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# Impacts of marine aquaculture at large spatial scales: Evidences from N and P catchment loading and phytoplankton biomass

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## ABSTRACT

While several studies point at off-shore aquaculture as a possible source of impacts on the local marine environment, very few have analysed its effects at large scales such as at the bay, gulf or basin levels. Similar analyses are hampered by the multiple sources of disturbance that may concomitantly affect a given area. The present paper addresses these issues taking the Gulf of Castellammare (Southern Tyrrhenian Sea) as an example. Nitrogen (N) and phosphorous (P) loads were calculated for the period 1970–2007, and compared to chlorophyll-a concentration as measured inside and outside the Gulf over the same period. Results indicate that N and P catchment loading has constantly decreased because of improved environmental management. Nevertheless, nutrient concentration in the Gulf has steadily increased since the establishment of aquaculture facilities in 1999. Chlorophyll-a concentration followed this trend, showing a marked increase from 2001 onwards. In the same period, chlorophyll-a concentrations measured inside and outside the Gulf have significantly diverged. As all the other possible causes can be ruled out, aquaculture remains the sole explanation for the observed situation. This paper demonstrates for the first time ever that off-shore aquaculture may affect the marine ecosystem well beyond the local scale and provides an additional element of concern to be kept into consideration when allocating oceans' space for new fish-farming activities.

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## 1. Introduction

Since the 1990s, coastal aquaculture has become a consolidated economic activity as a global alternative to land-based aquaculture (Beveridge, 1996), and has been the major cause of a rapid domestication of marine species (Duarte et al., 2007). Over the last two decades, along with the almost exponential increase in economic gains provided worldwide, coastal aquaculture has attracted a large interest either from marine scientists and policy makers for the potential detrimental effects of this industry on the surrounding marine environment (e.g., Munday et al., 1994; Pillay, 2004; Pusceddu et al., 2007; Holmer et al., 2008a,b; Borja et al., 2009). Most of the research carried out so far has, indeed, documented significant changes in the physical, chemical and biological attributes of sediments and the water column around off-shore aquaculture plants, and these include, among the others: i) increasing organic matter contents and compositional changes of the sediment below fish cages (e.g., Pusceddu et al., 2007); ii) altered inorganic

and organic chemistry of farm water and sediments (e.g., Sarà, 2007a,b,c; Šangulin et al., 2011); iii) altered abundance, biomass and biodiversity of micro- (La Rosa et al., 2004), meio- (Mirto et al., 2010) and macro-benthic (Kalantzi and Karakassis, 2006) communities; iv) modified distributional patterns of phyto- and microplankton abundance and production (e.g., Pitta et al., 1998). The extension of the abovementioned changes either in water and benthic variables has been observed generally at spatial scales ranging from meters to a few hundred meters from the edge of the pens, generally not exceeding 1–2 km from the rearing site (e.g., Gowen and Bradbury, 1987; Sarà et al., 2006; Pusceddu et al., 2007; Holmer et al., 2008a,b; Borja et al., 2009). Machias et al. (2005) showed that the presence of fish farms can increase the biomass of wild fish populations at a spatial scale larger than the immediate vicinity of fish cage, though with no clear impact on species diversity. However, to our best knowledge, information about aquaculture impacts on water and sediment variables at large spatial scale (e.g., at a basin scale) are practically not existent. Furthermore, three recent and independent meta-analyses of more than 1000 study cases have pinpointed the unevenly spatially resolved design of most of the reviewed experiments aimed at assessing the effects of

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**Fig. 1.** The study area; a) Map of the Gulf of Castellammare with symbols referring to the source of data as follows: grey circle = University of Messina, CNR Messina (1981); black circle = University of Messina, CNR Messina (1982); grey square = Sicilian Region/Dept. Of Territory and Environment (1984–1985); black square = CNR Castellammare (1990); grey triangle = Sarà G. PhD Thesis (1991–1993); black triangle = Dept. of Animal Biology and Marine Ecology, University of Palermo (1994–1995); grey reversed triangle = Mediterranean Oceanological Centre (2000–2001); black reversed triangle = Sarà G., unpublished data (2003–2005); grey hexagon = Sarà G., unpublished data (2003–2005); grey hexagon = Sarà G., unpublished data mare from which EMIS chlorophyll-a data were retrieved. The small arrow indicates the location of the Gulf of Castellammare.

aquaculture on a wide array of abiotic (i.e., temperature, salinity, pH, water turbidity, dissolved oxygen, inorganic nutrient) and biotic (e.g., chlorophyll-a, organic carbon and nitrogen, prokaryotic and phytoplankton abundance) variables in the water column (Sarà, 2007a,b,c).

The persistence of these gaps of knowledge is, on the one hand, partially due to the difficulty in disentangling, at large spatial scales, the ecological effects induced by coastal aquaculture from those induced by other natural (e.g., currents, circulation) and anthropogenic sources of environmental variability (e.g., urban and/or industrial agglomerates, terrestrial zootechny and agriculture). On the other hand, the sampling bias affecting many of the studies carried out on the impacts of aquaculture on the neighbouring environment (with a few exceptions, e.g., Pusceddu et al., 2007; Holmer et al., 2008a; Sanz-Lázaro et al., 2011) makes even more difficult task of identifying large-scale ecological effects of

aquaculture because of the presence of possibly confounding explanatory variables and uncontrolled sources of variability.

In spite of these premises, it could be hypothesised that waste nutrients released by fish-farming activities might co-act with N and P released in near-shore waters through continental run-off, rivers and seasonal streams, altogether promoting eutrophication of coastal waters (i.e., either in terms of increased nutrient contents, enhanced primary production and accumulation of algal biomass: Cloern, 2001). However, we still do not know yet whether additive loads released by aquaculture may trigger water eutrophication at a very large distance (>10 km) from the local area of waste release, or how the duration of exposure to aquaculture wastes may affect the ecological functioning of the receiving basin. As a consequence, large-scale mitigation of fish-farming impacts has been de facto neglected in the present management practices of coastal areas worldwide, thus posing a concern also in the light of the predicted tendency of fish-farming industries to move in off-shore waters, as the space in coastal waters is increasingly becoming a limiting factor (Holmer, 2010).

In this study, we aimed to evaluate, in an unprecedented manner: 1) whether and how N and P catchment loading may affect quasi-decadal variations in the pelagic phytoplankton biomass of a Mediterranean Sea Gulf (Gulf of Castellammare, Southern Tyrrhenian Sea), and 2) whether and how much additional N and P inputs released from off-shore fish farming in the same area have contributed to the observed variations. To achieve these objectives we: i) quantified the amount of N and P loads in the Gulf (using empirical and statistical data) coming from point (population, industry, terrestrial and marine farming) and non-point (uncultivated and cultivated soils) sources; ii) analysed the quasi-decadal fluctuations in phytoplankton biomass (expressed as chlorophylla concentrations in the seawater), and iii) analysed the difference in chlorophyll-a concentrations within the Gulf vs. those in open-sea waters, to understand if chlorophyll-a fluctuations occurred at the Gulf scale were due to local or basin-wide long-term changes of the whole Southern Tyrrhenian Sea.

## 2. Material and methods

## 2.1. The study area

The Gulf of Castellammare (Tyrrhenian Sea, surface  $\sim$  370 km<sup>2</sup>) is the widest inlet along the northern coast of Sicily (Fig. 1a). Bordered by rocky cliffs, it opens into shallow sandy shores with patches of rocky bottoms and interspersed seagrass beds. The area is influenced by limited continental inputs ( ~88,500  $\times$  10<sup>3</sup> m<sup>3</sup> y<sup>-1</sup>; about 600 times less than the Po River input in the northern Adriatic Sea and about 25 times less than the Simeto river inputs in the South Ionian Sea along the SE coasts of Sicily; Sarà et al., 1998, 2004, 2007; Modica et al., 2006). Those inputs are discharged by two of the four seasonal streams located in the central sector of the Gulf, namely the Nocella (13 km long, with a surface of about 15,000 ha) and the S. Bartolomeo (50 km long, 40,000 ha) streams (Fig. 1a). These two small rivers show only narrow fluctuations of discharge rates (on average  $0.32 \text{ m}^3 \text{ s}^{-1}$  and  $0.24 \text{ m}^3 \text{ s}^{-1}$  for the Nocella and S. Bartolomeo river, respectively; PTA, 2007) generally interrupted by seasonal rainfall episodes which cause short (i.e., a few days) flushing events (never exceeding 1.2 m<sup>3</sup> s<sup>-1</sup>; PTA, 2007). The whole drainage basin of the Castellammare Gulf includes 16 small municipalities (a total of about 175,000 inhabitants), of which six are located on the coast. The local population density is about 100 people per km<sup>2</sup> (ISTAT, 2005).

The economy of the Gulf catchment is based on the tertiary sector, including tourism and small industries (fishery processing), for about 60–70% of the local gross domestic product Agriculture,

pastoral, fish farming and fisheries account altogether for about 30-40% of the local gross domestic product (ISTAT, 2005; PTA, 2007). In the Castellammare Gulf, fisheries have played a major role in the local economy for many decades but only till 1990 (Badalamenti et al., 2000), when bottom trawling was banned from the area (Pipitone et al., 2000). One of the biggest Mediterranean aquaculture companies has been operating in the eastern area of the Gulf since 1999, producing about 600 t y<sup>-1</sup> of seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*) ever since (Mazzola et al., 2000; Sarà et al., 2007). Since 2000, a bluefin tuna farming industry (only fattening) located in the central sector of the Gulf (off the small harbour in Castellammare del Golfo; Fig. 1a; Messina et al., 2001) produces about 1000–1100 tons of farmed tuna per year (from July to December).

## 2.2. Long-term variations in chlorophyll-a, total N and P concentrations in the Castellammare Gulf and the adjacent open waters

We inventoried records of chlorophyll-a ( $\mu g L^{-1}$ ), total nitrogen  $(N, \mu M)$  and phosphorus  $(P, \mu M)$  concentrations in the upper (within 1 m depth) surface layer of the Gulf of Castellammare waters in the period 1970-2007. Data were obtained from research and oceanographic cruises carried out by several Italian Academic Institutions and Governmental Agencies as well as from our own data (Fig. 1a). Those data, consistently obtained using spectrophotometric techniques (chlorophyll-a: Lorenzen, 1965; total N and P: Valderrama, 1981), were averaged to obtain mean annual values and, though unevenly dispersed in space and time, used to assess inter-annual variations in the Gulf of Castellammare. Moreover, within the period 1998-2007, we inventoried also data of chlorophyll-a concentrations from five areas spread in the Southern Tyrrhenian Sea along the western coast of Sicily (Fig. 1b). These data, extracted from the Environmental Marine Information System (EMIS) published at http://emis.jrc.ec.europa.eu/4\_1\_gismap.php and maintained by the Joint Research Centre of the European Commission (2008), were used to compare inter-annual trends (1998–2007) in phytoplankton standing stocks inside and far from the Gulf of Castellammare (Fig. 1a,b).

## 2.3. Estimates of N and P loads entering the Gulf

Wasted loads (tons per year) of total N and P coming from the most important point (i.e., identifiable localized sources, such as resident and touristic cities/villages; industries; zootechny plants; fish farms; cattle, swine, sheep and goat farms) and non-point (i.e., diffuse sources, such as agriculture and uncultivated soils) sources were estimated according to the transformation coefficients proposed by Pagnotta and Barbiero (2003; Table 1) and adopted by the Italian Ministry of Public Health to assess the N and P loads along the Italian coasts. Since these coefficients were assumed to be constant across the year and since data about inputs coming from tourism are not available for the region, we are aware that our estimates of the N and P wastes entering annually the Castellammare Gulf from inland are affected by some uncertainty. Nevertheless, statistical data released by the Italian Statistical Institute (ISTAT), similarly to those released by Statistical Institutes of most Western European countries, are only available as annual means, so that we were not able to carry out our estimates on shorter (e.g., monthly or seasonal) temporal scales.

Data on resident population, industry, terrestrial farming and cultivated and non-cultivated soils in the study area were extracted from censuses carried out by the Italian Statistical Institute (ISTAT) in 1962, 1972, 1982, 1990, 2000 and 2007 (only population).

Estimates of nitrogen and phosphorus wastes released from seabream, seabass and bluefin tuna farms and entering the Castellammmare Gulf were based on individual gross metabolic waste outputs and coefficients of particulate and dissolved N and P released on a daily and biomass basis, as modelled in Piedecausa et al. (2010) and Aguado-Giménez et al. (2006). These models allow simulations of real rearing and husbandry practices. Gross metabolic waste output estimates for three cultivated species (i.e., seabream, seabass and tuna) in the Gulf were made on a nutritional basis, using the following bioenergetic model (Piedecausa et al., 2010):

$$\mathsf{C} = \mathsf{G} + \mathsf{E} + \mathsf{F}$$

where C is the nutrient (nitrogen, N and phosphorus, P) content in the food intake, G represents the retained nutrient as growth, E and F represent the excretory (dissolved) and faecal (particulate) losses, respectively. To calculate waste output coefficients of particulate and dissolved nitrogen and phosphorus on a daily and per biomass basis (mg N or mg P kg<sup>-1</sup> d<sup>-1</sup>), the nitrogen and phosphorus content in the diet and faeces, the feeding conversion rate and nitrogen and phosphorus digestibility were considered (Piedecausa et al., 2010).

## 2.4. Statistical analyses

Since we gathered data from very different sources, we were forced to analyse our hypotheses using a combination of different statistical techniques. Therefore, we used the t-Student (Sokal and Rohlf, 1981) to test differences in the measured variables among cruises and years, while the Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2001) was used to test the (null) hypothesis of no significant differences in chlorophyll-a concentrations between data collected during the cruises (spectrophometrically determined) and the data extracted from the EMIS GIS database (obtained by means of satellite sensors). The latter comparison was done separately for each year as well as for the data recorded before and after the launch of aquaculture activities in the Gulf. We applied the linear correlation approach to relate chlorophyll-a and nutrients concentrations (Sokal and Rohlf, 1981). The role of rainfall, water temperature and salinity on long-term (from 1980 to 2006) variations in chlorophyll-a annual mean concentrations was assessed by means of a distance-based multivariate multiple regression analysis with forward selection of

#### Table 1

Transformation coefficients used to calculate gross wastes from point and non-point sources into dissolved N and P wastes entering annually the Gulf. Modified from Pagnotta and Barbiero (2003) and Piedecausa et al. (2010).

	Waste coeffic	ient	Unit			
	Nitrogen	Phosphorus				
Point sources						
Resident and	6.16	0.92	g per person day <sup>-1</sup>			
touristic population						
Industry	27.4	0.09	g per employed day <sup>-1</sup>			
Cattle farming	7.51	1.01	g per individual day <sup>-1</sup>			
Swine farming	1.56	0.52	g per individual day <sup>-1</sup>			
Sheep and goat farming	0.68	0.11	per individual day <sup>-1</sup>			
Sebream farming <sup>a</sup>	298.9 (92.5)	12.2 (53.8)	mg kg <sup>-1</sup> fish <sup>-1</sup> day <sup>-1</sup>			
			(particulate)			
Seabass farming <sup>a</sup>	454.0 (21.5)	21.7 (50.7)	mg kg <sup>-1</sup> fish <sup>-1</sup> day <sup>-1</sup>			
			(particulate)			
Tuna farming <sup>a</sup>	852.0 (61.5)	65.5 (93.0)	$\mathrm{mg}\mathrm{kg}^{-1}\mathrm{fish}^{-1}\mathrm{day}^{-1}$			
			(particulate)			
Non point courses			er ar tour			
Non-point sources	5.40	0.07	MALEO MEL			
Uncultivated soil	5.48	0.27	g per nectare day			
Cultivated soll	43.8	1.04	g per day n			

<sup>a</sup> Mean values obtained considering different values for small (60 kg final weight) and big (140 kg final weight) tunas (from Piedecausa et al. 2010).

#### Table 2

Mean annual N loading (tons) entering the Gulf of Castellammare and percentages of each source to the total catchment. Data refer to the period 1970-2007.

	Tons per year						Percentage of total catchment				
	1970	1980	1990	2000	2007	1970	1980	1990	2000	2007	
Point sources											
Population	365.1	378.1	392.6	378.5	378.5	21.0	23.4	24.5	29.0	24.0	
Industry	37.0	38.8	48.2	40.0	40.0	2.1	2.4	3.0	3.1	2.5	
Seabass and seabream farm	0.0	0.0	0.0	0.0	84.9	0.0	0.0	0.0	0.0	5.4	
Tuna farm	0.0	0.0	0.0	0.0	190.0	0.0	0.0	0.0	0.0	9.5	
Cattle	15.4	21.7	13.4	4.9	4.9	0.9	1.3	0.8	0.4	0.3	
Swine	0.0	0.8	4.1	3.1	3.1	0.0	0.0	0.3	0.2	0.2	
Sheep and goat	0.0	7.8	16.1	7.6	7.6	0.0	0.5	1.0	0.6	0.5	
Non-point sources											
Cultivated	1306.5	1151.7	1115.9	858.0	858.0	75.2	71.3	69.5	65.8	54.3	
Not cultivated	14.6	15.5	15.3	12.1	12.1	0.8	1.0	1.0	0.9	0.8	
Total catchment	1738.5	1614.3	1605.5	1304.1	1579.0						
Total coastal towns	671.9	560.6	543.8	445.9	445.9						
% Coastal vs. catchment	38.7	34.7	33.9	34.2	28.2						
% Aquaculture vs. catchment	0	0	0	0	17.41						
% Aquaculture vs. coastal towns	0	0	0	0	61.65						

explanatory variables, using the DISTLM Fortran-written routine (McArdle and Anderson, 2001).

## 3. Results

## 3.1. Long-term changes in N and P loading from different sources

N wastes produced separately by all sources, with exception of agriculture, increased from 1970 to 1990 (by on average 15% for population, industry and zootechny), than all consistently decreased till 2007 (by about 30% on average; Table 2). P wastes derived from all sources increased in the period 1970-1990 (by on average 16% for population, industry and zootechny); P released by resident population and tourists decreased in 2000 (by 4% when compared with the preceding ten years average) while increased again in 2007. P released by industry, terrestrial farming and non-cultivated landscapes decreased from 1990 to 2007 (~32%; Table 3). N and P wastes derived from agriculture decreased almost constantly in the investigated period, with values in 2007 about 15% lower than in 1970. Generally, N and P wastes derived from diffuse sources (e.g., agriculture and non-cultivated landscapes) decreased almost continuously from 1970 to 2007 (by about 34% for both N and P), whereas wastes derived from point sources showed a biphasic temporal trend, increasing from 1970 to 1990 than decreasing again till 2007 (Fig. 2a,b).

Over the study period (1970–2007), the main sources of nitrogen and phosphorus were from cultivation (on average 1058 tons of N and 39.6 tons of P per year, representing  $\sim 67.2\%$  and 36.7% of total N and P inputs, respectively) and human population (378.5 tons of N and 56.9 tons of P per year, representing 24.4% and 52.4% of total N and P inputs, respectively).

In 1999, a fish farm producing about 500 tons per year of seabass (*D. labrax*) and seabream (*S. aurata*) was installed in the Gulf. While no data are available for the 1999, the total amount of N and P discharged every year from 2000 and 2007 in the Gulf by seabass, seabream and tuna fish-farming activities accounted up to 274.9 tons, representing about 17.4% and 34.2.5% of the relative N and P total inputs per year. These, in turn, represent about 51.5% and 122.0% of the N and P inputs derived from coastal discharge, respectively (Tables 2 and 3).

## 3.2. Temporal changes in chlorophyll-a in the Gulf

Dissolved nutrient concentrations in the Gulf, as extrapolated from field campaigns data, showed a steady increase in mean annual values from 1980 to date (Fig. 3). On the contrary, in the upper 10-m layer of the water column annual mean values of chlorophyll-a concentration, used here as a proxy of phytoplankton biomass, decreased between 1980 and 1990 (by 30%), and another significant (t-Student p < 0.05) drop was observed between 1991

#### Table 3

Mean annual P loading (tons) entering the Gulf of Castellammare and percentages of each source to the total catchment. Data refer to the period 1970–2007.

	Tons per year				Percentage of total catchment					
	1970	1980	1990	2000	2007	1970	1980	1990	2000	2007
Point sources										
Population	54.5	56.5	58.6	56.5	58.6	51.3	53.8	54.8	61.3	40.9
Industry	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Seabass and seabream farm	0.0	0.0	0.0	0.0	16.1	0.0	0.0	0.0	0.0	11.2
Tuna farm	0.0	0.0	0.0	0.0	33.0	0.0	0.0	0.0	0.0	23.0
Cattle	2.1	2.9	1.8	0.7	0.7	1.9	2.8	1.7	0,7	0.5
Swine	0.0	0.3	1.4	1.0	1.0	0.0	0.2	1.3	1,1	0.7
Sheep and goat	0.0	1.3	2.6	1.2	1.2	0.0	1.2	2.4	1.3	0.9
Non-point sources										ET.
Cultivated	48.9	43.1	41.8	32.1	32.1	46.0	41.1	39.0	34.8	22.4
Not cultivated	0.7	0.8	0.8	0.6	0.6	0.7	0.7	0.7	0.6	P-0.4
Total catchment	106.3	104.9	107.1	92.3	143.4					F. F. F.
Total coastal towns	44.1	41.0	41.7	37.2	37.2				M	L'AMA
% Coastal vs. catchment	41.5	39.1	39.0	40.3	25.9				\$ Q.	
% Aquaculture vs. catchment	0.0	0.0	0.0	0.0	34.2					8-
% Aquaculture vs. coastal towns	0.0	0.0	0.0	0.0	131.9				R 1 .01	



**Fig. 2.** Nitrogen (a) and phosphorus (b) loads released annually from point (resident and touristic population, industry and terrestrial farming) and non-point (agriculture and non-cultivated landscapes) sources in the Gulf of Castellammare.

 $(0.04 \ \mu g \ L^{-1})$  and 1995  $(0.01 \ \mu g \ L^{-1})$ . Chlorophyll-a concentrations have significantly risen again in the Gulf after 1998 (t-Student test between before and after 1998 values: p < 0.05), setting on constant values until 2001, when another sudden positive shift led to the maximum value over the entire study period (0.36  $\mu$ g L<sup>-1</sup> in 2006–2007) (Fig. 4). Such increase in phytoplankton biomass observed in the Gulf of Castellammare was not observed in any of the five areas outside the Gulf spread in the Southern Tyrrhenian Sea. Indeed, while in 1998 and 1999 chlorophyll-a concentrations increased consistently either in the Gulf and the open-sea area, showing not significant differences (PERMANOVA, p > 0.01; Fig. 4), from 2000 (about two years after the beginning of aquaculture operations), chlorophyll-a concentrations in the open-sea and the Gulf started to be significantly different (PERMANOVA, p < 0.05; Fig. 4). Accordingly, since 2000, chlorophyll-a concentrations followed the increase in N and P loading (Tables 2 and 3), and were significantly correlated to phosphate concentrations (Fig. 5).

## 4. Discussion

The most recent literature has provided consistent and repeated evidence that aquaculture can have remarkable effects on the surrounding environment with clearly detectable effects on the ecology of the benthos (Holmer et al., 2008a,b), but idiosyncratic effects on the ecology of the water column (Sarà, 2007a,b,c). Many of those investigations have proven that effects are detectable at a relatively short distance (i.e. hundreds of meters) from the farms, mainly because of the dilution effect promoted by the local currents (Sarà et al., 2006; Pusceddu et al., 2007; Mirto et al., 2010). However, given the well documented consistency of nutrient



Fig. 3. Annual means dissolved nitrogen (N tot,  $\mu$ M) and phosphorus (P-PO<sub>4</sub>,  $\mu$ M) concentrations in the surface waters of the Gulf of Castellammare from 1982 to 2007.

release from fish farms into the marine environment (Piedecausa et al., 2010) and the crucial importance of water currents in the dispersion of wastes (Pusceddu et al., 2007; Sanz-Lázaro et al., 2011), effects at large spatial scales may be reasonably expected in protected gulfs, bays and inlets, such as the area under scrutiny in this study.

We show here that the surface waters of the Castellammare Gulf have experienced large-scale and long-term variations in the nutrient and chlorophyll-a concentrations, mostly as the result of the chronic release of nutrient waste produced by local aquaculture. Between the 1970s and the 1990s, N and P loads from the Gulf of Castellammare catchment have constantly increased, though diminishing around 2000 (Tables 2 and 3). This is consistent with the socio-economic evolution of several Western Mediterranean and European countries over the last decades (Gürlük, 2009). The economic development of many Mediterranean coastal areas, led by tourism and resulting (among other effects) in the increased discharge of N and P into near-shore waters (Garau Taberner and Manera, 2006), was likely counteracted around 2000 by the effects of the European Union's (EU) Urban Wastewater Treatment Directive (91/271/EEC - 21 May 1991). The implementation and enforcement of the EU Directive has resulted in a significant amelioration of wastewater treatment before discharge into European rivers, and in measurable improvements in the water quality. This has in turn led to a decrease in point sources of N and P reaching near-shore waters in 2000. Contextually, agricultural runoff has significantly declined, reducing the discharge of N and P by at least 35% from the seventies to date (Tables 2 and 3). This has been probably due to the progressive shift of the local economy from agriculture to more profitable activities like tourism (Tyrakowski, 2004). Similar trends have been guite common in all North Mediterranean countries (Milesi et al., 2003), though in the North African countries the tendency appears to be inverse (e.g., agriculture, population and tourism are expected to increase till 2025, with effects still to be determined; Turley, 1999). Data for the Gulf of Castellammare do not strongly deviate from the common European scheme of the last decade. Indeed, suspended chlorophyll-a concentration was rather high in the 1980s, probably due to the almost total deregulation of N and P catchment loading, to the absence of wastewater treatment and to the intense bottom trawling activities carried out in the study area, which could have contributed to an enhanced mobilisation of benthic nutrients resuspended by fishing gears (Pusceddu et al., 2005). At the end of the eighties, important steps towards the correct management of natural resources in the catchment area included the regulation



**Fig. 4.** Chlorophyll-a ( $\mu$ g L<sup>-1</sup>) inside and outside the Gulf of Castellammare between 1982 and 2007. Data have been obtained from cruises (squares) and satellite data (filled circles, inside the Gulf; triangles, outside the Gulf). Open circles represent the average values between cruise and satellite data inside the Gulf. No chlorophyll-a from satellite data before 1998 are available.

and depuration of urban wastes released at sea and the ban on bottom trawling (since 1989; Pipitone et al., 2000). As a plausible immediate consequence, the Gulf waters dropped to an ultraoligotrophic status (i.e.,  $<0.1 \ \mu g$  chlorophyll-a L<sup>-1</sup>, according to the OECD scheme based on concentrations of chlorophyll-a; Zurlini, 1996; Sarà and Mazzola, 1997; Sarà et al., 1998), which lasted till 1999, when chlorophyll-a concentration in the surface waters of the Gulf steadily and significantly increased compared to open-sea baseline data sets (i.e., EMIS data). Between 2001 and 2007, the concentrations of chlorophyll-a in the surface waters of the Gulf were from 3 (0.15  $\mu$ g L<sup>-1</sup>, in 2001) to over 10 times (ca. 0.50  $\mu$ g L<sup>-1</sup>, as averaged for 2006–2007) higher than in the reference open-sea values. In the period 2004–2005 the concentration of chlorophylla in the eastern part of the Gulf was on average about 1.1  $\mu$ g L<sup>-1</sup>, but exceeded 1.5  $\mu$ g L<sup>-1</sup> (with frequent peaks over 3  $\mu$ g L<sup>-1</sup>) close to the aquaculture facilities (Sarà et al., 2009).

The divergent temporal patterns in chlorophyll-a concentrations in the two investigated systems might be attributed to a wide and complex array of explanatory variables and processes. Many reasons, indeed, could be invoked for explaining the rise in



**Fig. 5.** Relationship between chlorophyll-a and phosphates in the Gulf of Castellammare between 1980 and 2007.



Fig. 6. Temporal trend of rainfall in the Castellammare Gulf (PTA, 2007).

chlorophyll-a concentration in the surface waters of the Gulf which, among the others, include: i) increased terrestrial run-off due to rainfall; ii) increased release of nutrients from the sea bottom as affected by sediment resuspension induced by bottom trawling, iii) variations in global climate factors (e.g., temperature, storminess, etc.) and iv) increased nutrient loadings from local point and/or non-point sources. Over the last decades rainfall in the Gulf had a swinging trend (Fig. 6; data from SEDEMED II, 2009) and was accompanied by a general decrease of the number of wet days in Southern Italy (Brunetti et al., 2004). Nor rainfall or water temperature and salinity did explain significant proportions of chlorophyll-a variations in the Gulf (Table 4). Therefore, we can conclude that variations in the thermohaline conditions of the water column and in rainfall did not have crucial roles in modifying the trophic status of the Gulf waters. Bottom trawling was the main economic activity in the Castellammare Gulf until the ban in 1989 (Badalamenti et al., 2000; Pipitone et al., 2000). Disturbance of the seabed resulting from bottom trawling affects different ecosystem processes such as the rate and magnitude of nutrient regeneration (Pusceddu et al., 2005: Dounas et al., 2007). By cascade, plankton communities are expected to be influenced, which adds a pulsing component to local primary production (Dounas et al., 2007). The ban on bottom trawling in the Gulf (1989) could have led to a reduction of sedimentary nutrient contribution to the water column, so that we can exclude that the rise in nutrient and chlorophyll-a concentrations observed in the Gulf since 1999 was stimulated by bottom fisheries.

Along with a general temperature increase of sea surface waters, other climate-related factors like ocean acidification could invoked as additive macro-causes of the deviation of ecosystem functions (including primary production) from their common natural

#### Table 4

Results of the DISTLM analysis with permutations to test the role of rainfall, water temperature and salinity in explaining variations in chlorophyll-a concentrations in the period 1980–2006 in the Gulf of Castellammare. SS = sum of squares; pseudo-F = F statistic; P = probability level. Reported are the individual and cumulative percentages of variance explained by each variable.

Variable	SS	Pseudo-F	Р	Explained variance
				% Cumulative
Rainfall	0.010	0.708	0.402	4.81, 4.81
Water temperature	0.002	0.158	0.738	1,15 4 5.96
Salinity	0.004	0.224	0.704	1.72 7.68

patterns (IPCC, 2007). Although it has been shown that the Mediterranean Sea has experienced a significant increase in CO<sub>2</sub> water concentrations in the last 25 years (Touratier and Goyet, 2011), it must be noticed that, whatever the (physical or biological) mechanism involved, it can be reliably hypothesised that climate change in the study area couldn't have determined a general increase in primary production. On the one hand, this hypothesis is supported by the fact that an increase in sea surface temperature, as the one observed in the whole study area, could have led - under constant CO<sub>2</sub> concentration in the atmosphere, that is not the case (Touratier and Goyet, 2011) – to a decreasing CO<sub>2</sub> availability for primary producers (Harrison et al., 2005). On the other hand, phytoplankton possess C-concentrating mechanisms (counterbalancing CO<sub>2</sub> deficiency under warm temperature conditions; Giordano et al., 2005), and the eventual increase in CO<sub>2</sub> contents in the seawater may have had a mixture of relevant impacts on the availability of nutrients (Raven et al., 2005). Moreover, the temperature increase occurred in the whole Mediterranean Sea basin has considerably altered the water column stability and likely modified even the ecological response of deep fauna (Coma et al., 2009). Altogether these data suggest that if a change in primary production would have occurred in the study area, this has led most likely to a decrease or invariance rather than to an increase in chlorophyll-a concentrations in the Gulf. It must be noticed also that the expected diminished availability of nutrients under warmer conditions (Coma et al., 2009) might have favoured smaller phytoplankton taxa, with a consequently lower total primary production and C export to the sea bottom (Danovaro et al., 2000, 2001). Therefore, we conclude that changes in the nutrient loads from inland, rainfall, bottom trawling activities and other climate-related factors have had a minor role in explaining the increase in chlorophyll-a concentrations in the sea surface waters of the Gulf, as observed from 1999 onwards. Moreover, we would stress here that the huge and continuous in situ nutrient enrichment due to aquaculture in the Gulf could have reasonably been a major factor determining the observed divergence from the open-sea area. However, we also acknowledge that other local forcing factors (including circulation and altered food web characteristics), acting individually or synergistically, might have contributed to promote and maintain the observed shift in the productivity of Castellammare Gulf when compared with the adjacent open Tyrrhenian Sea. We also foster further research addressing these causes in future investigations. Nevertheless, the results of our study provide the first evidence that a long-term exposure of a certain coastal embayment to aquaculture wastes might result in a general modification of its trophic status (i.e. nutrient concentrations and phytoplankton biomass; sensu Pusceddu et al., 2009) at a spatial scale never considered before.

Our conclusions, on the one hand, give us the opportunity to stress the need of: i) improved bio-monitoring protocols to detect possible impacts of aquaculture at spatial scales larger than usual (i.e., >1-10 km from the farming structures) and ii) more appropriate temporal scales of investigation. On the other hand, our results provide an additional element of concern to be kept into consideration when, likely in the very next future, fish-farming activities will expand in off-shore waters, with a potentially expectable impact on the trophic status of vaster regions of the coastal oceans (Holmer, 2010).

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