OCCURRENCE OF THE POTENTIALLY TOXIC DINOFLAGELLATE *Ostreopsis ovata* ALONG THE APULIAN COASTAL AREAS (SOUTHERN ITALY) AND RELATIONSHIP WITH ANTHROPOGENIC POLLUTION

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ABSTRACT

The monitoring of the epi-benthic dinoflagellate *Ostreopsis ovata* was carried out during the summer seasons of 2007 and 2008 all along the marine coastal areas of the Puglia region (Southern Italy, Central Mediterranean). Eighteen monitoring sites were investigated in both years from June to September at 15 day-time intervals, collecting water samples in order to measure both the *Ostreopsis ovata* density and microbiological parameters (Fecal Coliforms and Streptococci). The microbiological parameters were measured as indirect indicators of pollution from anthropogenic sources. *Ostreopsis ovata* highest density values were estimated for both years in the monitoring sites located northbound from the town of Bari, on the southern Adriatic coast. Results from microbiological analysis showed a similar distribution pattern. The relationship between *O. ovata* abundance and microbiological pollution was investigated by using synthetic indices (*Ostreopsis*-Score and Bacteriological Quality Index respectively) and the correspondence between the dinoflagellate blooms and human-induced pollution pressures on these marine coastal areas was highlighted.

KEYWORDS: Harmful Algal Blooms, *Ostreopsis ovata,* Central Mediterranean Sea, Marine Coastal Area, human-induced pressure, Southern Italy.

INTRODUCTION

Harmful Algal Blooms (HABs) have occurred worldwide since old times, often due to "natural" hydrological and geo-morphological characteristics of different water bodies [1]. However, these phenomena seem to have recently increased, and the relationship with eutrophication and climate change has been hypothesized [2].

With regard to the Mediterranean sea, most algal species involved in marine blooms are planktonic [3, 4] but since the 1990s an increasing trend in the presence and abundance of benthic microalgae has been reported [5-7].

Most benthic microalgal blooms in the Mediterranean are related to the presence of the dinoflagellate *Ostreopsis ovata* Fukuyo [5, 6, 8-14], a species mainly distributed in tropical and sub-tropical geographical areas. This dinoflagellate is potentially toxic [15], and palytoxin-like compounds have been found in samples from Italian marine coastal zones [16, 17].

This species blooms during the summer – early autumn seasons in the Mediterranean (when high irradiance values and prolonged periods of calm seas occur, as well as the seasonal increase of seawater temperature), and mostly in marine coastal zones characterised by rocky bottoms and low circulation [9-11, 18].

Ostreopsis ovata blooms have been reported since the year 2000 along the Apulian coastal areas (Southern Italy, Central Mediterranean Sea) [9, 13], where it was considered as the main cause of several syndromes affecting people staying on the sea-shore during the summer season [13, 19].

The postulated interaction between the presence/ abundance of *O. ovata* and the recreational use of beaches and bathing waters was the main reason to carry out the monitoring of the benthic microalgae in the summer seasons 2007 and 2008 along the whole Apulian marine coastal areas. The Apulian Regional Agency for the Environmental Prevention and Protection (ARPA Puglia) was in charge of related activities.

MATERIALS AND METHODS

Ostreopsis ovata is a benthic species that colonises macroalgae, seagrass and often directly hard substrates

[18]. According to the monitoring purpose the Apulian marine coastal zones, characterised by rocky or mixed (sandy-rocky) bottoms, were selected only. Eighteen sites were chosen for the sampling surveys carried out during the summer seasons of 2007 and 2008 (Fig. 1). The sites are located both in impacted areas by anthropogenic pressures (urban zones, domestic discharges, etc.) and in not impacted ones.

The monitoring sites were investigated in both years from June to September at 15 day-time intervals, collecting seawater samples in order to measure both biological and microbiological parameters (*Ostreopsis ovata*, Fecal Coliforms and Streptococci). Seawater Surface Temperature (°C) was recorded during sampling also. The water quality control was mainly targeted to the monitoring of biological and microbiological parameters influencing human health and the use of the marine coastal areas for recreational purposes, and no data were collected on chemicals concentrations (including macronutrients). Nevertheless, the measured microbiological parameters were considered as indirect indicators of pollution from anthropogenic sources, being the presence of fecal coliforms and streptococci a signal of the sea water contamination due to the sewage discharges [20].

Water samples for the identification and quantification of benthic microalgae were collected close to the coast (0.5 – 1 m bathymetry), by sampling both the water column just under the surface (-0.4/-0.8 m depths) and close to the bottom; the water column was sampled by means of a sterile bottle, while the bottom water was collected close to the seabed using a 50 ml syringe with the tip cut (three replicates for each sample). The use of the syringe for the sampling of *O. ovata* population has to be considered as the simplest method to obtain reliable results (M. Abbate, ENEA La Spezia, Pers. Comm.), although other procedure are also available [21]. Sterile bottles were used for the water column samples to be analysed for the microbiological parameters.

The estimation of *O. ovata* density in the samples was carried out according to the Utermöhl method [22], using 10 ml phytoplankton sedimentation chambers observed by an inverted microscope (after 24 hours of sample sedimentation). Microbiological analysis were carried out according to the membrane filtration method (APAT-IRSA 7030-F for the Coliforms and ISO 7899-2:2000 for the Streptococci) [23].

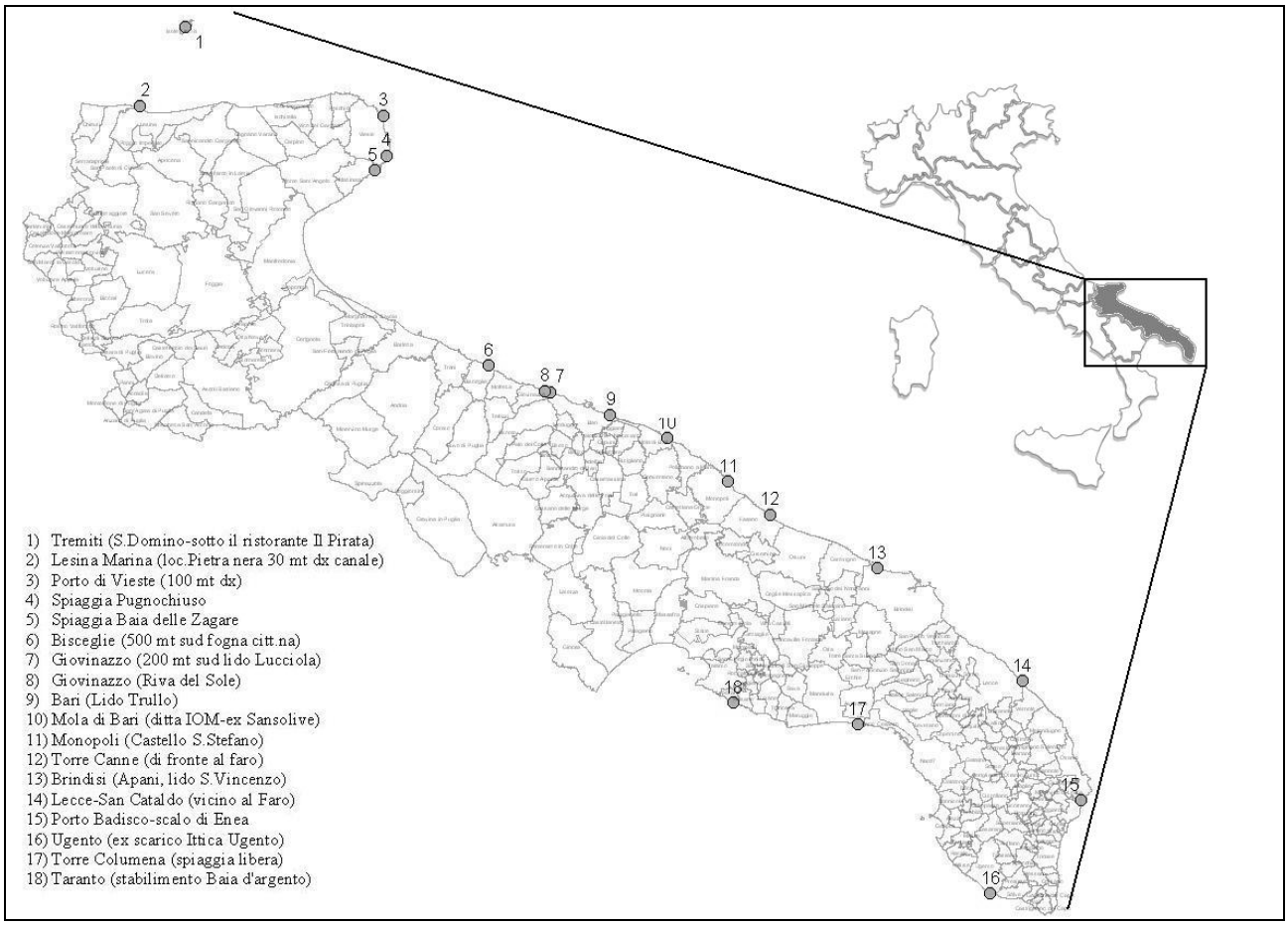

FIGURE 1 - *Ostreopsis ovata* monitoring stations during the 2007 and 2008 surveys.

TABLE 1 - *Ostreopsis ovata* density range and derived abundance classes.

Ostreopsis "bottom waters density"	1 - 5,000 cells/l	5,001 - 50,000 cells/l	50,001-100,000 cells/l	100,001-300,000 cells/l	> 300,000 cells/l
Abundance Class	**1**	**2**	**3**	**4**	**5**
Ostreopsis "water column density"	1-1,000 cells/l	1,001-5,000 cells/l	5,001-10,000 cells/l	10,001-20,000 cells/l	> 20,000 cells/l
Abundance Class	**1**	**2**	**3**	**4**	**5**

The data resulting from the analysis of the samples were processed in order to investigate site-specific presence and abundance of *O. ovata* and the relationship with potential human-induced pressures.

Several indicators, such as the Bacteriological Quality Index (BQI) [20] and the index for *Ostreopsis ovata* (O-Score, see below), have been calculated for each monitored site exploiting all data from the 2007/2008 summer surveys.

The BQI is considered as a condition indicator negatively correlated to the anthropogenic pressure on coastal areas (BQI value decreases when the impact from anthropogenic pressure increases), and it is computed according to the relative occurrence of fecal coliforms and fecal streptococci in marine waters (details of the method are reported in Melley et al. 2004 – [20]).

The index for *Ostreopsis ovata*, named *O-Score*, is based on the relative occurrence of five abundance classes of *Ostreopsis ovata* (Table 1). The benthic dinoflagellate density range for each abundance class was empirically chosen according to field observations (effects of the *O. ovata* population on the marine environment and human health) [13].

The *O-Score* value for each monitoring site was finally calculated using the simple formula reported below:

O-Score Value (per sampling site) =

$$\sum_{i=1}^{5} A_{(i)} * F_{(i)}$$

"A" is the vector of the abundance class from 1 to 5 [A = (1, 2, 3, 4, 5)], "F" is the vector of the frequency of each abundance class in the monitoring period [F = (X_1, X_2, X_3, X_4, X_5)], where $X_i = f_i/N$ (the ratio between the number of positive samples at the abundance class and the total number of samples; the value of the ratio between 0 and 1).

According to the index formulation, O-Score values can range from "0" (null presence of *O. ovata* in the investigated period) and "5" (all the samples classified in the highest abundance class).

The estimated values of both indices (BQI and O-Score) were compared for each monitoring site, and the results from the analysis were compared with the distribution maps of human pressure such as the coastal population and discharges of treated waste waters into the sea.

RESULTS AND DISCUSSION

During the summer seasons of 2007 and 2008 (from June to September) the benthic dinoflagellate *Ostreopsis ovata* was recorded at least once in 14 of the 18 sampling stations (Table 2); the highest density values were esti-

TABLE 2 - Presence and maximum recorded density of *Ostreopsis ovata* in bottom waters and water column samples (Apulian marine coastal areas, years 2007-2008).

SAMPLING STATION	LOCATION	*Ostreopsis ovata* reported presence in the samples (Yes/No)	*Ostreopsis ovata* maximum density			
			bottom waters		*water column*	
			cells/l	*month/year*	*cells/l*	*month/year*
1	*S.Domino-sotto il ristorante Il Pirata (FG)*	yes	256,000	sept./2008	5,000	aug./2007
2	*loc. Pietra nera 30 mt dx canale (FG)*	no	-	-	-	-
3	*porto di Vieste 100 mt dx (FG)*	no	-	-	-	-
4	*spiagga Pugnochiuso (FG)*	yes	400	aug./2008	-	-
5	*spiaggia baia delle zagare (FG)*	no	-	-	-	-
6	*500 mt sud fogna citt.na Bisceglie (BA)*	yes	421,200	sept./2007	4,900	sept./2007
7	*200 mt sud lido Lucciola (BA)*	yes	1,415,200	sept./2008	304,000	sept./2008
8	*Hotel Riva del sole (BA)*	yes	5,000,000	aug./2008	83,840	jul./2008
9	*Lido Trullo (BA)*	yes	1,054,400	sept./2008	137,920	sept./2008
10	*ditta IOM-ex Sansolive (BA)*	yes	1,222,400	sept./2007	140,480	sept./2008
11	*Castello S.Stefano (BA)*	yes	740,800	sept./2008	36,160	sept./2008
12	*Torre Canne di fronte al faro (BR)*	yes	192,000	sept./2008	16,000	sept./2008
13	*Apani lido S.Vincenzo (BR)*	no	-	-	-	-
14	*San Cataldo-vicino al Faro (LE)*	yes	11,604	aug./2008	-	-
15	*porto Badisco-scalo di Enea (LE)*	yes	23,280	sept./2008	120	sept./2008
16	*scarico Ittica Ugento a Punta Macolone (LE)*	yes	5,600	jul./2008	-	-
17	*spiaggia libera Torre Columena (TA)*	yes	1,800	jul./2007	-	-
18	*stabilimento Baia d'argento (TA)*	yes	160,000	aug./2008	7,680	aug./2008

TABLE 3 - Fecal coliforms and streptococci mean values in the water samples collected during the 2007 and 2008 surveys (Apulian marine coastal areas).

SAMPLING STATION	LOCATION	Fecal Coliforms C.F.U. mean value	Fecal Streptococci C.F.U. mean value
1	*S.Domino-sotto il ristorante Il Pirata (FG)*	1.9	0.8
2	*loc. Pietra nera 30 mt dx canale (FG)*	6.0	2.4
3	*porto di Vieste 100 mt dx (FG)*	11.1	2.8
4	*spiagga Pugnochiuso (FG)*	3.4	1.6
5	*spiaggia baia delle zagare (FG)*	3.8	0.8
6	*500 mt sud fogna citt.na Bisceglie (BA)*	32.4	26.3
7	*200 mt sud lido Lucciola (BA)*	20.1	15.8
8	*Hotel Riva del sole (BA)*	15.0	10.2
9	*Lido Trullo (BA)*	18.4	16.3
10	*ditta IOM-ex Sansolive (BA)*	19.6	17.9
11	*Castello S.Stefano (BA)*	21.3	17.9
12	*Torre Canne di fronte al faro (BR)*	2.3	4.8
13	*Apani lido S.Vincenzo (BR)*	2.7	2.5
14	*San Cataldo-vicino al Faro (LE)*	0.6	0.3
15	*porto Badisco-scalo di Enea (LE)*	0.2	0.3
16	*scarico Ittica Ugento a Punta Macolone (LE)*	1.5	1.0
17	*spiaggia libera Torre Columena (TA)*	0.9	1.1
18	*stabilimento Baia d'argento (TA)*	0.8	1.8

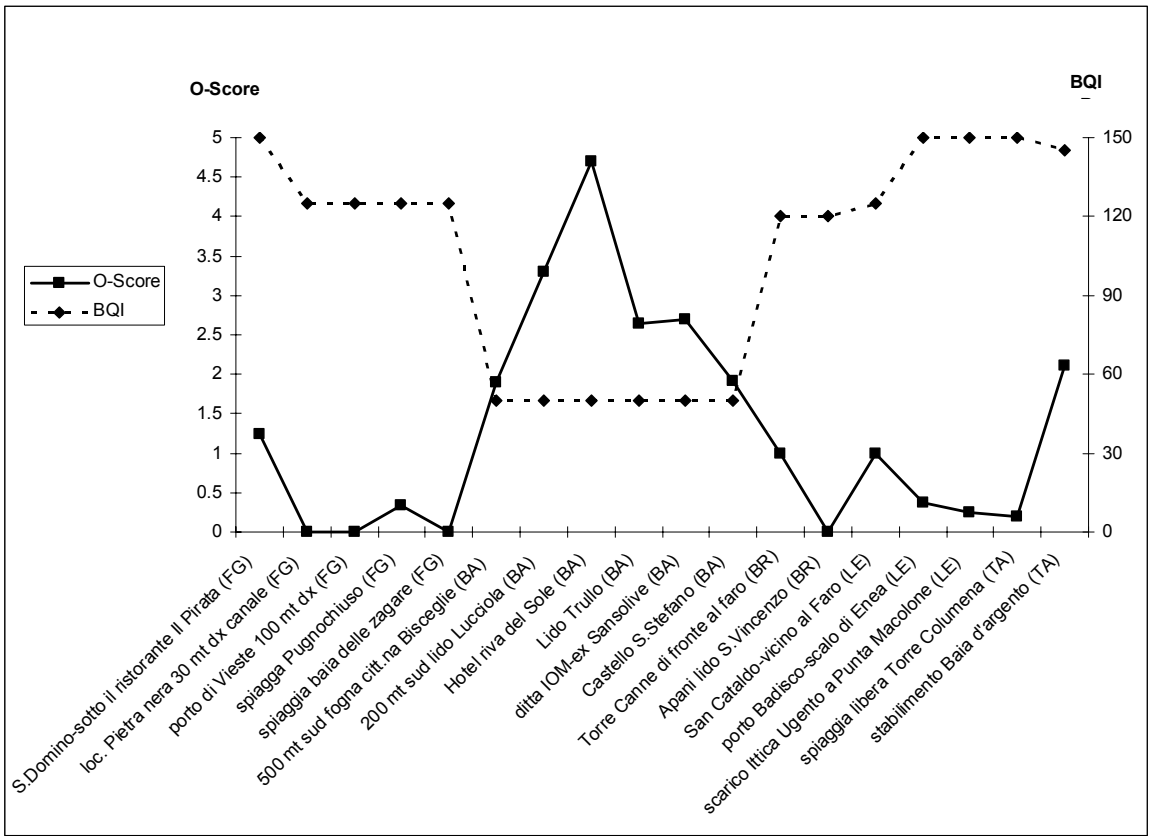

FIGURE 2 - Distribution of BQI and O-Score values per monitored site (Apulian coastal bottom waters, summer seasons 2007 and 2008).

mated for both the years in the monitoring sites located northbound from the town of Bari, the regional chief town located on the Adriatic coast. Results from microbiological analyses showed a similar distribution pattern, with higher bacteriological contamination of marine waters near the same town (Table 3).

Both the Bacteriological Quality Index and the *Ostreopsis* Score one were calculated for each sampling station according the data from the 2007 and 2008 monitoring (*O. ovata* relative abundance and bacteriological analytical results, respectively).

The 2007-2008 integrated values of BQI and O-Score per sampling site are represented in Figure 2. The two indices appear to be negatively correlated (r = - 0.76; α = 0,001). According to the reported results the abundance values of *Ostreopsis ovata* and frequency of blooms seems to increase in the monitoring sites characterised by the higher levels of microbiological contamination.

Clearly, the contamination/pollution of the marine waters depends on diffuse and/or point pressure sources. Human population density on coastal areas, and its related impact such as waste waters discharged at the sea (treated or not), can be considered as pressure factors and utilised in the environmental impact assessment from anthropogenic sources [24]. The World Health Organisation estimated the load of 54 g d^{-1} of BOD in the urban raw waste water as produced by each inhabitant-equivalent (IE) [25]. After treatment, the BOD removal percentage ranges between 75% and 95% as a rule [26], that means an estimated value of 27-135 kg d^{-1} of BOD discharged in the sea waters from 10,000 inhabitant-equivalents waste water treatment plant. At the same time the BOD value in the water bodies and streams is positively correlated to the nutrients concentration as it was reported in literature [27, 28]. Thus, in order to investigate the potential drivers for the presence and growth of *O. ovata* populations along the Apulian coasts the G.I.S. pictures of both the distribution of population density and the location/size (in terms of inhabitant-equivalent) of the sewage discharged at sea from waste water treatment plants were retrieved from the ARPA Puglia archives (Figures 3 and 4).

Looking at the map representation of the O-Score index values (Figure 5), it is worth noticing a fair overlap with the geographic distribution of the pressure factors.

The obtained results underline the postulated role of trophic load surplus in the marine waters from human activities [24, 29, 30] as a factor for the occurrence of *Ostreopsis ovata* blooms in the Apulian coastal waters, although other drivers can affect the presence and abundance of the species. The seawater temperature could play an important role, due to the preferential distribution of *O. ovata* in the tropical and sub-tropical geographical areas. In fact, most of the reported blooms occur during warmer months as a rule [13]. Nevertheless, the seasonal increase of the seawater temperature seems to be almost uniform in the whole investigated area, since the average SST values in all the monitoring sites resulted, during the summer seasons of 2007 and 2008, very close to each other. According to the monitored seasonal period, the recorded data don't support the hypothesis of inter-sites differences in the *O. ovata* occurrence due to the seawater temperature value (not significant correlation; α = 0,001) (Figure 6).

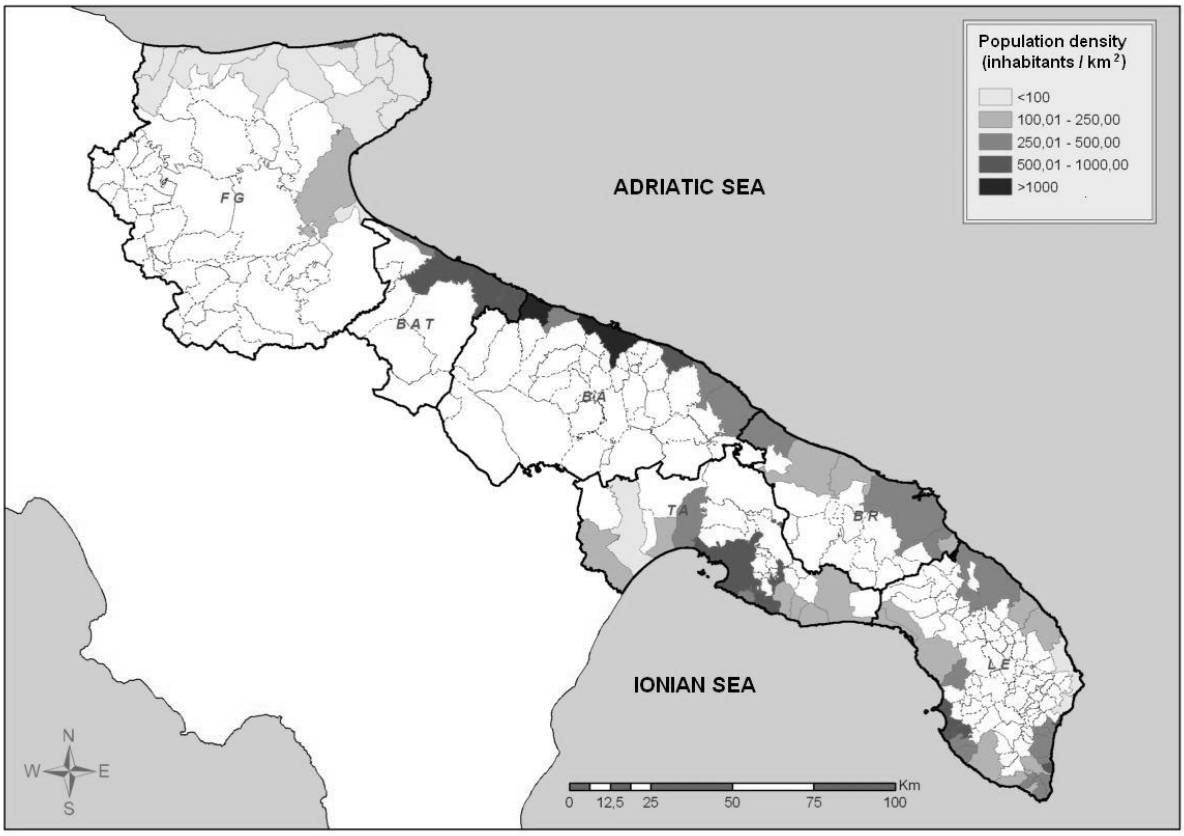

FIGURE 3 - Coastal population density in the Puglia region.

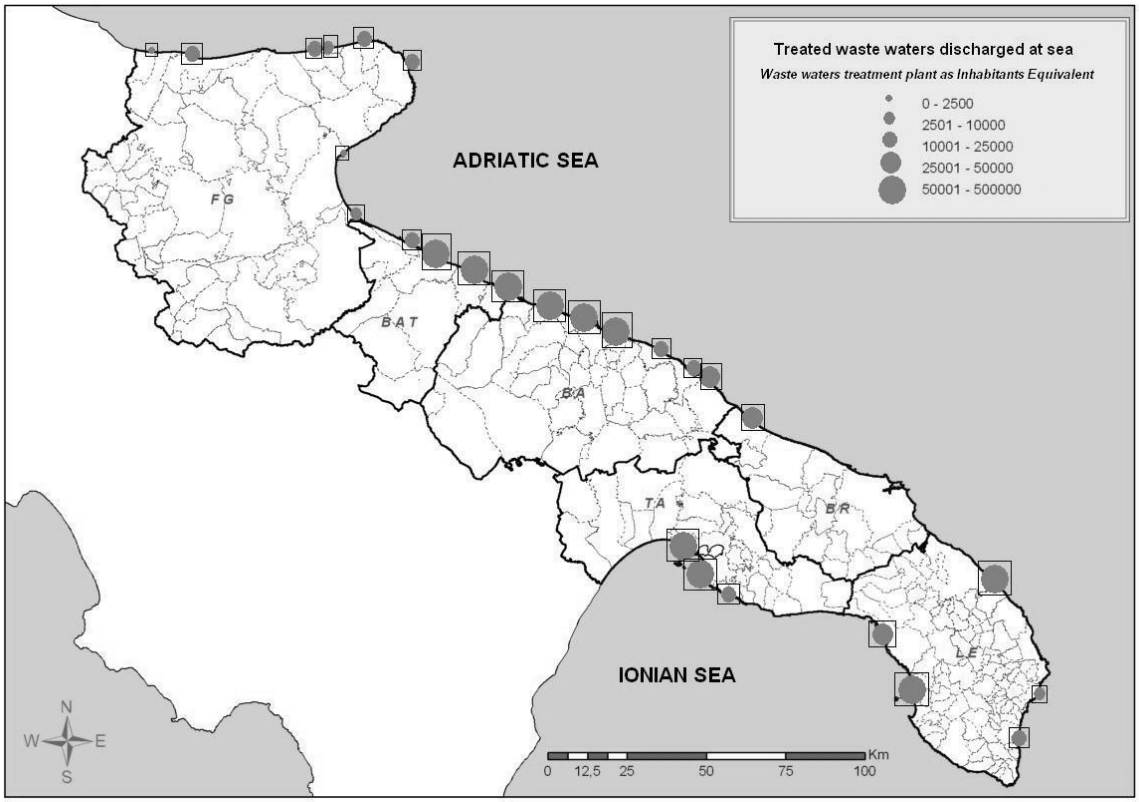

FIGURE 4 - Discharges at the sea of the Apulian waste waters treatment plants.

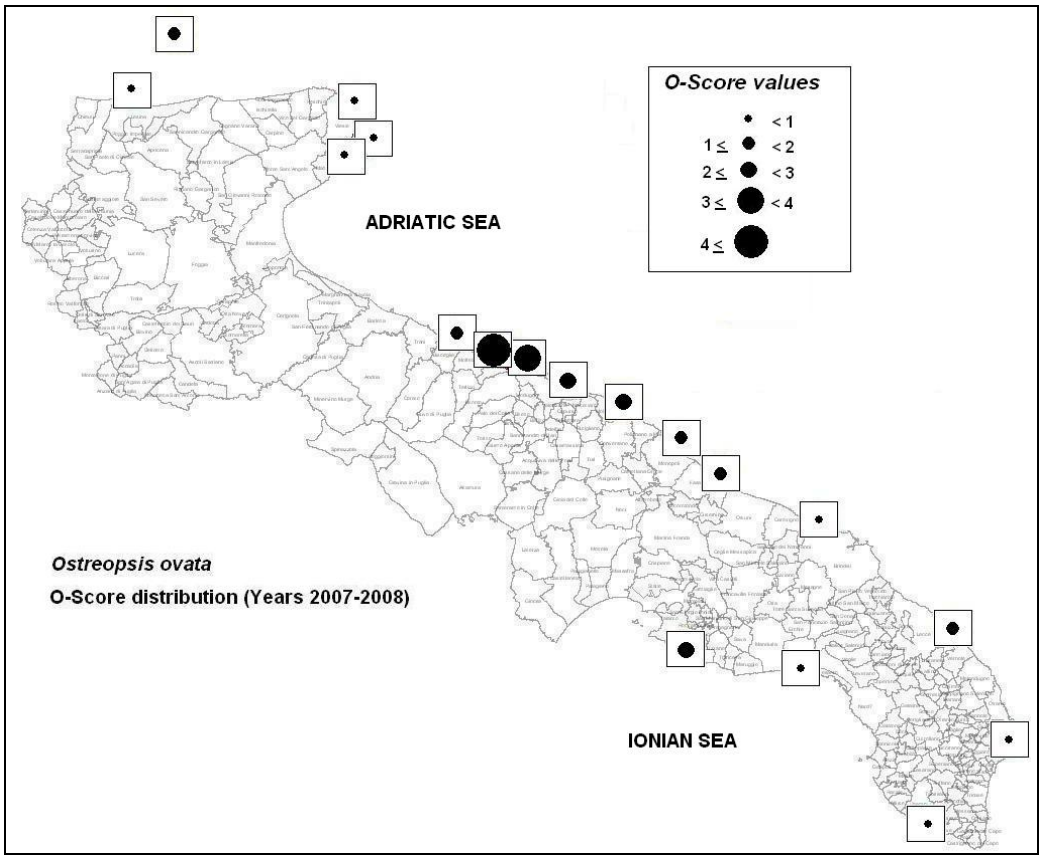

FIGURE. 5 - Map distribution of the O-Score values along the Apulian marine coastal area.

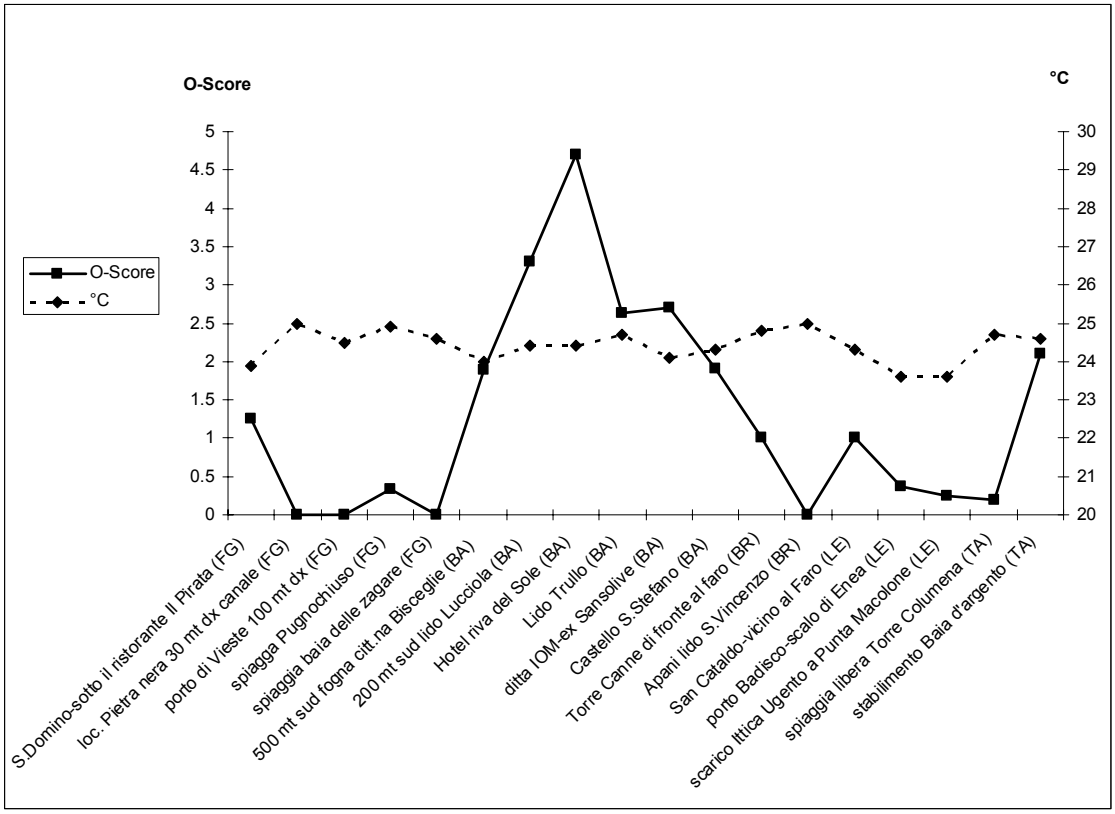

FIGURE 6 - Seawater average temperature (°C) and O-Score values per monitored site (summer seasons 2007 and 2008).

CONCLUSIONS

In the past decade the presence and blooms of *Ostreopsis ovata* have been increasingly reported in the Mediterranean basin [18], affecting human health [19, 31].

In Italy the emerging issue has been discussed at institutional level because of the environmental and health implications, and Guidelines for the monitoring and the management of the phenomena were published by ISPRA (Italian Institute for Environmental Protection and Research) [21] and the *Ministero della Salute* (Italian Health Ministry) [32].

Coastal areas of the Puglia region have been affected by *O. ovata* blooms since the year 2000 [9, 13]. According to the potential effects of the phenomena on the environment and public health, regular monitoring surveys were carried out since 2007.

Results highlighted a postulated correspondence between the occurrence of *O. ovata* and the trophic load discharged in the marine waters from anthropogenic sources. The load surplus was indirectly estimated looking at the distribution and size of sewages in the sea waters (in terms of inhabitant-equivalents – BOD values), according to the presumed role of coastal eutrophication for the increase of harmful algal blooms as reported in literature [2, 29]. Nevertheless, owing to the current lack of monitoring data on nutrients concentration in the sampling stations, the reported results have to be confirmed adopting new research protocols. However, the intraregional differences in the frequency and abundance of *Ostreopsis ovata* populations can also be related to the local geomorphological and hydrological features. In fact, the Apulian coasts are surrounded by two seas, the southern Adriatic and the north western Ionian, characterised by different water quality. The same parameters can change with natural variations from north to south and from east to west; as a rule the Southern Adriatic seawaters naturally range from mesotrophic to oligotrophic while the north western Ionian range from oligotrophic to ultraoligotrophic [33]. Thus, the occurrence of *O. ovata* blooms could be due to the synergistic effect of both natural features (hydrologic-geomorphologic characteristics) and the trophic loads from anthropogenic sources [29]. The hypothesis needs to be confirmed by specific research programs, using different sampling strategy and choosing a larger number of monitoring sites close to the urbanized areas as well as in uncontaminated zones, both the typologies characterised as much possible by the same basic hydrological and geomorphological conditions (seawater temperature, sea bottom substrate, etc.).

The data from the monitoring surveys were used to formulate and test for the first time the O-Score index. This index is based on the microalgal presence/abundance historical data with the aim to identify the "hot spots" for

blooms of *Ostreopsis ovata*. The first results from the use of such index seem to fulfill the requirements. According to the type and measure of the variables included in the formula, the synthetic index could be also useful for the comparison among Mediterranean areas despite the use of different monitoring designs.

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