

# **ECASA Study Site Report**

**Sounion**

**Greece**

**ECASA partners: UoC, UGOT**

**Authors:**

**Katerina Sevastou, Ioannis Karakassis**  
**University of Crete**

**Carina Erlandsson**  
**University of Göteborg**

## **Non Technical Summary** A 1-page summary in layman's language.

1. Sounion is located at the southern end of Attiki and it is open to the sea. However the existence of the islet Patroklos, near which the studied fish farm is located, creates a kind of strait, along which hydrodynamics are very intense. Maximum depth within the strait is 40m.
2. The cages are located in depths of approximately 16 m.
3. The site has been surveyed by IMBC in 1995 (two times), 1996 (once), 2003 (once), and by UoC in 2006 (once, ECASA survey) and in 2007.
4. One benthic and one water column transect were sampled during ECASA survey. Both transects were established towards the direction of the main current and they shared four stations in common, 0, 25, 50 m from the edge of the cages, plus the control station, ca 1 km upstream. In addition, the benthic transect included two stations at a distance of 5 and 10 m from the cages while the water column transect included two more stations at a distance of 100 and 200 m from the cages.
5. Sediment samples were collected using plexiglas corers of internal diameter 9.4 cm for macrobenthos use and 4.4 for the rest of the analyses (LOI, CHN, Chla, PSA, TOP, meiofauna). Water column samples were collected using Niskin bottles. Phytoplankton bioassay experiments were set up for five days at selected stations of the water column transect.
6. Measurement of nutrients, POC and PON concentrations in the water column was not an efficient method in tracing nutrient loading due to fish farming. The phytoplankton bioassay experiment was performing better, though not ideally, in detecting environmental effects on water column.
7. The coarse sediments of the site had very low levels of organic carbon and organic nitrogen (CHN analysis) as well as of organic matter (LOI methodology). The values of these variables were higher near the cages but they decreased away from the farm. The opposite pattern was observed for chl *a* concentrations in the sediment.

8. No gradient of redox potential was detected along the sediment transect.
9. Several of the macrobenthic univariate indices, such as macrofauna density, the indices Shannon, Evenness and AMBI, could detect a gradient of fish farm impact across the sampling transect.
10. Likewise, the meiofaunal variables N/C ratio and density of copepod nauplii were shown to be very useful for detecting fish farm environmental effects.
11. According to the index AMBI, the macrobenthic community under the cages was moderately disturbed, while further away as well as at the reference station the benthic community was characterized as slightly disturbed.
12. MOM model was used for estimating the environmental effect of the fish farm on Zone A. The model indicated that this site is well suited for fish farming due to the high current speed in the strait that is created between the islet of Patroklos and the land of Attiki.
13. The holding capacity of the farm is estimated to be about 720 tonnes/year, which is above the present production of 290-340 tonnes/year. The estimated holding capacity may further increase by separating the net pens even further and place them in single rows perpendicular to the main current direction.
14. Among the various indicators tested in this site, the following have been pointed out through the present study as the most suitable tools for monitoring environmental effects of fish farming in highly hydrodynamic areas of coarse sediments:
  - CHN analysis of organic carbon and nitrogen in surficial sediments
  - organic matter content as determined by LOI in surficial sediments
  - AMBI index
  - Shannon diversity
  - Pielou index  $J'$  of Evenness
  - macrofauna density
  - meiofauna Nematodes/Copepods ratio
  - meiofaunal copepod nauplii density.



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# **1 Introductory background statement**

## **The Company**

Kastelorizo S.A has 2 marine sites, one at Sitia-Crete and one at Sounion-Attiki with an average output for the period 2003-2005 of approximately 800 tonnes/year and 300 tonnes/year respectively. The company owns one hatchery (Sitia-Crete) as well, which is located at Sitia.

## **Field campaign**

In July 2006 a field survey was carried out by members of the Marine Ecology Laboratory of the UoC at one of the fish farm sites of Kastelorizo S.A. The site lies on the north coast of the islet Patroklos, which is located westerly to cape Sounion. Fieldwork lasted for a week (26/6-2006 – 1/7/2006), during which the field workplan that was established for the greek sites has been followed. In particular, the following activities have been carried out:

- Sediment sample collection (scuba-diving)
- Water column sample collection
- Secchi depth measurement
- CTD deployment
- Phytoplankton bioassay experiment

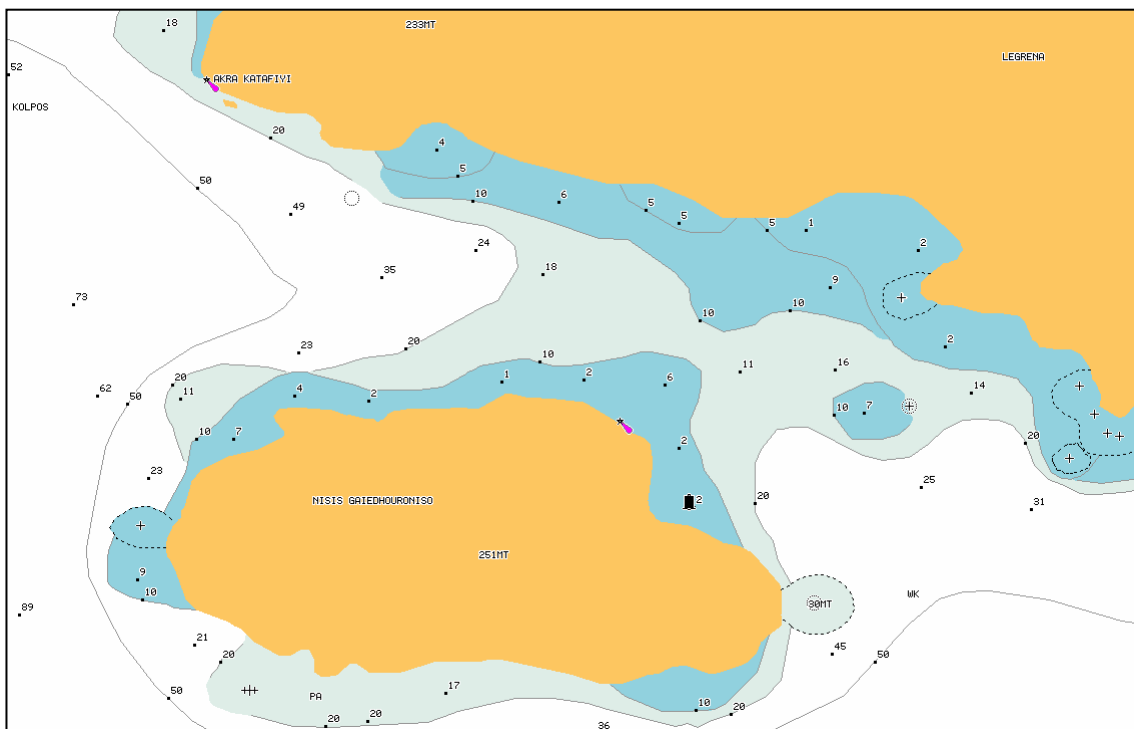
Prior to this visit as well as during the field campaign, UoC has been in contact with the manager of the fishfarm, Dimitra Koutavaki.

Sample processing and further laboratory analysis were carried out at the premises of the Hellenic Centre for Marine Research (HCMR) at Anavissos following the methodologies described in the Book of Protocols.

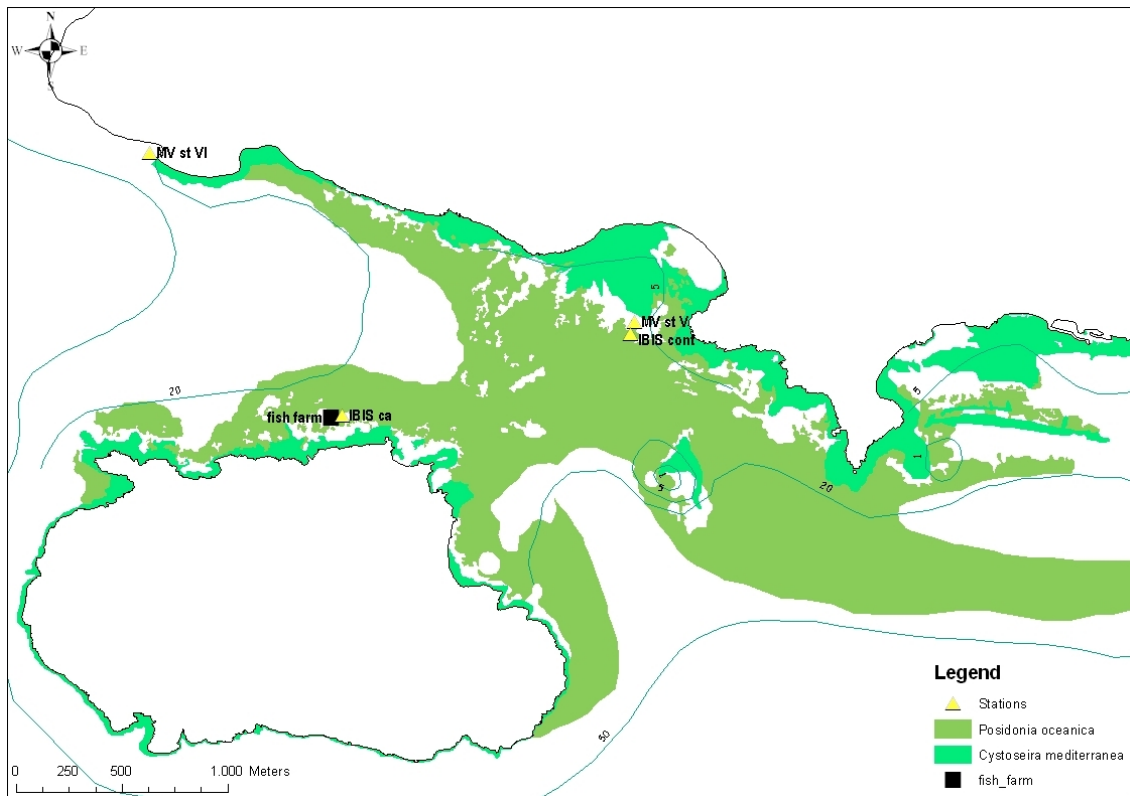
## **2 Site description**

### **2.1 Location**

Although most of the southern part of Attiki, where Sounion is located, is open to the sea, the existence of the islet Patroklos at the south-west of the area creates a kind of strait (**Figure 2.1**) along which hydrodynamic conditions are very intense. Current measurements that have been made periodically at the site by IMBC from July 1995 to January 1997, for at least a period of 2 months each time, indicated the existence of strong currents (average speed 3.4 cm/s, maximum 30.2 cm/s) mainly along the east-southeast axis. That leads to a homogenized water column throughout the year, with a minimum temperature of 14° C in winter and a maximum of 27° C in summer. Maximum depth in the strait is 40 m, while near the fish farm cages the depth ranges between 12-17 m. The area is characterized by very coarse sand and extensive meadows of *Posidonia oceanica* and to a lesser degree of *Cystoseira mediterranea* (**Figure 2.2**). Despite the presence of seagrass meadows, the area supports an intense local fishery. Records of the critically endangered mediterranean species of monk seal *Monachus monachus* exist for the area.



**Figure 2.1.** Map of the broader area of the studied site (courtesy of HCMR).



**Figure 2.2.** Map of seagrass habitats in Sounion area constructed under MedVeg project (courtesy of HCMR).

## **2.2 Proposed management strategy: biomass, medicines, chemicals, cycle, feed inputs, growth measurements.**

The production cycle for the fishes that reach a commercial size of 350 – 400 g is 14 – 15 months, while for only a small number of fishes that are grown up to 800 – 1000 g the production cycle is increased to approximately 2 years. The expected FCR is estimated between 1.8 and 2.0.

## **2.3 Physical farm logistics, including type of gear used (Cages, long lines, rafts...), moorings, access, lighting and anti-predator measures (use maps, and diagrams).**

During the ECASA survey the fish farm at Sounion consisted of:

- 4 circular cages of 100m perimeter
- 8 circular cages of 60m perimeter
- 4 circular cages of 50m perimeter
- 3 rectangular cages 6X6m
- 2 rectangular cages 12X12m

The cages nets extend down to 15m depth.

### **3 Results of ECASA field studies: Indicators and Models applied and evaluated.**

#### **3.1 Background to field programme: dates, staff, boats, stations sampled, etc.**

The ECASA field survey at Sounion was carried out during the period 26/6 – 1/7/2006 by the following members of UoC: Katerina Sevastou, Nafsika Papageorgiou, Ioanna Kalantzi, and it was supported by two divers, Sotiris Kiparissis and Ioulios Glambedakis. The fieldwork was according to the plan proposed for the greek sites. More precisely, two transects were established along the direction of the predominant current (160° NW-SE), one for water column measurements and one for sediment sample collection. With regard to the water column transect, five stations were located at the following distances from the edge of the cages: 0, 25, 50, 100, 200 m. The sediment sampling stations were located at 0, 5, 10, 25, 50 m distance from the edge of the cages. Besides the three stations that both transect shared in common (0, 25, 50 m) they also shared the same reference station which was located upstream at a distance of approximately 1 km. Basic information on the sampling stations is provided at **Table 3.1**.

Fieldwork involved the following activities:

**Sediment sample collection (scuba-diving).** Plexiglas corers of 4.4 cm internal diameter were used for collecting sediment for POC, PON, POP, LOI, chlorophylls and granulometry analyses as well for the *in situ* measurement of Redox. Sediment cores were sliced in 1 cm layers down to a depth of 12 cm and stored in -20°C. 3 extra sediment cores were collected for meiofauna analysis. 5 replicated sediment cores were collected with bigger diameter corers (Ø 9.4 cm) for use in macrofauna analysis and were sieved in the field through a double set of sieves (upper: 1mm mesh sieve size, lower: 0.5mm mesh sieve size) for testing the BFI index.

**Water column sample collection.** Water samples were collected using Niskin bottles for measuring concentrations of dissolved nutrients (ammonia, nitrate, phosphate, silicate), POC, PON and chlorophylls.

**Secchi depth measurement** for estimating the water turbidity

**CTD deployment** for estimating the vertical profile of water temperature, salinity and chlorophyll at different distances from the cages.

**Bioassay experiment** set up for assessing nutrient levels around the fish farm.

**Table 3.1.** Information on stations and sampling during ECASA survey

Station (Distance from the cages, m)	Depth (m)			Water Column sampling date	Bioassays deployment	Bioassays retrieval	Sediment sample collection
		Latitude	Longitude				
0	16,7	37 39.577	23 57.303	30/60/2006	26/6/2006	1/7/2006	29/6/2006, 10:10:00
5	16,9	37 39.578	23 57.305				27/6/2006, 9:50:00
10	16,1	37 39.579	23 57.307				27/6/2006, 9:50:00
25	16,9	37 39.581	23 57.313	30/60/2006	26/6/2006	1/7/2006	29/6/2006, 10:10:00
50	17,1	37 39.585	23 57.323	30/60/2006	26/6/2006	1/7/2006	27/6/2006, 17:00:00
100	15	37 39.592	23 57.343	30/60/2006			
200	15	37 39.608	23 57.384	30/60/2006			
Reference	12,5	37 40.176	23 56.543	30/60/2006	26/6/2006	1/7/2006	29/6/2006, 15:20:00

### **3.2 Sampling methods and materials, analytical methods. (Refer to the book of protocols for detailed methods)**

Field and laboratory work was carried out according to the appropriate methodologies described in the Book of Protocols.

### **3.3 Models used and their parameterization.**

#### **3.3.1 The MOM model**

The model estimates the environmental effects of fish farming on local scale (zone A). The MOM model can be used to calculate the holding capacity (TPF-Total Fish Production) of an area for fish farming. The model calculations are based on requirements on the water quality for fin fish in the cages, and for the benthos beneath the cages. The environmental effects of fish farming on the surrounding water and on the water quality in the farm are calculated as oxygen and ammonium concentrations in the farm and oxygen concentrations at the bottom below the farm. The model contains four sub-models; a fish model, a cage water quality model, a dispersion model, and a benthic model. The model also calculates the nutrient release from the farm to the surface water. The holding capacity is expressed as the minimum of the TPF based on oxygen and ammonium concentrations in the cages and oxygen concentration of the bottom water (i.e. of TPFO<sub>2</sub>, TPFNH<sub>4</sub>, TPFbent). Besides TPF, outputs of the model are also: the theoretical food coefficient, energy content of the

food, the length of the production cycle assuming optimal conditions at the site, median weight of the fish during the production cycle, maximal carbon flux to the sediment, outlet of dissolved and particulate matter per tonne of fish (expressed as nitrogen and phosphorus). The model results can thus be used to possibly increase the holding capacity at a site. The two basic criteria in the MOM model are:

1) The accumulation of organic matter under and in the vicinity of the fish farm must not result in extinction of the benthic macro fauna ( $O_2 > 2$  ml/l). This condition is met if the flux of organic matter from the farm is adjusted to local dispersion conditions so that the decomposition capacity of the benthic system is not exceeded.

2) The water quality in the net pens must meet the needs of the fish. This means that the concentration of oxygen is kept above the threshold level and that the concentration of ammonium and other potentially harmful substances are kept below the threshold values. These conditions can be met if the respiration of and emissions from the fish are adjusted to the rate of water renewal in the net pens.

#### Current characteristics

Three different characteristics of the current speed are needed to calculate the environmental effects in and below the farm:

1)  $U_{min}$  - the dimensioning current speed in the surface water which is calculated based on the longest time it takes for the water to pass through the group of cages. It is estimated from current measurements at the level of the farm. This dimensioning current speed is used to calculate the lowest/highest concentration of oxygen/ammonium expected in the net pens.

If the current measurements are performed before the establishment of the farm or away from the farm, a reduction factor should be applied simulating the effect of current resistance caused by the cages and algae growing on the net pens.

2)  $U_{std}$  - the fluctuation component of the current which is calculated as the standard deviation of the current component perpendicular to the farm below the cages. The fluctuation component determines the spreading of organic matter from the farm. The fluctuating component is also used to estimate whether resuspension of sediment will occur in the farm area, which is the case for  $U_{std} > 3.5$  cm/s. A value of

Ustd > 3.5 cm/s suggests that current speeds of more than 10 cm/s occur in the area (Stigebrandt and Aure 1995), which have been found to be the threshold current speed value for resuspension of organic matter from fish farms (e.g. Cromey et al. 2002).

3)  $U_{bent}$  – the dimensioning current in the bottom layer which is calculated as the minimum mean speed determined from the bottom current record. This characteristic is used to estimate the minimum oxygen transport from the water to the sediment below the cages.

### Model input data

#### *Background*

Three groups of cages are located along the coast of Sounion on the southern side of the strait between the islet of Patroklos and the main land. The dominating current is in the direction of the strait, which is ESE (100 degrees) with a mean speed of 6.5 cm/s at 10 m depth. The value of  $U_{std}$  at this site is high and above 3.5 cm/s, indicating only temporary sedimentation of organic matter below the farm. 80 % of the production at this site is sea bream, but we have no information in which of the cage groups the two species are farmed. The MOM model is run on three cage groups with sea bream in group 1 and 2, and sea bass in group 3 which is somewhat larger. The water depth is 17 m. The cage groups are run separately. The number of cages in each group and the farm layouts are presented in **Table 3.2**.

The total production of the farm is 290-340 ton/year, with a production cycle of 14-15 months for an end weight of 350 g. The fish (80% sea bream) are given feed with protein content of 43-56%, and the real food conversion,  $FCR_{real}$ , ratio is 2.0.

**Table 3.2.** Farm layout

Site	No. cages	No. rows	diameter/ side length (m)	Spacing (m)	Water depth (m)	Reduction of flow factor (0-1)	Farm direction (deg)
Sounion 1	9	3	19 (d) & 13	20	17	0.8	90
Sounion 2	12	2	12	2	17	0.8	90
Sounion 3	15	2	15	2	17	0.8	90

**Table 3.3.** Surrounding water characteristics

Site	Sal.	Temp.	Ox.	Amm.	<b>Currents</b>	$U_{min}$	$U_{std}$	$U_{bent}$
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	(psu)	(C)	(mg/l)	(mg/l)	(cm/s)			
Sounion 1	38	14-25	5	0.003		0.7	5.8	1.1
Sounion 2	38	14-25	5	0.003		0.45	5.8	1.1
Sounion 3	38	14-25	5	0.003		0.55	5.8	1.1

**Table 3.4.** Fish data (from Cephalonia, Greece, Magill (Thetmeyer) et al. 2006)

site	Fish	Start weight (g)	End weight (g)	Protein cont. (0-1)	Fat cont. (0-1)	Sinking speed of faeces (cm/s)
Sounion 1	Sea bream	10	380	0.2	0.04	0.5
Sounion 2	Sea bream	10	380	0.2	0.04	0.5
Sounion 3	Sea bass	10	380	0.2	0.04	0.7

**Table 3.5.** Food data

	Protein cont. (0-1)	Fat cont. (0-1)	Carbohydrate cont. (0-1)	Ash cont. (0-1)	Sinking speed of faeces cm/s	FCR <sub>teo</sub> /FCR <sub>real</sub>
Sounion 1	0.43	0.22	0.02	0.07	8	1.29/2.0
Sounion 2	0.43	0.22	0.02	0.07	8	1.21/2.0
Sounion 3	0.43	0.22	0.02	0.07	8	1.25/2.0

## 3.4 Results.

### 3.4.1 Water

The water column at the vicinity of the fish farm cages and at the reference station was very well homogenized during the time of CTD deployments (**Table 3.6**), while the use of Secchi disk indicated very transparent waters down to the bottom of all the water column stations.

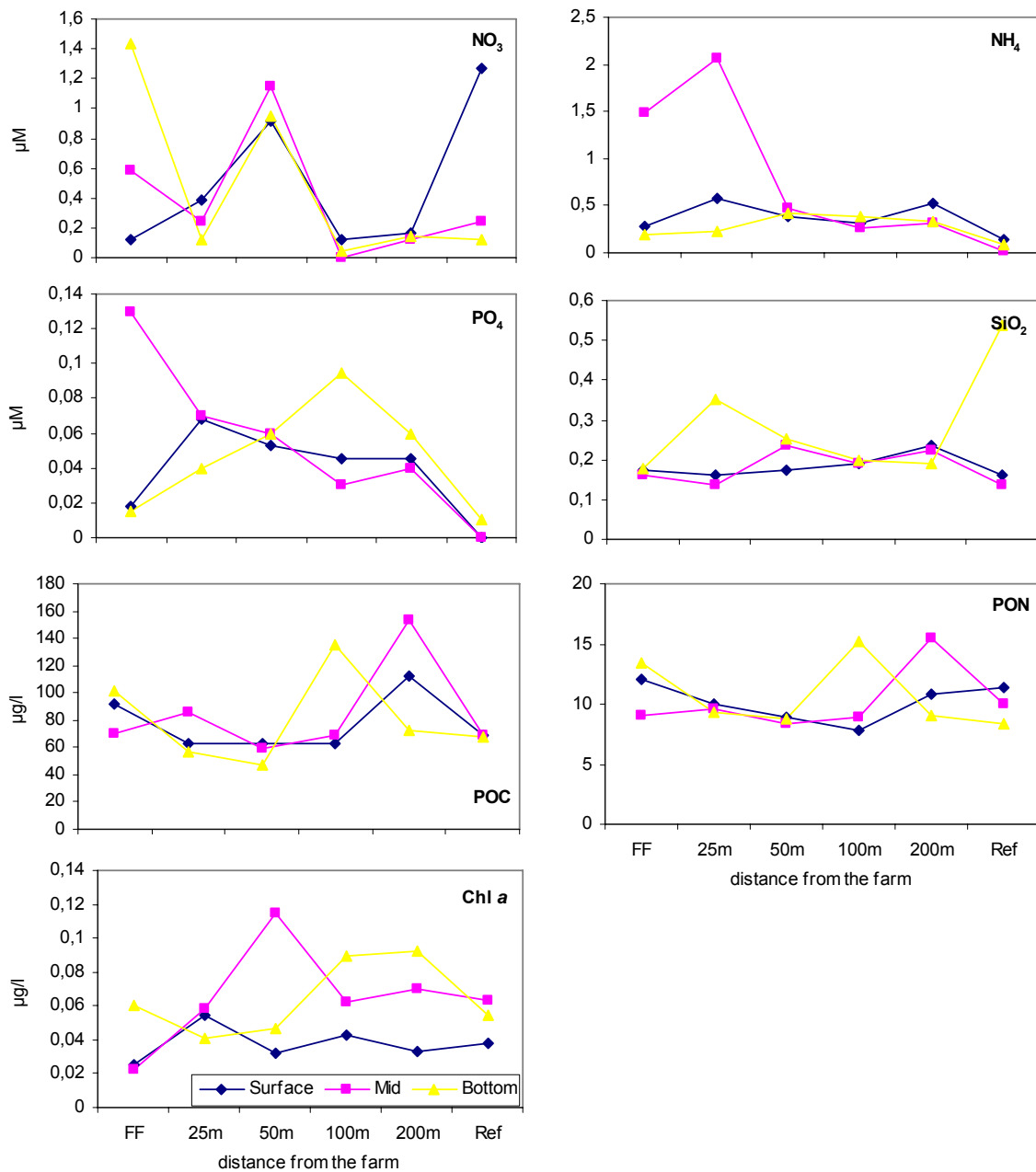
Nutrient, POC, PON and chlorophyll concentrations were measured at three different depths in the water column (**Table 3.7**); however no obvious trend could be detected for any of the measured variables with regard to both their horizontal and vertical distribution (**Figure 3.1**).

**Table 3.6.** Minimum, average and maximum values of temperature, salinity and particulates in the water column of the sampling stations.

Distance from the farm	FF	25m	50m	100m	200m	Reference
<i>Temperature (°C)</i>						
Minimum	23,13	23,12	23,11	23,15	23,24	23,09
Average	23,45	23,48	23,47	23,52	23,62	22,50
Maximum	23,79	23,79	23,82	23,80	23,86	23,66
<i>Salinity (‰)</i>						
Minimum	37,11	37,13	37,18	37,22	37,26	37,28
Average	36,21	36,51	36,59	36,22	37,04	36,09
Maximum	37,55	37,63	38,93	37,81	38,18	37,70
<i>Particulates (umol/m<sup>2</sup>)</i>						
Minimum	555,30	309,30	274,60	515,30	517,00	266,20
Average	955,22	763,69	413,79	1011,48	952,27	615,35
Maximum	4556,00	4549,00	1211,00	4798,00	2601,00	1516,00

**Table 3.7.** Nutrient, chl-*a*, POC and PON concentrations at three different water column depths (Surface: -1m, Mid: ~ 10m, Bottom: 1m above the bottom) at different distances from the cages

		Distance from the farm					
		FF	25m	50m	100m	200m	Reference
WATER COLUMN DEPTH	<b>NO<sub>3</sub> (µM)</b>						
	Surface	0,12	0,38	0,91	0,12	0,17	1,27
	Mid	0,59	0,24	1,15	0,00	0,12	0,24
	Bottom	1,44	0,12	0,95	0,05	0,15	0,12
	<b>NH<sub>4</sub> (µM)</b>						
	Surface	0,29	0,58	0,38	0,31	0,53	0,15
	Mid	1,48	2,05	0,47	0,27	0,31	0,01
	Bottom	0,19	0,23	0,42	0,39	0,34	0,08
	<b>PO<sub>4</sub> (µM)</b>						
	Surface	0,02	0,07	0,05	0,05	0,05	0,00
	Mid	0,13	0,07	0,06	0,03	0,04	0,00
	Bottom	0,02	0,04	0,06	0,10	0,06	0,01
	<b>SiO<sub>2</sub> (µM)</b>						
	Surface	0,17	0,16	0,17	0,19	0,23	0,16
	Mid	0,16	0,14	0,23	0,19	0,23	0,14
	Bottom	0,18	0,35	0,25	0,20	0,19	0,54
	<b>Chl a (µg/l)</b>						
	Surface	0,02	0,05	0,03	0,04	0,03	0,04
	Mid	0,02	0,06	0,11	0,06	0,07	0,06
	Bottom	0,06	0,04	0,05	0,09	0,09	0,05
	<b>POC (ug/l)</b>						
Surface	91,99	62,26	62,61	63,39	111,95	69,20	
Mid	69,91	85,75	59,34	69,01	153,58	68,26	
Bottom	100,95	56,42	47,69	134,94	72,45	67,99	
<b>PON (µg/l)</b>							
Surface	12,09	10,04	8,88	7,79	10,82	11,39	
Mid	8,97	9,57	8,35	8,85	15,51	10,02	
Bottom	13,44	9,38	8,78	15,23	9,04	8,39	



**Figure 3.1.** Nutrient, chl-*a*, POC and PON concentrations at three different water column depths (Surface: -1m, Mid: ~ 10m, Bottom: 1m above the bottom) for each of the water column sampling stations.

### 3.4.2 Sediment

The well oxygenated sediments of the area (**Table 3.8**,  $E_h > 0$  for all stations) consist of very coarse sand with a relatively high percentage of gravels, which is decreasing with increasing distance from the cages but reaches its maximum at the control station (>30%). (**Table 3.8**, **Figure 3.2**).

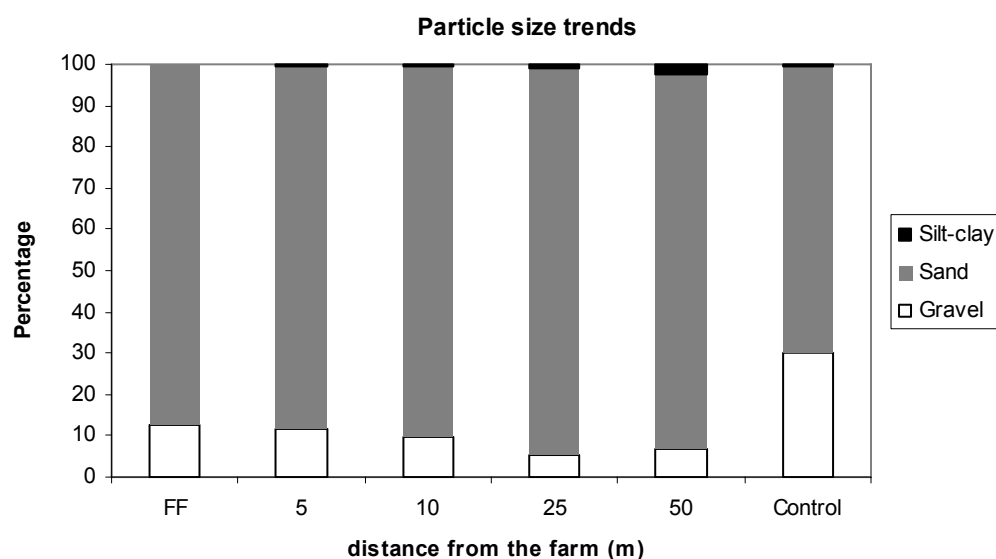
Both organic carbon, nitrogen and phosphorus concentrations in the upper layer of the sediment (0-3cm) are lower at the reference station and peak close to the cages (under

the cages, at 5m and at 10m respectively) (**Table 3.8**). Chl *a* values are lower at the reference station as well, however, its concentrations increase with increasing distance from the cages (**Table 3.8**).

**Table 3.8.** Sediment variables

Station	Gravel (%)	Sand (%)	Silt-clay (%)	Eh (0-2cm) (mV)	TOC (0-3cm) (µg/g)	TON (0-3cm) (µg/g)	TOP (0-3cm) (µg/g)	Chl-a (0-3cm) (µg/g)
<i>FF</i>	12,71	87,20	0,09	194,67	7,94	1,37	0,15	0,84
5	11,65	87,78	0,58	264,50	16,61	1,11	0,20	0,73
10	9,52	89,83	0,65	63,50	10,06	0,71	0,33	1,11
25	5,46	93,75	0,79	353,00	7,71	0,76	0,20	1,26
50	6,74	90,69	2,57	213,50	8,26	0,88	0,14	1,56
<i>Control</i>	30,10	69,49	0,41	381,00	1,42	0,29	0,01	0,40

Organic matter (OM) content as determined by LOI was very low in the sediment of all stations (2.5 – 6.5 %) (**Table 3.9**); however, OM content was quite higher near the cages and decreased at a distance > 50 m, therefore indicating the existence of a slight gradient of impact due to fish farming. Rp values ranged between 0.57 – 0.83 indicating sediments characteristic of protein and polyphenolic compounds (Kristensen 1990).



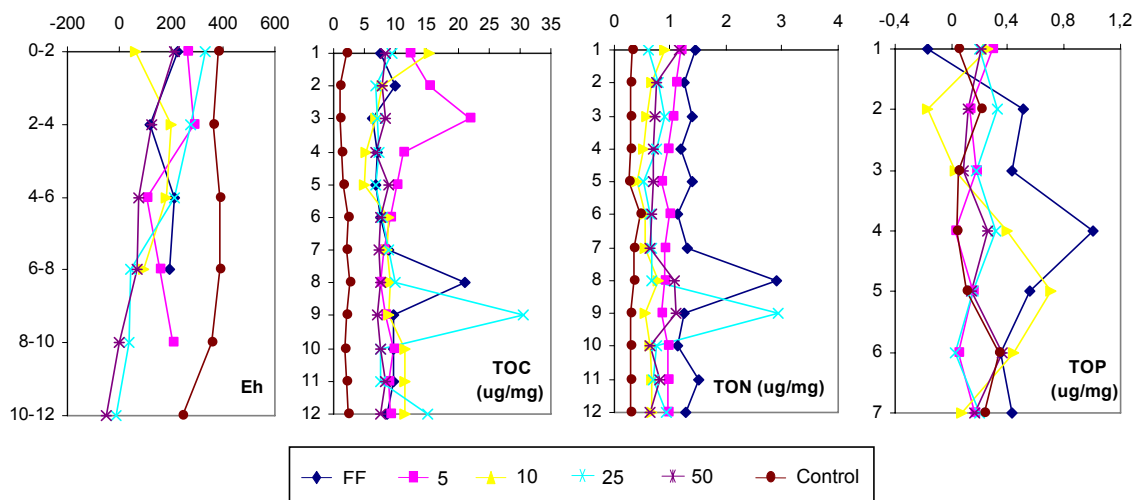
**Figure 3.2.** Particle size trends across the sampling transect and at the reference station.

**Table 3.9.** Percentage of organic matter in the sediment (using LOI methodology).

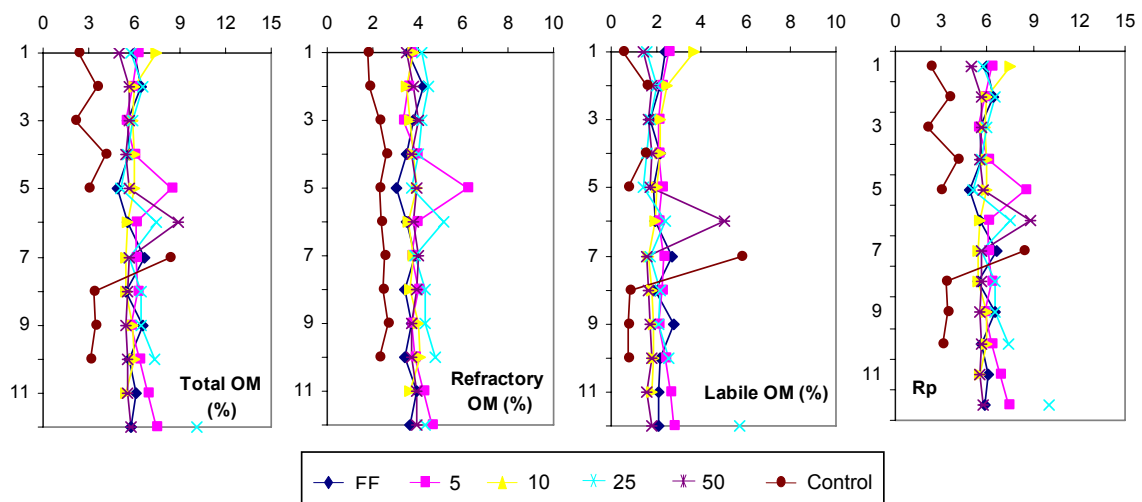
	Distance from the farm					
	<i>FF</i>	5	10	25	50	<i>Control</i>
<b>Total Organic Matter</b>	5,99	5,86	6,45	6,06	5,42	2,64
<b>Refractory</b>	3,86	3,56	3,66	4,27	3,78	1,98
<b>Labile</b>	2,13	2,30	2,79	1,79	1,64	0,66
<b>Rp</b>	0,64	0,61	0,57	0,71	0,70	0,83

### Vertical profile

The vertical profiles of Redox, TOC, TON, TOP and organic matter in the sediment were studied at all stations (**Figures 3.3, 3.4**). Sediments of all stations were very well oxygenated down to a depth of 10 to 12 cm as it was indicated by Eh values. Organic carbon and nitrogen values were similar at all sediment depths though higher values at one deeper sediment layer were recorded for stations 0, 5 and 25m. No pattern was revealed for the vertical profile of TOP and OM.



**Figure 3.3.** Vertical profile of Redox, TOC, TON and TOP for each of the sampling stations.



**Figure 3.4.** Vertical profile of total organic matter, refractory and labile percentages and Rp index for each of the sampling stations.

### 3.4.3 Macrofauna

97 macrofauna species were found in Sounion, 41% of which belong to Polychaeta (Figure 3.5). This was also the dominant taxon in terms of abundance (Table 3.10). Density ranged between 13 985 ind/m<sup>2</sup> at the control station and 85 063 ind/m<sup>2</sup> at a distance of 10 m from the edge of the cages. The most dominant species and their density changes across the sampling transect are presented in Table 3.11. Polychaeta species predominated at all stations, however, close to the cages (stations 0 and 5 m) an oligochaeta species was the most dominant one. Although *Capitella capitata* contribution to the macrofauna abundance was low at all stations (< 17%), and it was not encountered at the control station, its density was quite higher close to the cages (stations 5 and 10 m), indicating thus more disturbed conditions near the fish farm.

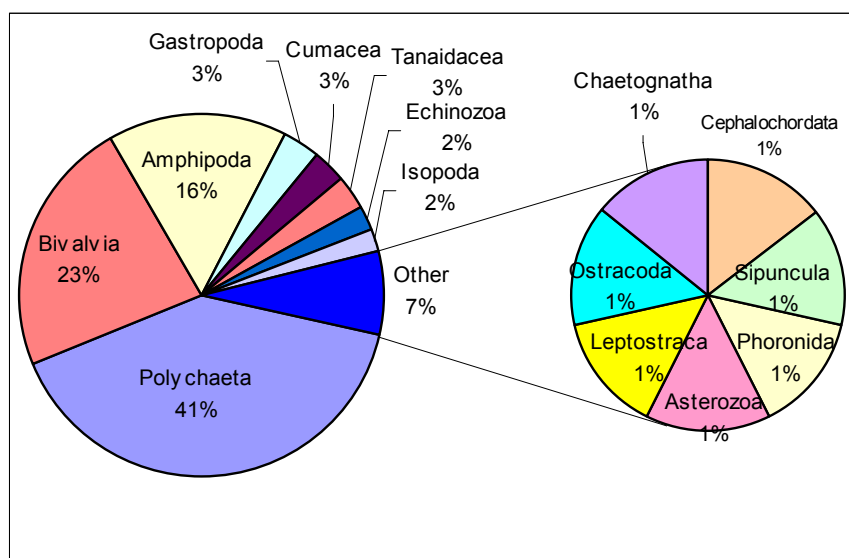


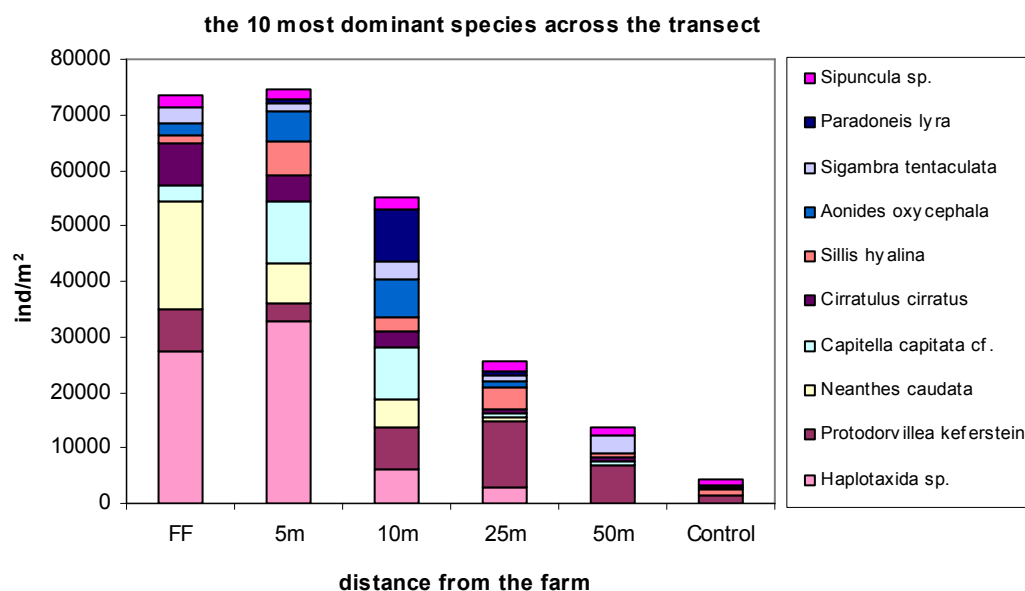
Figure 3.5. Contribution of major taxa encountered in Sounion fish farm.

Table 3.10. Density of macrofauna taxa (ind/m<sup>2</sup>, sum of 5 replicates).

Taxon	Distance from the farm						TOTAL
	FF	5m	10m	25m	50m	Control	
Bivalvia	2595,16	6776,24	14561,71	6199,54	7208,77	1585,93	38927,34
Gastropoda			144,18	576,70	288,35		1009,23
Polychaeta	54642,45	55651,67	65167,24	32871,97	24077,28	7641,29	240051,90
Phoronida	432,53	432,53	144,18	432,53			1441,75
Asterozoa		576,70	144,18	288,35			1009,23
Echinozoa				865,05	144,18		1009,23
Amphipoda	144,18	1441,75	1153,40	5190,31	5334,49	2883,51	16147,64
Cumacea		576,70	865,05	2739,33	720,88	144,18	5046,14
Isopoda		288,35	144,18			144,18	576,70
Leptostraca		288,35		144,18	144,18		576,70
Ostracoda		288,35	144,18	1297,58	1009,23	288,35	3027,68
Tanaidacea	144,18		144,18	432,53	144,18		865,05
Chaetognatha			432,53				432,53
Cephalochordata				144,18	144,18	288,35	576,70
Sipuncula	2162,63	1874,28	2018,45	1874,28	1585,93	1009,23	10524,80
<b>TOTAL</b>	<b>60121,11</b>	<b>68194,93</b>	<b>85063,44</b>	<b>53056,52</b>	<b>40801,61</b>	<b>13985,01</b>	<b>321222,61</b>

**Table 3.11.** Density of the 10 most dominant species at Sounion fish farm (ind/m<sup>2</sup>, sum of 5 replicates).

Species	Distance from the farm						TOTAL
	FF	5m	10m	25m	50m	Control	
Haplotaxida sp.	27249,13	32727,80	6055,36	2739,33			68771,63
Protodorvillea kefersteini	7785,47	3316,03	7641,29	11966,55	6776,24	1297,58	38783,16
Neanthes caudata	19319,49	7064,59	5046,14	865,05	144,18	288,35	32727,80
Capitella capitata cf.	3027,68	11245,67	9227,22	720,88	720,88		24942,33
Cirratulus cirratus	7352,94	4757,79	3027,68	576,70	576,70		16291,81
Sillis hyalina	1730,10	6055,36	2595,16	4036,91	720,88	865,05	16003,46
Aonides oxycephala	1874,28	5334,49	6776,24	1009,23	144,18	432,53	15570,93
Sigambra tentaculata	2883,51	1730,10	3171,86	1297,58	3171,86	144,18	12399,08
Paradoneis lyra	144,18	576,70	9515,57	576,70		144,18	10957,32
Sipuncula sp.	2162,63	1874,28	2018,45	1874,28	1585,93	1009,23	10524,80



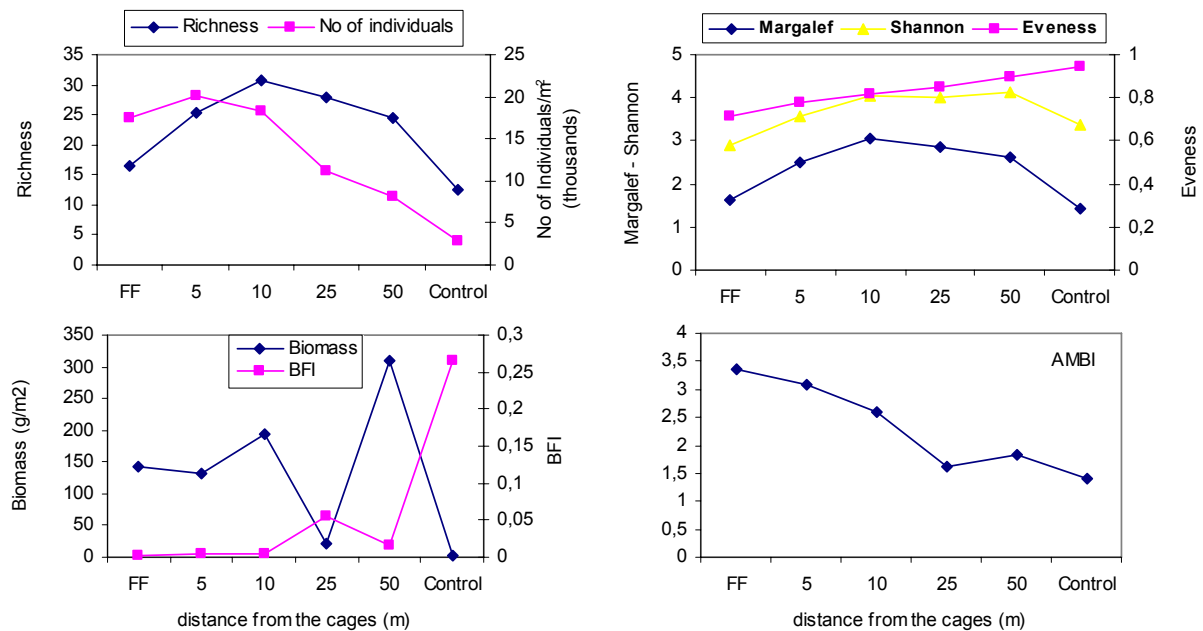
**Figure 3.6.** Density of the 10 species dominating in Sounion fish farm.

Diversity indices such as Richness, Shannon and Margalef were quite low very close to the fish cages (0 and 5 m) as well as at the control station, while maximum value was calculated at 10 m distance from the edge of the cages for Richness and Margalef and at 50 m for Shannon, an index which was recorded to increase with increasing distance from the cages (**Table 3.11, Figure 3.6**). The same trend was also observed for Pielou's evenness index  $J'$ , while the opposite pattern was recorded for macrofauna density and AMBI values. AMBI index indicated slightly disturbed conditions even at the control station, nevertheless, higher level of disturbance was recorded only under the cages (5 and 10m), where macrbenthic community was characterized as moderately disturbed. It is worth noting that the first order

opportunistic species (EG-V) are at all stations at very low percentages (<18%), even under and very close to the cages (0 and 5m stations), where second order opportunistic species and indifferent species dominate. Thus, we may conclude that even near the farm the macrobenthic community is rather at an intermediate stage of ecological succession.

**Table 3.12.** Macrofaunal variables for Sounion fish farm. (EG: Ecological group).

Index	Distance from the farm					
	FF	5m	10m	25m	50m	Control
Density (ind/m <sup>2</sup> )	17474,05	20184,54	18223,76	11159,17	8160,32	2797,00
Biomass (g/m <sup>2</sup> )	143,92	131,89	193,11	22,85	309,33	1,86
Richness	17	25	31	28	24	12
Margalef	1,62	2,48	3,04	2,86	2,60	1,44
Evenness	0,72	0,78	0,82	0,85	0,90	0,94
Shannon	2,89	3,57	4,04	4,00	4,12	3,39
BFI	0,00	0,01	0,00	0,05	0,02	0,27
AMBI	3,36	3,08	2,58	1,63	1,84	1,39
EG-I (%)	8,20	16,00	21,80	23,80	31,00	46,30
EG-II (%)	24,70	29,00	22,20	53,80	36,20	24,20
EG-III (%)	7,70	11,70	28,40	13,70	17,50	18,90
EG-IV (%)	54,40	26,00	16,70	7,00	13,10	10,50
EG-V (%)	5,00	17,30	11,00	1,70	2,20	0,00



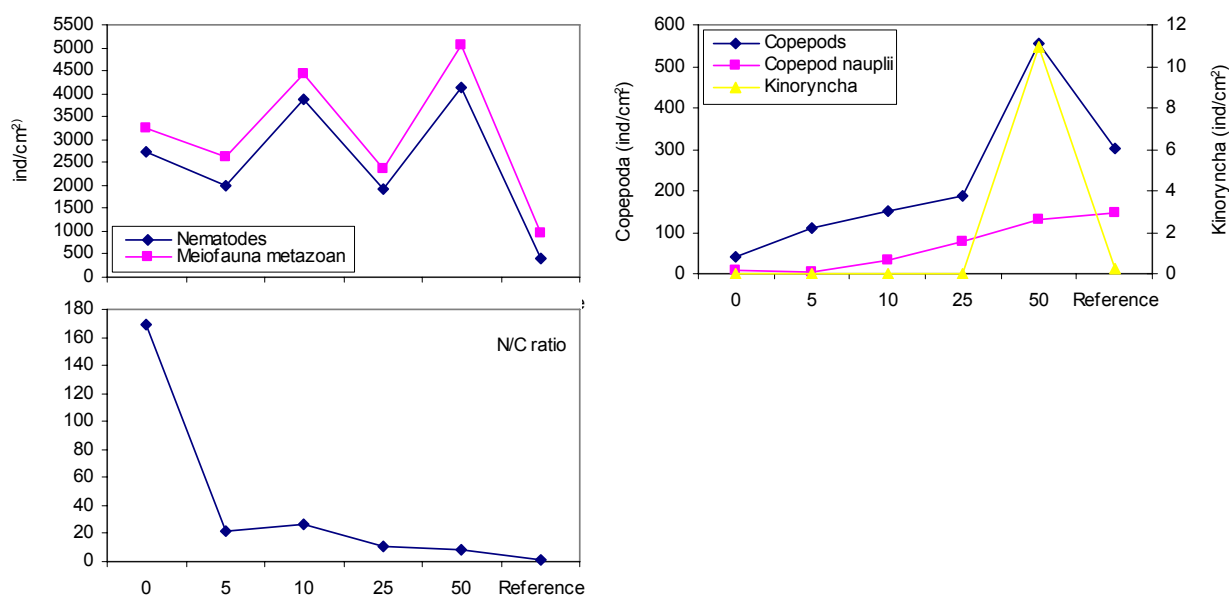
**Figure 3.7.** Macrofauna variables and indices across the sampling transect at Sounion.

### 3.4.4 Meiofauna

The abundance of the major meiofaunal metazoan taxa as well as the Kinoryncha density and the Nematodes/Copepods ratio (N/C ratio), which have been suggested by some researchers as tools for assessing organic pollution (Raffaelli et al. 1991, Mazzola et al. 2000) are presented in **Table 3.13**. As it was expected, meiofaunal density showed the same spatial pattern as nematode abundance (**Figure 3.8**), with very low values (<1000 and 400 ind/cm<sup>2</sup> respectively) at the control station and higher density at 50 m distance from the cages. Copepod and copepod nauplii density appears to increase with increasing distance from the cages (**Figure 3.8**), while Kinoryncha are absent up to a distance of 25 m from the cages, reaching their maximum density at the station of 50m. N/C ratio is very high (>160) under the cages, but further away it decreases greatly and at the control station it does not exceed 1.5 (**Figure 3.8**).

**Table 3.13.** Meiofauna variables across the sampling transect.

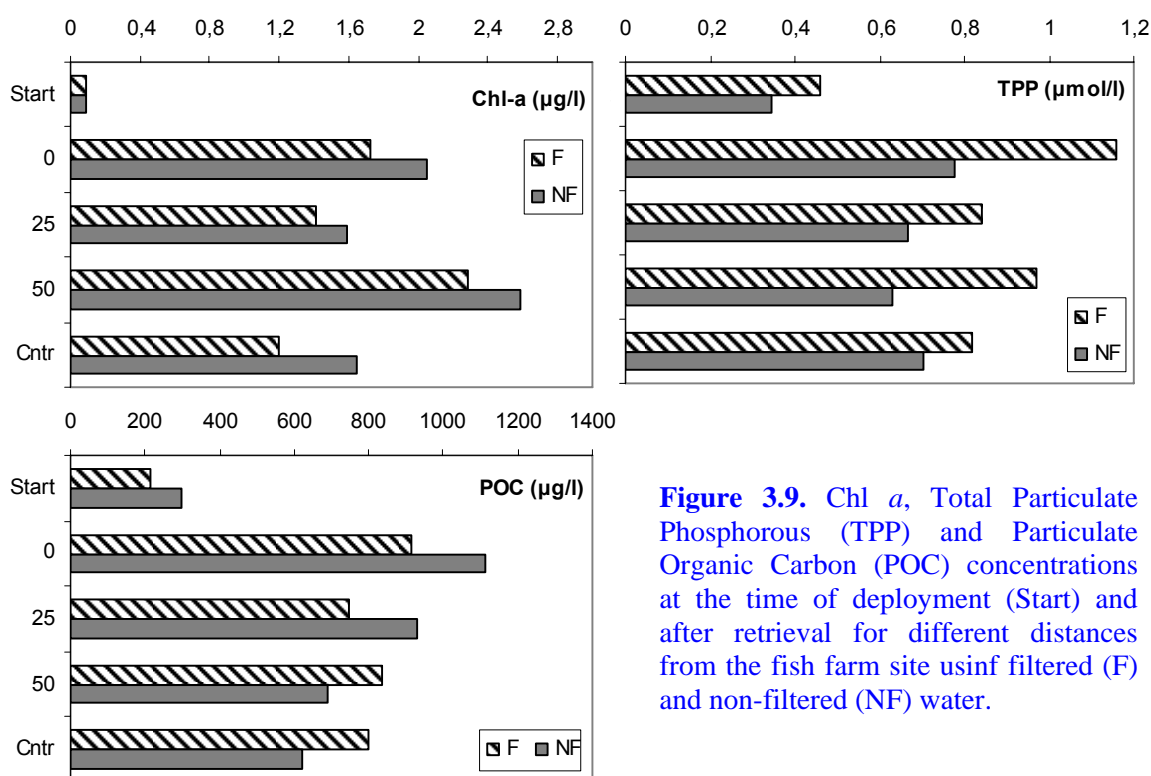
Abundance (ind/cm <sup>2</sup> )	Distance from the farm					Reference	Total
	FF	5	10	25	50		
Nematodes	2719,30	1985,96	3859,65	1925,44	4135,96	399,78	2504,35
Copepods	39,47	111,84	150,22	187,50	557,02	301,32	224,56
Copepod nauplii	8,77	4,39	31,80	78,95	129,39	148,25	66,92
Kinoryncha	0,00	0,00	0,00	0,00	10,96	0,22	1,86
N/C	168,52	22,20	26,10	10,35	7,98	1,40	39,43
<i>Meiofauna metazoan</i>	3263,16	2617,54	4423,25	2365,13	5072,37	961,18	<b>3117,11</b>



**Figure 3.8.** Density of metazoan meiofauna and its major taxa and N/C ratio.

### 3.4.5 Bioassay experiment

Two treatments of bioassays were deployed *in situ*, one for which the water used in the dialysis bags was filtered through a mesh size of 25  $\mu\text{m}$  to remove grazers and one with untreated water. For both treatments, Chl-a, Particulate Organic Carbon (POC) and Total Particulate Phosphorous (TPP) values have increased compared to the initial concentrations (**Figure 3.9**). The concentrations of POC and TPP decrease with increasing distance from the cages for non filtered water, however, when filtered water was used both POC and TPP values decrease up to a distance of 25 m from the cages but they appear to increase again at the station of 50 m. The same pattern is observed for Chl *a* concentrations regardless of the water treatment.



**Figure 3.9.** Chl *a*, Total Particulate Phosphorous (TPP) and Particulate Organic Carbon (POC) concentrations at the time of deployment (Start) and after retrieval for different distances from the fish farm site using filtered (F) and non-filtered (NF) water.

### 3.4.6 The MOM model

The water quality at this site is good but since the smaller cage groups are placed along the direction of the main current the through flow is not optimized and the limiting criteria is in fact the water quality in the cages at this site. The larger cage group have 3 rows which also limits the through flow. With the values of the dimensioning current ( $U_{\text{min}}$ ) shown in **Table 3.3**, the longest flushing times are 2 h

20 min for Sounion 1, and 1h 40 min for Sounion 2 and 3. The oxygen concentration of the surrounding water is 5 mg/l due to the high temperature and salinity, and with the configuration of Sounion 1 the calculated holding capacity at the site is about 720 tonnes/year, and only about 300 tonnes/year with the configuration of Sounion 2 and 3. The reason for the larger holding capacity shown for Sounion 1 is the longer distance between net pens and it can be increased further with even more separated net pens, which will increase the through flow and thereby the holding capacity of the site.

The outlet of dissolved nutrients from the cage groups per 1 tonne of fish production are 8 kg of phosphorus (P), and 45 kg of nitrogen (N) for Sounion 1-3, and are calculated based on the requirement of the fish and the amount of protein in the food, where most of the nutrients are tied up. The excessive nutrients can be reduced by reducing the part of protein in the feed. The outlet of dissolved nutrients is hard to validate, but at this site bioassay studies showed a local increased of chl *a* concentrations at and in the vicinity of the farm (0-50 m) compared to the reference station further out in the strait, but no effects on B or C scale were shown (see **chapter 3.4.5**).

The outlet of particulate matter from the farm per 1 tonne of fish production are with the estimated holding capacity; 10/11/8 kg (P), 58/64/47 kg (N), 137/125/130 kg of faeces, and 706/794/738 kg of wasted food at Sounion 1/2/3. The wasted food was calculated based on the theoretical food conversion ration FCR<sub>teo</sub> and the food conversion ratio given by the farmer FCR<sub>real</sub> (see **Table 3.5**). FCR<sub>teo</sub> is based on the requirement of the fish and type of feed given. The figures show a slightly smaller sedimentation rate from Sounion 1 than below the other cage groups as a result of the cage group configuration with rather large distances between the net pens. The model results show that most of the outlet of particulate matter from the cages does not settle at the bottom due to the large current speed and the oxygen concentration at the bottom is above the critical level of 3 mg/l. ECASA observations at the site show that the density (No of individuals) increases below the cages, while the diversity decreases (see **chapter 3.4.3**). This indicates an enrichment of organic matter below the cages compared to the surrounding bottom, but a well oxygenated bottom, which agree with the model result.

The production cycle of this site is 14-15 and the model predicts 16.5 month for the fish to reach the weight of 380 g with an initial weight of 10 g, and with a median weight of 110-116 g during the production cycle. The larger median weight at this site indicates a faster growth, which is also seen in the shorter production cycle of the site. The faster growth is probably due to the higher temperatures in the waters of Greece.

### **3.5 Evaluation of Indicator Performance**

#### Water column Indicators

Similar to the second greek site investigated within ECASA project, the traditionally used water column variables (nutrient, chl *a*, POC, PON concentrations) were not efficient estimators of the fish farm impact on the surrounding water body. Moreover, the phytoplankton bioassay experiment which appeared to perform very well at Cephalonia fish farm, could not detect very clearly a gradient of impact at Sounion, especially when filtered water was used for removing grazers.

#### Sediment Indicators

The widely used in monitoring studies redox potential did not reveal any trend of impact at the studied site. Likewise, chl *a* concentrations in the sediment increased with increasing distance from the cages, a pattern opposite to the one which would have been expected in case of a fish farm impact, and therefore could not be considered as a suitable indicator for detecting fish farm effects at such type of environments. On the other hand, measurement of TOC and TON concentrations in the sediment as well as total organic matter measured by LOI, seem to be quite useful parameters as they appear to rather decrease away from the farm.

#### Benthic indicators

Among the macrobenthic univariate indicators used in this study Shannon, Evenness and AMBI indices as well as macrofauna abundance, were efficient in detecting a gradient of environmental impact across the sampling transect, while the use of macrofauna biomass seem to be not efficient. With regard to meiofauna, the highly criticized N/C ratio and the abundance of the sensitive to environmental disturbance copepods nauplii are pointed out as very helpful tools in detecting fish farm impacts in such ecosystems.

### **3.6 Evaluation of Model Performance**

The outlet of dissolved nutrients calculated by the model is hard to validate, but at this site bioassay studies showed a local increase of chl *a* concentrations at and in the vicinity of the farm (0-50 m) compared to the reference station further out in the strait, but no effects on B or C scale were shown (see **chapter 3.4.5**).

The model results show that most of the outlet of particulate matter from the cages does not settle at the bottom due to the large current speed and the oxygen concentration at the bottom is above the critical level of 3 mg/l. ECASA observations at the site show that the density (No of individuals) increases below the cages, while the diversity decreases. This indicates an enrichment of organic matter below the cages compared to the surrounding bottom, but a well oxygenated bottom, which agree with the model result.

### **3.7 Site specific conclusions**

Several of the sediment and benthic indicators that have been used for assessing the environmental state of the studied site indicated that there is an impact on the area due to fish farming, nonetheless, this impact is very local, limited within the immediate vicinity of the cages (Zone A), extending up to a distance of 5 – 10 m from the edge of the cages. However, a few of those indicators, namely AMBI and chl *a*, POC and TPP concentrations in the water column as determined after deployment of bioassay experiments, indicated that the reference station, which fall within Zone B scale, is slightly disturbed, at levels similar to those detected for stations located close to the fish farm (25-50 m distance from the edge of the cages). However, this fact can not be attributed directly to the presence of the farm in the area since other activities (e.g. fisheries, urbanisation) might synergistically contribute to the observed disturbance. Furthermore, the area is characterised as highly dynamic in terms of hydrography and therefore, such a disturbance could be as well attributed to the physical disturbance the ecosystem experiences. No effects resulting from the fish farm are anticipated for Zone C.

The use of MOM model at Sounion site indicated that this site is well suited for fish farming due to the high current speed in the strait between the islet of Patroklos and the main land of Attiki. The holding capacity of the farm with the configuration of

Sounion 1 and with both MOM criteria fulfilled (see **chapter 3.3.1**) is 720 tonnes/year, which is above the present production of 290-340 tonnes/year. The configuration of the farm is not optimal and thus, the holding capacity of the site may be increased by separating the net pens even further and place them in single rows perpendicular to the main current direction. One may also decrease FCR<sub>real</sub> at this site. The protein retention of the fish with FCR<sub>real</sub>=2.0 is only 23 %, therefore, the protein content in the fish food can also be reduced considerably.

No conflicts between the fish farm and other human activities occur currently at the area. Furthermore, the wild fish market does not seem to be sufficient for covering the area's needs and hence the company seems to substantially support the fish food market of the area. Nonetheless, an increase in fish farming companies in the specific area might provoke a conflict with the highly developed tourism industry of the area, since it is quite possible that it will alter the landscape from an aesthetic point of view.

More importantly, the development of aquaculture in the specific area will definitely place a risk on the protected seagrass habitats of the area. Therefore, though it might not be realistic to cease the operation of the existing fish farm company, it is essential in terms of ecosystem conservation, not to allow the expansion of aquaculture industry in this area.

## **4 Acknowledgements**

## **5 References**

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- Raffaelli D, Karakassis I, Galloway A (1991) Zonation schemes on sandy shores: A multivariate approach. *Journal of Experimental Marine Biology and Ecology* 148:241-253

## 6 Appendix 1: Environmental data.

Study environment	File type	Parameter(s)	Headings	Location	Prepared by
water column	Excel	CTD	ST_CODE; DATE; TIME_GT; LAT_DEGR; LAT_MIN; LON_DEGR; LON MIN; SPINDLE; DATUM; Z_DEPTH; Z_SAMPLE; TEMP; SAL; PAR; CHLA	C:\Katerina\ECASA\Data\water\CTD.xls (worksheet Sounion)	Katerina Sevastou
	Excel	CHN	ST_CODE; DATE; TIME_GT; LAT_DEGR; LAT_MIN; LON_DEGR; LON MIN; SPINDLE; DATUM; Z_DEPTH;	C:\Katerina\ECASA\Data\water\water column variables.xls (worksheet Sounion)	Katerina Sevastou
	Excel	Nutrinets	Z_SECCHI; Z_SAMPLE; NITRITE; NITRATE; AMMONIUM;		
	Excel	Chl a	PHOSPHATE; SILICATE; CHLA; POC; PON; C/N		
Bioassay experiment	Excel	CHN	Code; A/A; Site; Distance; Replicate; Treatment; Volume; N	C:\Katerina\ECASA\Data\water\data_bioassay.xls	Katerina Sevastou
	Excel	Chl a	(ug); C (ug); C (ug) transformed; N (ug/l); C (ug/l); N (mol/kg);		
	Excel	TPP	C (mol/kg); Chla (mg/l); Phaeop. (mg/l); CPE; TPP (umol/l); PIP		
	Excel	TOP	(umol/l); POP (umol/l)		
Sediment	Excel	Eh	Code; Season; Site; Distance; Replicate; Layer; T (°C); Eh (mV); Eh standardised	C:\Katerina\ECASA\Data\raw data\Redox.xls	Katerina Sevastou
	Excel	CHN	Code; Season; Site; Distance (m); Replicate; Layer; sample weight; ug N; ug C; ug N/mg; ug C/mg; C/N; ug N/kg; ug C/kg; N (mol/kg); C (mol/kg); CN; remarks	C:\Katerina\ECASA\Data\sediment\sediment_over all variables.xls (Worksheet CHN)	Katerina Sevastou
	Excel	LOI	Code; Season; Site; Distance (m); Replicate; Layer; Wc; W0; W 250; W 500; PI; PII; Rp; % labile OM; % refractory OM; total OM; Ws	C:\Katerina\ECASA\Data\sediment\sediment_over all variables.xls (Worksheet LOI)	Katerina Sevastou
	Excel	Phosphorus	Code; Season; Site; Distance (m); Replicate; Layer; TP (mg/g); IP (mg/g); OP (mg/g); TP (mol/kg); IP (mol/kg); OP (mol/kg); Notes	C:\Katerina\ECASA\Data\sediment\sediment_over all variables.xls (Worksheet P)	Katerina Sevastou
	Excel	Chl a	Code; Season; Site; Distance (m); Replicate; Layer; Chla (ug/g); Phaeop. (ug/g); CPE (ug/g)	C:\Katerina\ECASA\Data\sediment\sediment_over all variables.xls (Worksheet Chl-a)	Katerina Sevastou
Benthos	Excel	macrofauna abundance	sample code; site; distance (m); replicate; sieve; phylum; subphylum; class; order; family; Genus; GenusSpecies; abundance (raw data); density (in/m <sup>2</sup> )	C:\Katerina\ECASA\Data\raw data\1EA1macrofauna.xls	Nafsika Papageorgiou
	Excel	macrofauna biomass	code; site; distance (m); replicate; sieve; phylum; class; family; Genus; GenusSpecies; abundance; biomass	C:\Katerina\ECASA\Data\raw data\Species (abundance+biomass).xls (worksheet Sounion)	Nafsika Papageorgiou
	Excel	macrofauna biomass	site; distance (m); replicate; g(0,5mm); g(1mm); Total; BFI	C:\Katerina\ECASA\Data\raw data\Total biomass.xls	Nafsika Papageorgiou
	Excel	macrofauna diversity indices	station; S; N; d; J'; H'(loge); H'(log2); 1-lambda; N1; N2; Ninf	C:\Katerina\ECASA\Data\macrofauna\indices_Sou nion.xls	Katerina Sevastou
	Excel	meiofauna	sample code; participant; location; year of study; month; site; sampling gear; sample size; distance from cages (m); replicate; Nematoda; Soft bodied; Copepoda; Copepodites; Copepoda nauplii; Cops; Total Cops; Polychaeta; Oligochaeta; Ostracoda; Kinoryncha; Tardigrada; Halacaroida; Amphipoda; Cnidaria; Tanaidacea; Notes	C:\Katerina\ECASA\Data\meiofauna\meiofauna.xls	Katerina Sevastou