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Marine Biogeography of the Mediterranean Sea: patterns and dynamics of biodiversity

From the Tropics to the Adriatic: *Coolia monotis* Meunier, *Ostreopsis lenticularis* Fukuyo and *Prorocentrum mexicanum* Tafall, toxic algae in sea water and marine organisms along the coast of the province of Bari

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Key words: *Coolia monotis, Ostreopsis lenticularis, Prorocentrum mexicanum*, Toxic algae, South-Western Adriatic Sea.

SUMMARY

Towards the end of summer 2001 and again in September 2002, a sudden death of sea organisms occurred along the coast from Molfetta to Polignano (southern Adriatic). At the same time many bathers, after swimming in the sea or quite simply sunbathing on the beach of the same coastal tract, showed respiratory problems, high body temperature and skin disorders. In order to discover the cause of these phenomena, samples of seawater and dead sea organisms were taken. An analysis of phytoplankton revealed the presence of three toxic dinoflagellates: *Prorocentrum mexicanum* Tafall, never found before in the Mediterranean, *Ostreopsis lenticularis* Fukuyo, never found before in Italian waters and *Coolia monotis* Meunier, found for the first time in the southern Adriatic. Anatomic and histopathologicalexaminations performed on cuttlefish and white bream revealed disturbances of the cardiac, respiratory, muscular and digestive apparatus. The toxic algae found in water samples appeared to have been the cause not only of the symptoms found in bathers, but also of the deadly inflammatory process noted in the sea organisms examined.

INTRODUCTION

The increase in anthropic activity due to fish farming, sea transport and tourism, has put coastal seawaters at risk of increasing pollution, eutrophycation and introduction of allochthonous species. There has been an increase in episodes of microalgae blooming, with a particular increase in the presence of harmful species, a process known as HABs (Harmful Algal Blooms).

From the end of August to the beginning of September 2001, and September 2002 a sudden death of sea organisms was noted along the coastal tract that stretches from Molfetta to Polignano. This event was accompanied, during the same period, by another one: numerous bathers, who had swum or sunbathed along that stretch of coast, began to suffer from respiratory disorders, high body temperature and skin problems. In order to verify the presence of harmful agents that might have been responsible for the phenomena mentioned above water samples and samples of sea organisms from that area were taken.

Particular attention was paid to the examination of phytoplankton to identify toxic algae. It is widely known that HABs phenomena are caused by approximately 80 species of microalgae, able to produce substances which are potentially harmful to man. In fact, they can cause sudden death of both wild and farmed fish, molluscs and crustaceans, and consequently cause problems to tourism and economy (Sournia, 1995; Zingone and Oksfeld Enevoldsen, 2000).

In the Adriatic sea, about 20 toxic species from the phytoplankton have been identified, belonging to the genera *Alexandrium, Coolia, Dinophysis, Gymnodinium, Goniaulax, Noctiluca, Ostreopsis and Prorocentrum* (Tolomio and Cavolo, 1985; Moro and Andreoli, 1991; Viviani, 1991; Cabrini et al., 1992). Furthermore, studies of phytoplankton present in the southern Adriatic (Rizzi et al., 1994; Rizzi and Aprea, 1998) have revealed the presence of species belonging to the genera *Alexandrium, Dinophysis, Gymnodinium, Goniaulax* and *Prorocentrum*, but in such low concentrations as not to be toxic to either marine life or humans.

This paper reports the results of analyses carried out on water and animal samples taken at Mola di Bari, Lama Palomba (Fig. 1), an area in which the sudden death of marine life and the occurrence of ill bathers were particularly evident.

Fig. 1 - Molfetta-Polignano: the area affected by the phenomenon of sudden death of marine life and symptoms felt by bathers

MATERIALS AND METHODS

Samples of seawater were taken from several centimetres below the water surface and bacteriological analyses were carried out in the laboratory using the MPN method (Ministero della Sanità, 1978). Tests for nitrogen azote and ammoniacal azote, mineral oils and surface-active substances were also run and methods indicated by Stickland and Parson (1972) were used.

Phytoplankton was analysed in seawater samples and water samples conserved inside sea urchins (*Paracentrotus lividus* Lamarck). In particular the sea urchins used were specimens clearly suffering from some disturbance such as aculei pointing downwards or missing on various parts of the theca (Fig. 2).

The water was fixed with 4% neutralised neutralized formaldehyde following the Utermhöl method (Zingone et al., 1990).

Some examples of fish and cephalopods were gathered, already dead, from the sea surface. After careful macroscopic examination, the samples were fixed in 10% salt formalin set in paraffin and processed using common histological techniques. Sections of 5-6 μm, obtained by means of a microtome, were stained with haematoxylin-eosin, PAS and Masson's trichrome for anatomic and histopathological examination.

Fig. 2 - Condition upon death of sea urchin (*Paracentrotus lividus* Lamarck) sampled at Mola di Bari, September 2001

RESULTS

The results of the chemical-physical and bacteriological examination of water samples (Tab. I) reveal only a slight pollution from faecal bacterial and cannot be considered responsible for the sudden death of sea organisms and/or the disturbances suffered by bathers.

Tab. I - Results of chemical-physical and bacteriological analysis of sea water sampled at Mola di Bari

Temperature	27.5 °C
pH	8.2
Dissolved oxygen	120% saturation
N- NO_2	< 0.2 mg/l
N-NH_4	< 0.1 mg/l
Mineral oil	< 0.5 mg/l
Tensioactive substances	< 0.2 mg/l
Total coliforms	23 in 100 ml of water
Faecal coliforms	13 in 100 ml of water

The most common species found in the phytoplankton samples from the seawater and the water conserved inside sea urchins (sampled in September 2001), were the diatoms *Pleurisosigma* sp., *Cylindrotheca closterium* and *Pseudo-nitzschia pseudodelicatissima*. Furthermore, the presence of 3 toxic dinoflagellates was noted: *Prorocentrum mexicanum* Tafall (Fig. 3) never found in the Mediterranean before, *Ostreopsis lenticularis* Fukuyo (Fig. 4) never found in Italian waters before and *Coolia monotis* Meunier (Fig. 5) never found in the southern Adriatic before.

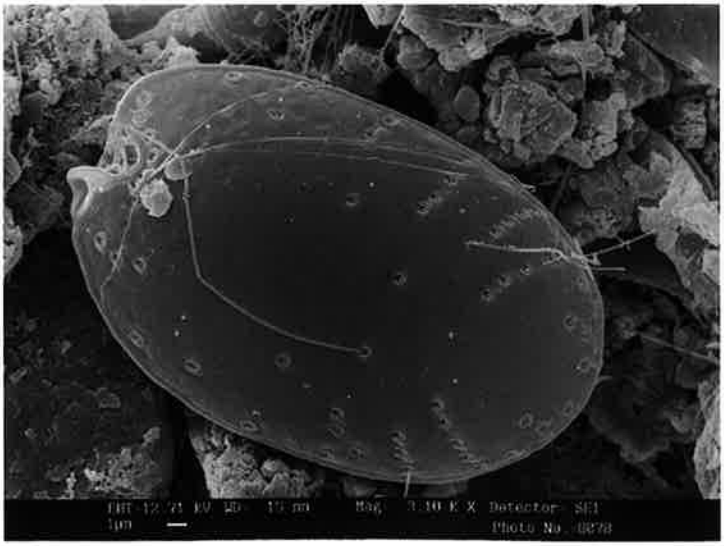

Fig. 3 - *Prorocentrum mexicanum* Tafall

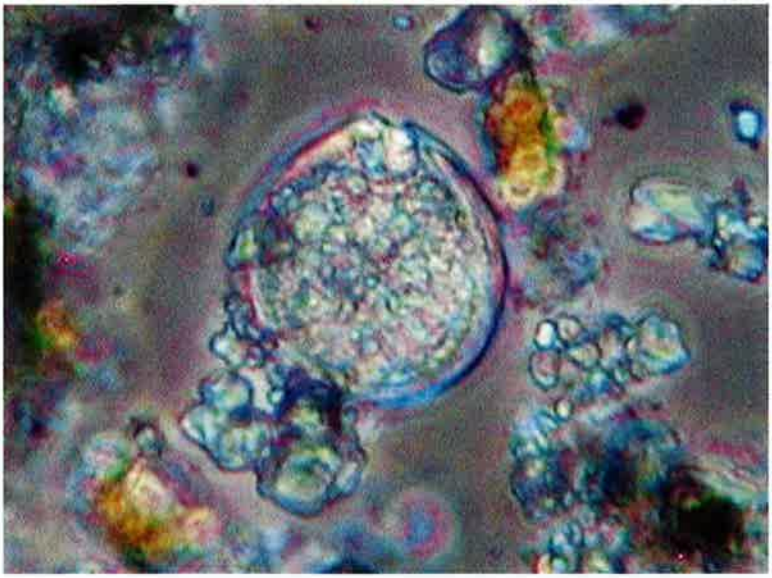

Fig. 4 - *Ostreopsis lenticularis* Fukuyo

Fig. 5 - *Coolia monotis* Meunier

Through the quality-quantity analysis of samples taken in September 2002, it was establish the presence, once again, of *C. monotis*, with a concentration of 116 cell/1, and *O. lenticularis*, with a particularly high concentration of 5000 cell/1.

A description of the three dinoflagellate species follows.

Fig. 6 - Muscle tissue of *D. annularis* bright red cause congestion

Fig. 7 - *S. officinalis* with signs of inflammation

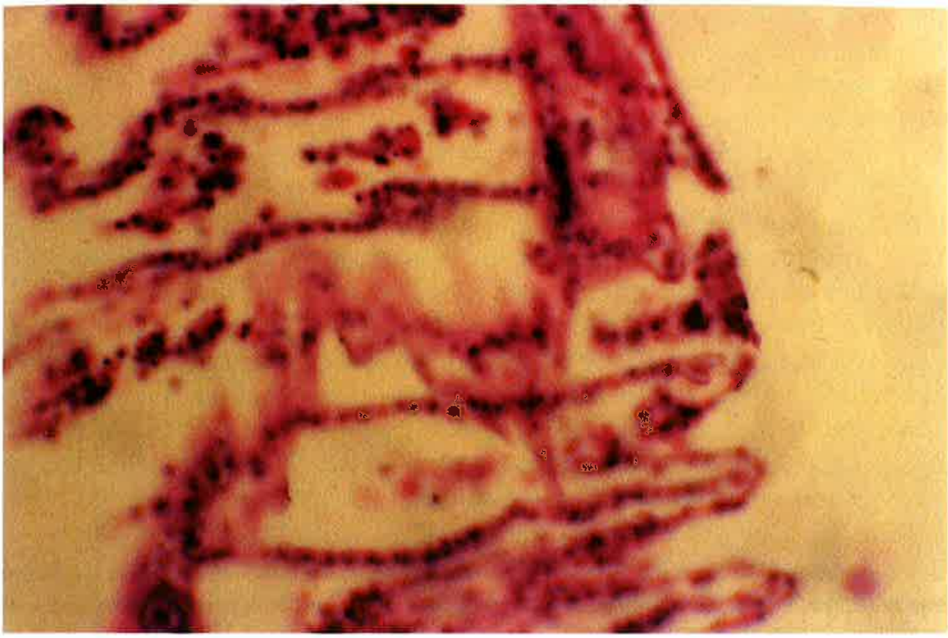

Fig. 8 - Alteration of gill epithelium of *S. officinalis* (dye haematoxylin eosin)

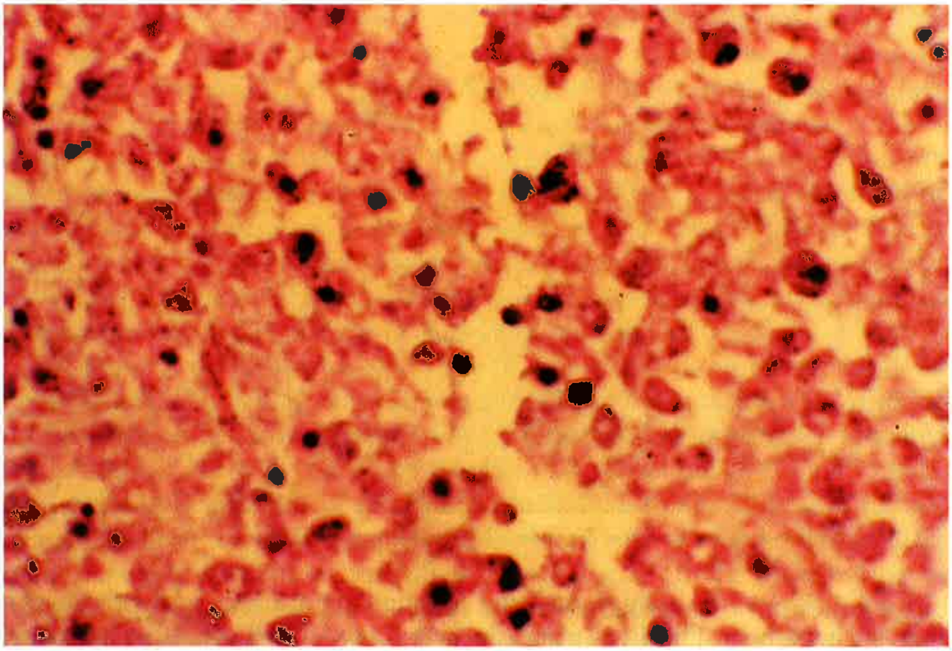

Fig. 9 – Liver oedematous of *D. annularis* (dye haematoxylin eosin)

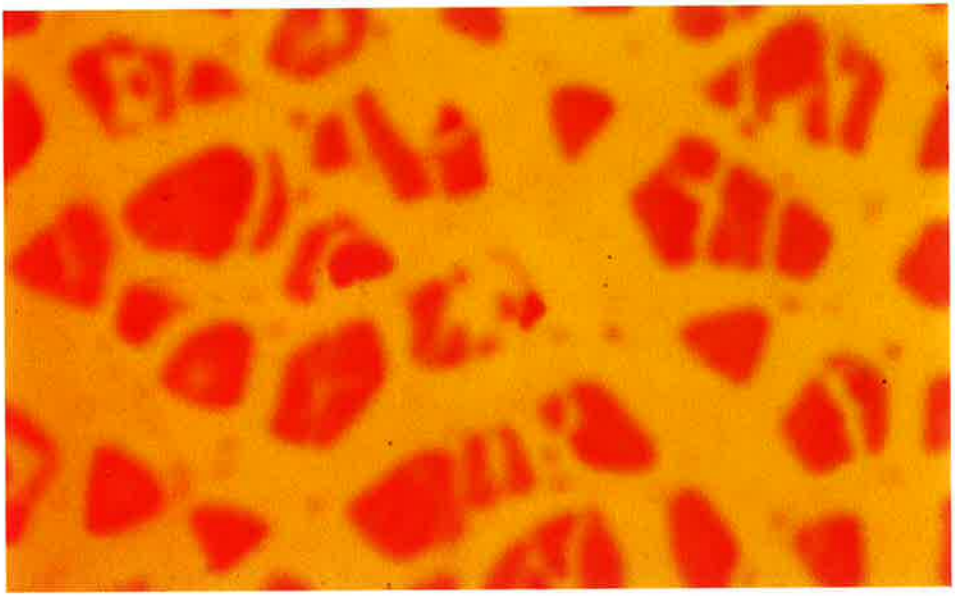

Fig. 10 - Muscle fibres of *D. annularis*: the lack of colour of the fibres has to be attributed to the phenomenon of imbibition (dye haematoxylin eosin)

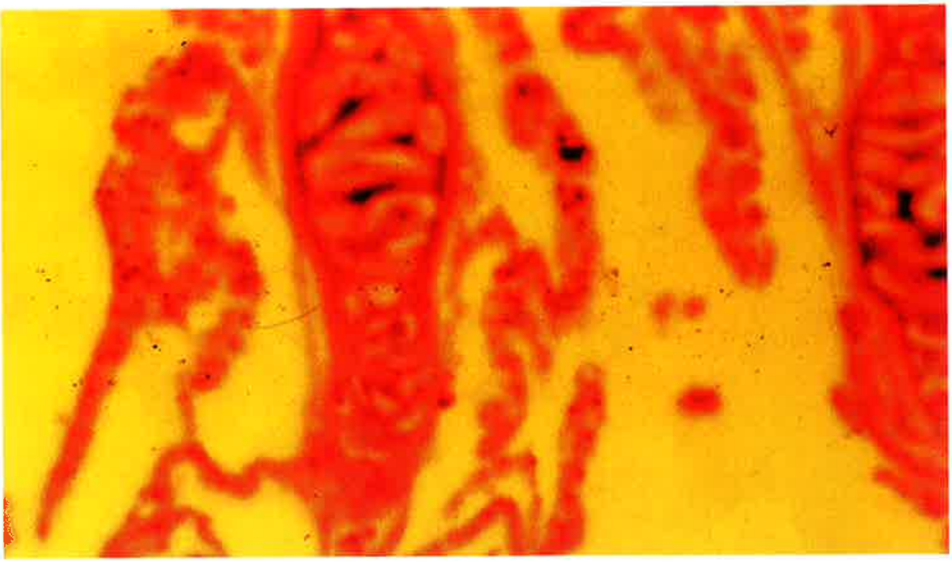

Fig. 11 - Changes in the gill epithelium of *D.annularis* (L.) (dye haematoxylin eosin).

Coolia monotis: the body is small and rounded, the epitheca is slightly smaller than the hypotheca. The thecal surface is covered by well defined plates, irregularly arranged. The thecal surface is smooth and covered by large pores. A distinct oblong plate is located on the epitheca. This plate is positioned off center and containes an apical pore complex, about 12 µm long, that is slightly curved and endowed with a long slit, which have two supporting costal. Round pores are evenly spaced between the costae. Due to its large size, the apical pore complex is easily observed using a light microscope and is useful for identification. Cell size of *C. monotis* ranged from 20 to 50 µm in diameter and 23 to 45 µm in length. Cells have a short longitudinal flagellum, approximately 20 µm long. Finally, cells are photosynthetic and contain many golden-brown discoid chloroplasts.

Ostreopsis lenticularis: the body is lenticulate and antero-posterior compressed, without spines or horns. The epitheca and hypoteca are nearly equally sized and composed of asymmetrical plates. All the thecal plates have many trichocyst pores and additional minute ones scattered all over, which is species-specific and differentiates *O. lenticularis* from the other *Ostreopsis* species. Cells have a dorso-ventral diameter of 65-100 µm and a transdiameter of 57-63 µm. This species is photosynthetic. The body is filled with many golden-brown chloroplasts, except the ventral beak, which is transparent; a large nucleus and, sometime, a large red vacuole are located in the dorsal half of the body. *O. lenticularis* is similar to *O. siamensis* as far as size, but is distinguishable from the latter by the presence of fine pores densely scattered all over the thecal plates.

Prorocentrum mexicanum: the body is oval (length 38-40 µm; width 22-25 µm). It is covered by two large valves and seven or eighth small plates. The left valve is flat, whereas the right one has a slight indentation at the anterior end. Both valves have many trichocyst pores, most of which radially arranged in rows from the center and perpendicularly to the valve margin. Anteriorly a small spine (2-3 µm), with a wing, is present. The cell has a nucleus in the posterior half.

As far as the marine organisms involved in the sudden death phenomenon, an initial visual examination of the body detected disturbances which typically reveal the death as being occurred long before. Indeed, muscle tissue revealed a loss of consistency, was easily detached from the dorsal structure, and was bright red due to congestion (Fig. 6). In the cuttlefish examined, the edema and the imbibitions revealed clear signs of inflammation (Fig. 7).

Anatomic and -histopathological examinations carried out on the cuttlefish *Sepia officinalis* L., and on white bream *Diplodus annularis* (L.) revealed disturbances of the cardiac, respiratory and digestive apparatus, which appeared to have been caused by toxic substances. The epithelium of the gills of the cuttlefish was uneven due to exudation, which tends to dilate the gill structure (Fig. 8); the liver revealed widespread edema along with the presence of vacuoles with irregularly distributed coartic hepatocytes (Fig. 9). In the white bream the muscle fibre showed evident signs of severe suffering with imbibitions and fragmentation of the fibres themselves (Fig. 10); the epithelium of the gills was uneven due to exudation provoked by the dilation of the gill structure (Fig. 11).

CONCLUSIONS

The existence and spread of harmful microalgae in Italian waters might be, according to Hallegraeff and Boch (1991), the consequence of fish-farming and increase in sea transportation. Nevertheless, Tognetto et al. (1995) believe that it is difficult to assert that these species have been introduced into Italian waters by ships or through the importation of fish and seafood (molluscs) from tropical regions. They hypothesize, along with Margalef (1994), that such species are indeed indigenous Mediterranean species that have simply not been noted before. This hypothesis agrees with Penna's observations (2001) and with those of Giacobbe et al. (2001), who carried out studies on genetic variability of *Coolia monotis* and *Ostreopsis* spp. clones. They found similarities between these microalgae with those from various other Mediterranean areas, and differences with species of Atlantic-tropical origin.

In Italy, *C. monotis,* which produces the toxin "cooliatoxin" with its haemolytic effect (Giacobbe et al., 2000), was first identified in the Venice Lagoon (Tolomio and Cavolo, 1985) and along the Eastern Sicilian coastline (Gangemi, 2000).

The microalgae *Ostreopsis* was discovered in Italian waters. Tognetto et al. (1995) reported the presence of *O. ovata* in the Tyrrheanian Sea. The presence of this species was later confirmed along the Apuan coastline by Sansoni et al. (in press). Di Turi et al. (2002) identified *Ostreopsis,* presumably *ovata,* in the Southern Adriatic Sea. Gangemi (2000) noted that along the Eastern Sicilian coastline the dinoflagellate *Ostreopsis* existed, the author, however, was not able to identify the species. Tindall et al. (1990) consider that *O. ovata* is not toxic underlined, however, following Tindall et al. (1990) that the *O. ovata* species is not toxic.

Ostreopsis lenticularis has never been described in Italian waters. Its harmful qualities, due to the neuro-toxic action of ostreotoxin, was proved by experiments on rats by Tindall et al. (1990).

The presence of *Prorocentrum mexicanum* was unexpectedly noted. This dinoflagellate, which produces a fast-acting toxine with ahaemolytic effect, has not been previously found in the Mediterranean. Until now, it had been found in the Caribbean (Faust, 1995), the Virgin Islands (Carlson and Tindall, 1985), the Hawaiian waters (Carbonnière), Canada (Levasseur et al., 2003), Puerto Rico (Ballantine et al., 1988), Mexico (Gárate-Lizárraga et al.), Colombia (Medina), Vietnam (Nguyen et al.), Tasmania (Pearce et al., 2001) and in Australia (Murray and Patterson).

Thus, while it has not been possible to carry out tests on the toxins yet, the presence of dinoflagellates, both in marine organisms and sampled water, may suggests that they are responsible for the sporadic episodes of sudden death of sea creatures and the symptoms felt by bathers in recent years. Further analyses are necessary in order to establish the toxic-level of the species sampled along the coast, which is regularly monitored; the sporadic nature of the events, and the frequency of toxic species belonging to the *Ostreopsis, Coolia* and *Prorocentrum* genera in the micro-phytoplankton population.

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