CHANGES IN BENTHIC MEIOFAUNAL ASSEMBLAGES IN THE VICINITY OF FISH FARMS IN THE EASTERN MEDITERRANEAN

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ABSTRACT. – The response of meiofaunal communities to seabed organic enrichment was investigated in three fish farms within Greek coastal waters. Sampling took place during November 1995 and April 1996. Samples taken at stations close to farms showed a significantly higher abundance (by a factor of 2–5) than those taken at the control sites during both seasons. This increase was mainly attributed to nematode density and was more conspicuous at farms with a silty substrate rather than at coarse sediment ones. The use of the BIOENV method showed that Redox, Total Organic Carbon and ATP concentrations in the sediment were the factors best explaining the multivariate patterns in the meiofaunal community. It is suggested that analysis of meiofauna may provide a sensitive tool for detecting early signs of benthic organic enrichment near fish farms.

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RÉSUMÉ. – La réponse de la méiofaune à l'enrichissement en matière organique du fond de la mer a été étudiée dans la région de trois fermes de pisciculture dans les eaux grecques côtières en novembre 1995 et en avril 1996. Pendant les deux saisons, les échantillons pris à proximité des fermes montrent une abondance de la méiofaune significativement plus élevée (d'un facteur de 2-5) que celle des échantillons des stations témoins. Cette augmentation, liée à la densité des Nématodes est plus nette dans les substrats vaseux que dans les substrats grossiers. La méthode BIOENV a permis de montrer que les concentrations du Carbone Organique Total et de l'ATP des sédiments sont les facteurs qui expliquent le mieux les modèles multivariés de la méiofaune. Il est suggéré que l'analyse de la méiofaune peut représenter un outil sensible pour la détection des signes précoces d'enrichissement organique benthique à proximité des fermes de pisciculture.

INTRODUCTION

Seabed organic enrichment is the most widely encountered impact of cage fish farming (Gowen & Bradbury 1987, Iwama 1991). A small proportion of food supplied to fish is retrieved through harvest, whereas a considerable amount reaches the seabed either as unconsumed food pellets or as excrements. For salmonids, it has been calculated that 29% of carbon supplied through fish food (Hall *et al.* 1990) is lost in particulate form and deposited on the sea bottom. Effects of this particular type of enrichment have been assessed for sediment geochemistry (Holby & Hall 1991, Holmer 1991, Hall *et al.* 1992, Hargrave *et al.* 1993, Karakassis *et al.* 1998) and macrobenthic communities (Brown *et al.* 1987, O'Connor *et al.* 1989, Weston 1990, Kupka-Hansen *et al.* 1991, Karakassis *et al.* 1999), showing similar results re-garding macrofaunal succession but significant differences regarding spatial extent of impacts.

A review on impacts of aquaculture showed that, until the middle of the 90s, little was known on impacts of fish farming in the Mediterranean (Munday *et al.* 1994) where farming of sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) has grown exponentially during the last 20 years. During the last five years, progress has been made in understanding these processes in the Mediterranean. A series of papers have been published regarding the impact of fish farming on water column chemistry and parasites (Papoutsoglou *et al.* 1996, Black & MacDougall 2002), the effects on nutrients and plankton (Pitta *et al.* 1999, Karakassis *et al.* 2001), the effects on seagrass (Delgado *et al.* 1999, Ruiz *et al.* 2001), the dynamics of sediment accumulation beneath fish farm cages (Karakassis *et al.* 1998), the effects on sediment geochemistry and benthic organisms (MacDougall &

Black 1999, Mazzola *et al.* 1999, Karakassis & Hatziyanni 2000, Karakassis *et al.* 2000, Mazzola *et al.* 2000, Karakassis *et al.* 2002) and the recovery process of the benthos after cessation of fish farming (Karakassis *et al.* 1999).

Most of the field studies on the response of meiofaunal communities to organic enrichment in northern temperate marine areas have dealt mainly with sewage pollution (Coull & Chandler 1992), whereas only a few studies have investigated the response of meiobenthic communities in relation to organic enrichment due to fish farming (Sugunan & Pillai 1984, Lorenzen *et al.* 1987, Ólafsson *et al.* 1995, Duplisea & Hargrave 1996, Mazzola *et al.* 1999, Mazzola *et al.* 2000). In the Mediterranean, and particularly in the warm oligotrophic Eastern basin, meiofauna studies are rather scarce and the few, which have addressed the impact of organic enrichment in this type of marine environment (Gowing & Hulings 1976, Lampadariou *et al.* 1997, Papadopoulou *et al.* 1998), deal with the effects of sewage, which is a mixture of pollutants rather than organic enrichment alone.

In the present paper, data on the response of meiofauna to organic enrichment are reported from three commercial fish farms within Greek coastal waters. In this context, the present paper provides information that could improve our understanding of processes related to anthropogenic disturbance (such as the organic enrichment from aquaculture) as well as the response of meiofauna to such effects in the oligotrophic conditions of the eastern Mediterranean. In particular, subtle changes in sediment organic content are investigated, in order to assess the performance of meiofaunal studies as a tool for detecting early signals of environmental change.

MATERIALS AND METHODS

Description of areas studied: Sampling was carried out during two seasonal cruises (November 1995 and April 1996) aboard the RV 'Philia'. Three fish-farms were visited (Fig. 1), two in the eastern Ionian and one in the Aegean Sea, henceforth referred to as Cephalonia, Ithaki and Sounion, respectively. Detailed description of these sites, as well as data on water column variables, sediment chemistry and macrofaunal communities have been reported elsewhere (Pitta *et al*. 1999, Karakassis *et al*. 2000). The sediment in Cephalonia was silty (silt and clay accounted for more than 60%) corresponding to the biocoenose of 'terrigenous mud' (or VTC) after Peres (1967). The other two sites had coarse sediment typical of the 'Amphioxus sand' biocoenose described by Peres (1967) as "coarse sands and fine gravels under bottom currents". In Cephalonia bay and Sounion, farms were located in areas with a bottom depth of less than 20 m, whereas the Ithaki farm was situated over a steeper bottom at a depth ranging from 20 to 30 m. At all three sites, samples for meiofaunal analysis were taken near cages, 25 m away from their edge and downstream of the water current main direction. A control station was selected 1 km upstream from cages at a similar depth and sediment type.

Meiofauna: At each station, three replicates were taken by sub-sampling a Smith McIntyre grab (Somerfield *et al.* 1995), with a plastic corer (4.7 cm internal diameter). Each sub-sample was taken from a different replicate grab to avoid pseudoreplication (Hurlbert 1984). Samples were sectioned into two layers: 0–2 and 2–4 cm depth. Before preservation in 4% neutralized formaldehyde solution, meiofauna sediment samples were first placed in 6% magnesium chloride solution to promote tissue relaxation. Back in the laboratory, samples were sieved through 500 and 45 μm mesh, and the fauna from the fraction remaining on the 45 μm sieve was extracted, depending on the sediment type, by a combination of decantation (ten times) and triplicate
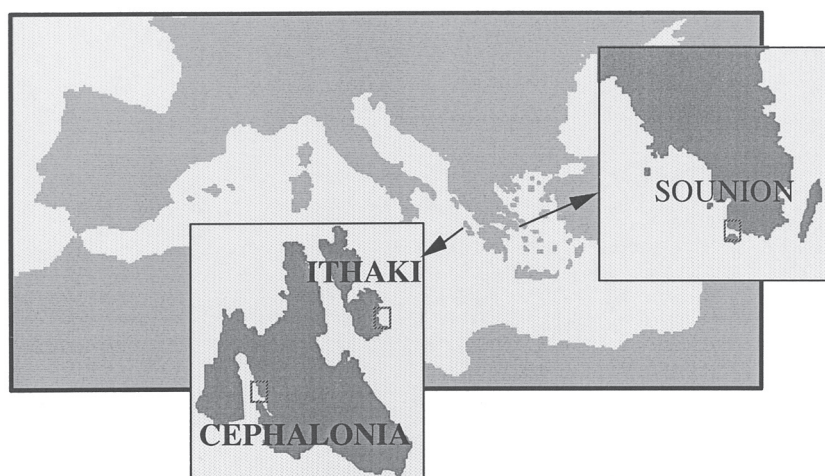

Fig. 1. – Map showing the location of the three fish farms investigated.

centrifugation in Ludox TM {density 1.18 g cm^3; (Heip *et al.* 1985)}. Regarding foraminifera, the extraction method described above collects only allogromiids quantitatively, whereas 'testate' (presumably hard-shelled individuals) remain in the sediment (Schwinghamer 1981). Thus, only results for soft-bodied foraminiferans are presented in this study. All extracted meiobenthic animals were counted and identified to higher taxa under a stereomicroscope after being stained with Rose Bengal (0.5 g l^{-1}).

Statistical analyses: Data analysis followed standard methods described by Clarke (1993), using the PRIMER software package (Plymouth Marine Laboratory). Taxon diversity was calculated by means of the log$_2$ Shannon-Wiener index and Hill's diversity indexes (Hill 1973) N_0, N_2 and N_∞, which are recommended for meiofaunal assemblages (Heip *et al.* 1988). Faunal samples were subjected to cluster analysis and non-metric multidimensional scaling (MDS) ordination (Field *et al.* 1982). Faunal data for this and subsequent multivariate analyses were square root transformed. Such transformations were necessary to allow for the relatively high dominance in some samples. A two-way crossed analysis of similarities (ANOSIM) was used to test for statistically significant effects of the factors 'site' and 'sampling date' on meiobenthic assemblages. The relationship between measured environmental variables and faunal community structure was first explored by BIOENV analysis (Clarke & Ainsworth 1993). In BIOENV, Euclidean distance similarity matrices of data from environmental variables are correlated with Bray-Curtis similarity matrices computed from faunal data. These correlations are repeated for all possible permutations and combinations of the eight measured environmental variables. The highest correlation determined indicates those variables which, combined, best agree with patterns observed in the faunal MDS. Ordinations of all the environmental data and those variables, which gave the best correlations with the faunal data, were carried out using correlation based principal components analysis (PCA). Prior to ordination, the environmental variables with the exception of Redox data were log$_{10}$ (x+1) transformed. The effect of both 'disturbance' and 'sampling time' on the abundance and taxon diversity was investigated at each of the three farms using a two-way analysis of variance (ANOVA) and the Bartlett's and Cochran's tests were used to test for homogeneity of variance. Tests were performed for the total meiofaunal abundance as well as for its components i.e. nematodes, copepods, foraminifera and the group 'others' (which comprised the remaining taxa). Paired a posteriori comparisons of density and taxon diversity estimates were carried out using the Tukey test. Prior to the analysis of variance, all abundance data were log$_{10}$ (x+1) transformed.

RESULTS

Abiotic data

Redox measurements obtained at 2 cm (Table I) never displayed negative values and were, with the exception of both stations from Cephalonia, within background values for uncontaminated sediments. All three sites showed different distribution patterns of the various environmental variables measured. Significantly higher values near the cages were observed at Sounion, during both sampling events for ATP and chlorophyll-*a* concentrations. CPE values were higher in November only (Table I). A similar pattern was observed in Ithaki where ATP and chlorophyll-*a* increased dramatically by a factor of 9–28 for ATP and 3-12 for chlorophyll-*a*. In Ithaki, CPE values were also significantly higher at the station near the cages (Table I). Contrarily in the near cage stations of Cephalonia, significantly higher values were measured for particulate organic carbon only (Table I).

The PCA ordination plot of sampling stations based on geochemical variables (Fig. 2A) showed a clear separation of the silty sites of Cephalonia from the stations sampled at the other two areas. It also showed (with the exception of Cephalonia) a pattern that can be related to proximity to the cages (Fig. 2B).

Table I. – Geochemical parameters of the sediment measured at 25m distance from the cages and at the control station.

	Cefalonia				Ithaki				Sounion			
	25m		Control		25m		Control		25m		Control	
	Nov	Apr	Nov	Apr	Nov	Apr	Nov	Apr	Nov	Apr	Nov	Apr
RPD 0-2cm (mV)	16	38	65	30	358	251	267	479	396	348	186	378
RPD 2-4cm (mV)	5	24	53	12	298	242	235	383	370	315	100	305
TOC (%)	1,77	1,49	1,29	1,27	0,54	0,38	0,38	0,54	0,29	0,23	0,63	0,34
TON (%)	0,18	0,15	0,17	0,15	0,05	0,05	0,04	0,06	0,04	0,04	0,06	0,05
Chl α (μg/g)	2,3	1,6	2,2	2,3	3,5	1,7	1,1	0,1	2,8	1,8	1,3	1,7
Phaeop. (μg/g)	13,4	10,5	11,9	9,6	5,1	2,8	3,7	1,6	3,2	1,9	3,3	1,9
ATP (ng/g)	2894	1514	4421	1361	11834	13725	1300	502	5778	4363	3519	3149
CPE (μg/g)	15,7	12,1	14,1	11,9	8,6	4,5	4,8	1,7	6,0	3,7	4,6	3,6

RPD: Redox potential discontinuity layer; TOC: Total organic carbon; TON: Total organic nitrogen; Chl α: Chlorophyll-α; Phaeop: Phaeopigments; CPE: Chloroplastic pigment equivalent
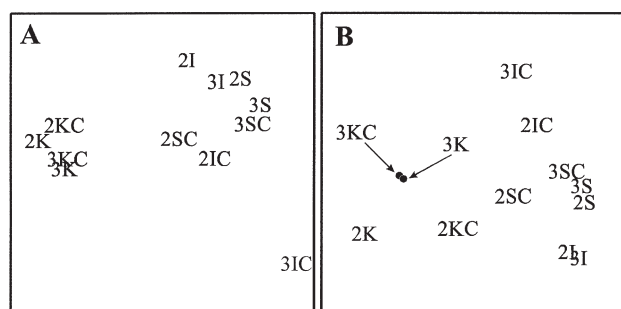
Fig. 2. – PCA ordinations of the various environmental variables. Numbers refer to sampling events (2, November; 3, April). Letters refer to sites (K, Cephalonia; I, Ithaki; S, Sounion; C, Control station). A, PCA ordination with all the measured environmental variables; B, PCA ordination with only Redox 2-4cm, TOC and ATP, which showed the highest rank correlation with the faunal abundance similarity matrix in BIOENV

Faunal data

Meiofaunal density in the top 4 cm of the sediments ranged from 440 to 1137 ind/10 cm^2 at the control stations and from 1415 to 3309 ind/10 cm^2 at the impacted stations. Meiofaunal densities encountered at the control stations in all the three

sites were within ranges reported from other areas in the eastern Mediterranean (Hulings 1971, Dinet 1976). Nematodes were the most abundant taxon (Fig. 3) at all stations ranging between 59.7 and 89.9%. Copepods were the second most abundant taxon at Sounion and Ithaki, ranging between 3.1 and 16.3%, while in Cephalonia foraminifera ranked second ranging between 4.7 and 32.4%. Densities of the most abundant meiofaunal taxa comprising on average more than 2% of the total fauna, are presented in Fig. 3.

Total density of all meiobenthic taxa and mean percentages of the most abundant taxa for all sampling sites are presented in Table II. At all three sites and during all seasons, the total density of meiobenthic taxa increased near the cages by a factor ranging from 2.5 to 4.2 ($P \leq 0.01$ in all three areas). Nematodes and copepods jointly contributed largely to this increase of the total abundance ($P < 0.01$ for Cephalonia and Ithaki and $P < 0.05$ for Sounion). Furthermore, in Ithaki the less abundant taxa such as tardigrades, priapulids and some temporal meiofauna, contributed also significantly to the increase of the total abundance ($P < 0.05$). Between the two sampling events, November and April, no significant differences were detected either in the total abundance or in any of the other groups. In contrast, meiofauna group diversity was
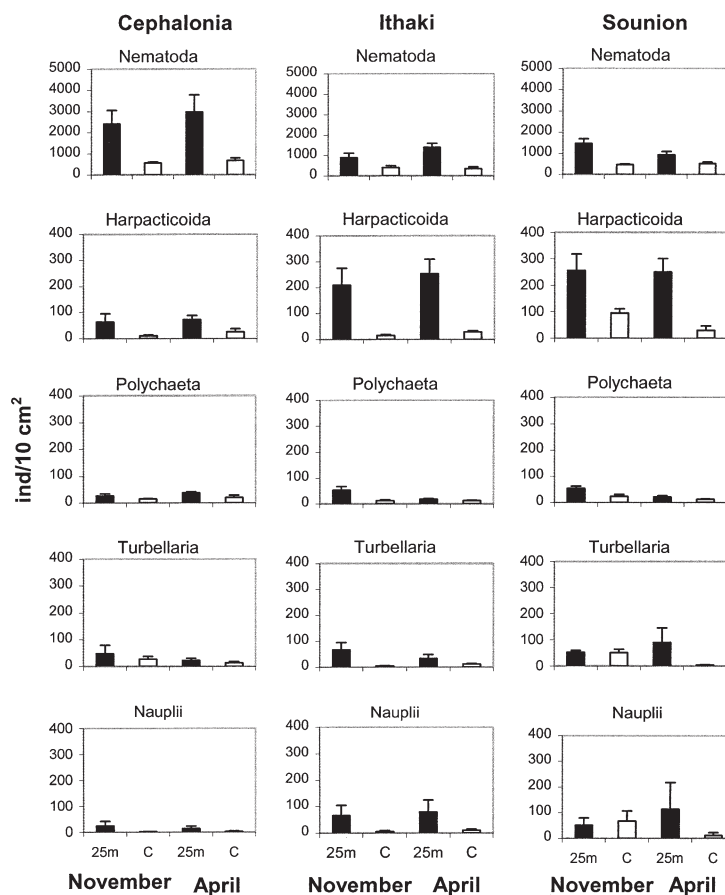

Fig. 3. – Average number of major meiobenthic taxa at the control sites (C) and at 25 m distance (25) from the cages for the three different fish farms. Values shown are mean numbers of individuals per 10 cm^2 ± SE.

Table II. – Total meiofaunal density (mean number of ind./10 cm^2) and mean percentage of most abundant taxa (copepods include both adults and nauplii; Foraminifera include only those retrieved by the extraction method) at 25 m distance from the cages and at the control stations.

	Cephalonia				Ithaki				Sounion			
	25m		Control		25m		Control		25m		Control	
	Nov	Apr	Nov	Apr	Nov	Apr	Nov	Apr	Nov	Apr	Nov	Apr
Total (ind/10cm^2)	2862	3309	757	1137	1415	1854	479	440	1939	1541	780	607
Nematoda (%)	84,1	89,9	74,3	59,7	63,3	75,6	84,9	77,3	75,7	60,4	60,1	84,8
Copepods (%)	2,2	2,2	1,7	2,2	14,8	13,7	3,1	6,3	13,2	16,3	12,1	4,8
Foraminifera (%)	8,5	4,7	13,9	32,4	6,2	2,9	6,7	7,4	2,3	4,3	4,6	4,8

significantly different for all diversity indexes only in Cephalonia (Table III), where the 25 m station displayed lower taxon richness than the control station. The other two areas did not show any significant differences in group diversity between the stations close to and away from the cages. Again, between the two sampling events, no significant differences were detected in taxon diversity for all three investigated sites.

The resulting dendrogram of the cluster analysis is represented in Fig. 4A. All control stations were separated first by this method with the exception of the November control station from Sounion (2SC), which clustered together with the 25 m stations from Sounion and Ithaki. The plot resulting from the MDS ordination is very similar with the cluster analysis and is represented in Fig. 4B. The spatio–temporal pattern in change of meiofauna community pattern is similar in all three areas. Samples taken near the cages clustered at one side of the graph whereas all control stations are clustered on the other side of the graph (Fig. 4B); furthermore, Cephalonia is clearly separated from the

other two sites, as was also shown from the cluster analysis. The ANOSIM two–way crossed significance test confirmed that meiofaunal assemblages differ significantly between the control and the 25 m stations at the 5% level (disturbance effect: R=0.83, $P<0.05$; site effect: R=0.64, $P<0.05$).

A high correlation was found between Redox 0–2 cm and Redox 2–4 cm as well as between Phaeopigments and CPE. Therefore, according to the method suggested by Clarke & Ainsworth (1993), the former variable in each pair was excluded from the BIOENV analysis. All variables

Table III. – Top, significance of two-way ANOVA on diversity of meiofaunal groups between the 25 m and control stations from three replicates and two sampling events. Bottom, summary of results from BIOENV analysis. K =environmental variables used each time to correlate biotic and abiotic similarity matrices. Faunal abundances were square root transformed and environmental variables were $\log_{10}(x+1)$ transformed.

Diversity:	Shannon	Hill N_0	Hill N_2	Hill N_{inf}
Ithaki	ns	ns	ns	*
Sounion	ns	ns	ns	ns
Cephalonia	*	*	*	*

* P <0.05; ns, not significant

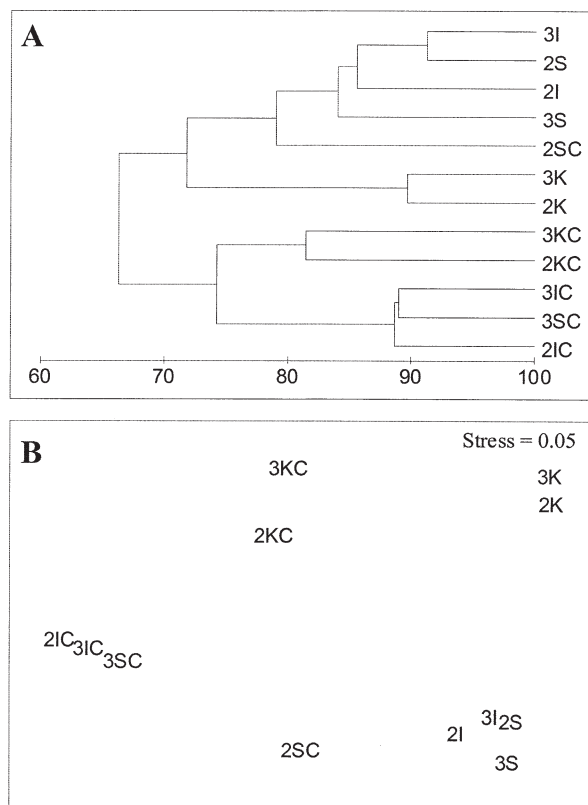| k	Best combination
1	TOC
	0,21
2	TOC, ATP
	0,37
3	**Redox 2-4cm, TOC, ATP**
	0,37
4	Redox 2-4cm, TOC, ATP, CPE
	0,33

Fig. 4. – Dendrogram (A) for group average clustering and MDS (B) ordination of Bray-Curtis similarities based on square root transformed taxon abundance data for the three areas investigated. Numbers refer to sampling events (2, November; 3, April). Letters after numbers refer to sites (K, Cephalonia; I, Ithaki; S, Sounion). The third letter C refers to the control station and where C is absent the 25 m station is mentioned.

were transformed with Log_{10} (x+1). BIOENV results are summarized in Table III. The highest rank correlation between multivariate patterns of the geochemical and meiofaunal data (Table III) was obtained with three variables only i.e. Redox 2–4 cm, TOC and ATP. The PCA ordination of these three variables (Fig. 2B) gives a particularly good match to the MDS ordination of the faunal data (Fig. 4B) with the exception of the 25 m station from Cephalonia, which is plotted together with the control stations from the same site.

DISCUSSION

The density of the most important meiofaunal taxa varied both between the control and impacted stations and between the two sampling periods, but the major component of this variation was related to the spatial factor. In all three sites during all seasons and with all sediment types, the total density of meiobenthic taxa was found to increase at the enriched stations close to the cages. Similar cases of increased densities as a response to sediment enrichment have also been found in laboratory experiments and field studies (Vidakovic 1983, Gee et al. 1985).

Disturbance of macrobenthic communities that, in some cases, lead to total absence of macrofauna have been reported in relation to salmon farming in the North Atlantic (Rosenthal & Rangeley 1988, Kupka-Hansen et al. 1991) and the Baltic Sea (Holmer & Kristensen 1992). However, Karakassis et al. (2000) when studying the macrofauna at the very stations sampled in the present study, found that the stations located at 25 m from the cages showed little change in comparison to the respective control sites, whereas stations closer to the cages (at distance of 0, 5 and 10 m) showed considerable change in macrofaunal abundance, biomass and community structure. They also showed that even under the cages, there was no 'azoic' or heavily polluted zone as defined by Pearson & Rosenberg (1978). On the other hand, studies on fish-farm impacts on meiofaunal assemblages have produced rather confusing results. For example, Duplisea & Hargrave (1996) investigating the response of meiofauna to organic enrichment from a salmon fish farm and numerous other studies involving other types of organic loadings (Coull & Chandler 1992), have found no clear trend in total meiofaunal abundance with changes in sediment organic enrichment. Therefore, Duplisea & Hargrave (1996) concluded that farm impact on meiofauna cannot be identified by examination of the abundance alone. Contradictory to the above are the results from Mazzola et al. (1999), who reported that changes in the sediment conditions beneath a fish farm cage determined a significant reduction of the total meiofaunal density. The results of the present study are in agreement with Mazzola et al. (1999) in that the meiofaunal assemblages were clearly affected near the cages, although the observed pattern was opposite. It should be mentioned, however, that while Mazzola et al. (1999) sampled beneath the cages, our cage station was placed at a distance of 25 m, which may be an area near the ecotone point. Unfortunately, in the present study no data were available from distances closer than 25 m to the cages; however, if this area indeed approaches the ecotone point then an increase in densities is expected (Pearson & Rosenberg 1978).

Concerning meiofauna density estimates, many workers have expressed severe reservations about the quality of samples recovered from various grab samplers (see review by Fleeger et al. 1988) and box corers (Bett et al. 1994). They cause a bow wave effect, which displaces the sediment surface and thus may result in an underestimation of the densities. However, other authors found no evidence of a significant bow wave effect on meiobenthic densities (Thistle 1983, Thistle & Sherman 1985). Furthermore, Somerfield et al. (1995) argued that it may be possible to obtain useful results using a less appropriate sampler under favorable sampling conditions (i.e. shallow water depth, calm conditions). In addition, in another study undertaken at a fish farm (Lampadariou unpubl data), where grab samples were compared with diver collected samples, it was found that, though differences between samplers were observed, they were not consistent and moreover abundance estimates from grabs were, in some cases, even higher. Thus, taking also into account the intra-station variability and the fact that the densities from all replicate samples taken near the cages were consistently higher, it was felt that, if samples are taken carefully, the use of a grab could represent an alternative survey method that gives reliable results. Under these circumstances, the results of the present paper showed that meiofaunal data seem to reflect more readily even subtle changes in the sedimentary environment and therefore meiofauna seems to be quite a reliable tool for detecting early signs of environmental change.

Although the response, in terms of meiofaunal abundance, was similar at all three sites showing significantly higher values near the cages, this was not observed in terms of taxon diversity, with the exception of Cephalonia where the Redox regime of the sediment was more severely affected by the deposition of the organic material. As mentioned above, within meiofauna as a whole, there is generally a decrease in species diversity and an increase in abundance with increasing organic enrichment, a process which supports the 'paradox of enrichment' (Hockin 1983). There is, however, a differential response within each major component of the

meiofauna. At low levels of organic enrichment, Gee *et al.* (1985) showed that the chemical composition of the sediment is not altered. Thus, increases in species richness and abundance are probably a direct result of enhancement of both the quantity and variety of food resources available for exploitation by those species with the reproductive potential to take advantage of them. However, at high levels of organic enrichment, as in the case of Cephalonia, the chemical composition of the sediment is altered to such an extent that, in the harpacticoid assemblage for example, only those species living on, or above, the surface of the sediment are able to survive. In the nematode assemblage, on the other hand, we might expect an increase in abundance at the surface layer of those anaerobic species, which normally live below the redox discontinuity layer (Warwick & Gee 1984). Such changes may have occurred at the three investigated areas, indicating that a variety of different factors, including other environmental factors such as grain size and salinity, may play an important role in the distribution of the meiofauna (Fenchel 1969, Schwinghamer 1981, Decho *et al.* 1985). It also indicates that changes in meiofauna structure along a pollution gradient are not simply a function of distance from the pollution source.

Warwick & Clarke (1991) emphasized that multivariate methods, compared to univariate and graphical methods, offer the advantage of high sensitivity. The multivariate analysis performed in the present study clearly showed community changes between the impacted and the control sites and the overall pattern seemed to be correlated with variables affected by the deposition of organic material. However, the correlation between community structure and the geochemical variables used in the BIOENV analysis was rather weak (0.37), indicating that the multivariate patterns of meiofauna are governed by much more complicated factors than the handful of geochemical variables included in our sampling program, despite the fact that these included all the variables suggested for monitoring the effects of fish farming (GESAMP 1996).

The analysis of community data at higher taxonomic levels, as carried out in the present study, has been found to be appropriate for detecting meiobenthic response to organic enrichment (Heip *et al.* 1988, Herman & Heip 1988). It has also been shown that for macrofauna, multivariate patterns are almost identical in different types of gradients and different locations around the world, regardless of the level of taxonomic discrimination (Ferraro & Cole 1990, Somerfield & Clarke 1995, Peterson *et al.* 1996, Olsgard *et al.* 1997, 1998, Rumohr & Karakassis 1999, Karakassis & Hatziyanni 2000), indicating little 'information loss' with decreasing taxonomic effort. This is particularly important for reducing the costs of routine monitoring, or reallocating resources towards more

spatially extensive or temporally intensive sampling programs where needed. The data from the present paper seem to indicate that the analysis of meiofauna may provide a useful means for assessing the effects of organic enrichment due to fish farming more readily than macrofauna. However, there is a need for more detailed studies on the comparative performance of the two approaches in order to establish standards for monitoring.

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