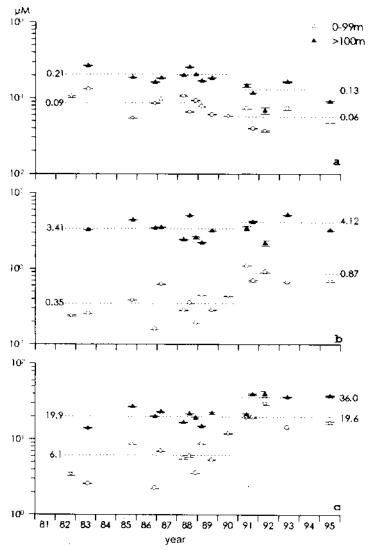


Figure 4. Mean concentrations of P-PO<sub>4</sub> (a), N-(NO<sub>5</sub> + NO<sub>3</sub>) (b) and mean N P values (c) in all the cruises carried out in the Northern Tyrrhenian Sea and in the two layers, General means for the periods 1982–1990 and 1991–1995 are indicated.

The recent Tyrrhenian phosphorus deficiency (since 1991, Nair *et al.*, 1994), is quite different from that of Adriatic. In the latter, the high N/P ratio is a chronic characteristic also related to the high nutrient supply from rivers. The increase of the N/P ratio in the surface Tyrrhenian waters appears independent from anthropic sources and related to the increase of N/P in the LIW due to a variation of dissolved nitrogen, phosphorus and N/P ratio and related to temperature variations.

Detailed examination of the spatial distribution of temperature, salinity, nutrients and N/P ratio in the core of the LIW from 1982 up to 1998, was carried out in the different Mediterranean areas (Table 1). The variation is mostly evident between 1991 and 1995, even though it is complicated by the superimposition of temporal and spatial variability. Nevertheless, the great number of data allows us to hypothesize a thermohaline and trophic change along a temporal scale (Figure 4), produced in the LIW that, originated in the Levantine basin and circulating from Eastern to Western Mediterranean, could affect surface and intermediate layers all over the Mediterranean Sea.

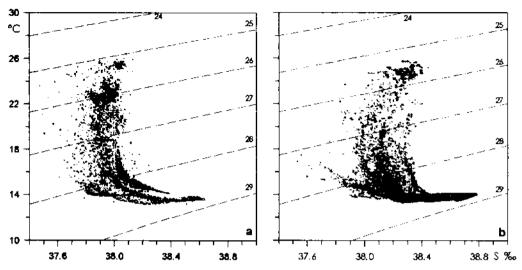


Figure 2. T-S diagrams (γ<sub>t</sub> is also reported). Data from 1982 to 1995 summer cruises; (a) before 1990 (June September); (b) after 1990 (May September). S‰ = salinity.

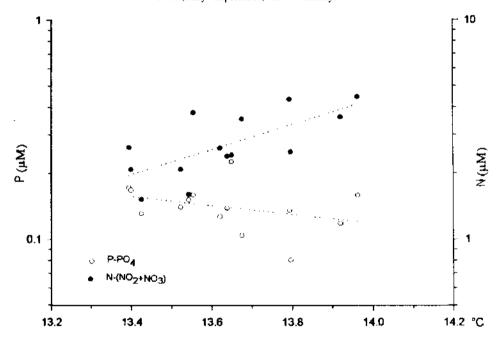


Figure 3. Nutrient concentrations vs. temperature in the LIW in the Northern Tyrrhenian Sea during 1982–1995. Correlation coefficients (r) of N and P regressions are respectively 0.69 and -0.35.

It seems possible to relate the transient variation suggested by the shape of the fluctuations (Figure 4) and mostly evident in the Central Mediterranean, to the change that occurred in the Levantine Basin between 1987 and 1995 (Roether *et al.*, 1996). In these waters there was the sinking of denser waters and the consequent raising of the deep and intermediate layers, with a perturbation of the water circulation at a wider spatial scale (Malanotte-Rizzoli *et al.*, 1999) that could affect the characteristics of the Tyrrhenian Sea.

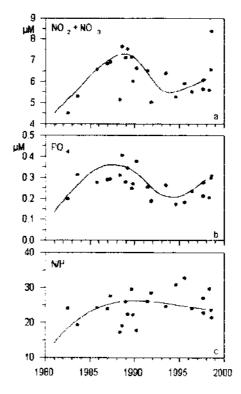


Figure 4. Temporal distribution of the means of N-(NO<sub>5</sub> + NO<sub>5</sub>) (a), P-PO<sub>4</sub> (b) and of the N P ratio (c) in the LIW from various Mediterranean areas. (Manual fitting curves).

No direct relationship exists between mucilage invasion and the observed changes in marine environment, unless it is that they are natural events without anthropic interference. The challenge for nature conservation is that of the preservation of all environmental conditions, as they are or can be, as are necessary for humankind. The natural destruction of ecosystems must be stopped: no matter if mucilages and consequential changes are for ages recurrent natural events. The future goal is to avoid the damage they cause, even though our present knowledge does not suggest anything to restore the previous properties of these enormous water masses.

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