Marine Pollution Bulletin xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Long-term monitoring of fish farms: Application of Nematode/Copepod index to oligotrophic conditions

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ARTICLE INFO

Keywords:
Tenerife
Canary islands
Meiofauna
Nematodes
Copepods
Fish farming
Impact
Seasonal

ABSTRACT

Interannual variability (2003–2008) of meiofaunal assemblages were analyzed in sediments beneath fish cages (Impact group) and in areas not affected by aquaculture activities (Control group). Organisms responded with spatial and seasonal variation in meiofauna assemblages, with an abrupt increase of abundances in locations beneath fish cages throughout the study period. This increase was greater during the last sampling year (2008) and mainly due to high abundances of nematodes. Univariate analyses showed differences between control and impacted sites at both sites, however, only significant variations were found in Los Gigantes, which are consistent with seasonal meiofauna variations throughout the study period. These results are partially explained by differences in current velocity between both sampling areas. The Ne/Co index showed the same trend and it seems to be a reliable index in sediment slightly affected by aquaculture wastes. This index is especially recommended in oligotrophic areas (e.g. Canary Islands) where meiofaunal assemblages are poorly represented in terms of abundances.

1. Introduction

Aquaculture industry, which use floating cages for growing fish near of coastal line, can produce changes in the natural environment (Holmer et al., 2008), threatening the environmental quality of coastal zones and generating conflicts between aquaculture and the conservation of marine habitats. Therefore, farmers are obliged to carry out monitoring programs on environmental affections to ensure that the aquaculture enterprise run within the laws, regulations and rules of the specific country. For example, the European Community Directives relevant to marine aquaculture propose the establishment of a monitoring program of water quality with Environmental Quality Objectives and Environmental Quality Standards to be achieve (Water Framework Directive; 2000/60/EC). Other directives are also implicated in the integration of aquaculture management within the management of the whole coastal zone, through Integrated Coastal Zone Management, and in certain procedural formalities involved in the setting up of aquaculture activities, such as the requirement for Environmental Impact Assessment in the licensing procedures for aquaculture developments (Fernandes and Read, 2001). Deleterious effects of fish farming could be minimized or negated by adopting environmental safeguards, including regulatory control and monitoring procedures. Mariculture is monitored in most European countries; however, there is no overall system of monitoring and control that is widely applicable throughout Europe (http://www.lifesciences.napier.ac.uk/maraqua/).

To achieve Environmental Quality Objectives, long-term monitoring programs using environmental indicators should be an important tool for coastal managers, helping to interpret the effects of fish farming by comparison of impact versus control locations. Regarding to aquaculture, one of the most important aspects to be monitored is the organic enrichment of benthic communities. This organic enrichment of the sediments beneath fish cages is a direct result of the sedimentation of particulate waste products (uneaten food pellets and fish faeces) from the fish farm (Karakassis, 2005; Holmer et al., 2008) and has a remarkable effect on benthic communities. The behavior of the particulate organic matter released into the water column, and therefore the environmental effects, will depend on hydrographic conditions, bottom topography and geography, linked to the fish stocking density, the amount of feed utilized and the number of years of farm operation (Maldonado et al., 2005; Belias et al., 2007). The detectable effects on the fauna often diminish with distance from the farm (Kalanzi and Karakassis, 2006; Pusceddu et al., 2007) and can range from irrelevant to important (Tomasetti et al., 2009; Mirto et al., 2010).

The monitoring program of fish farm wastes on the benthic environment has mainly been assessed in terms of changes in macrofaunal community structure and sediment chemistry (Carroll et al., 2003). Quantitative macrofaunal analysis proved to be a very sensitive method in the detection of environmental effects from

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doi:10.1016/j.marpolbul.2012.01.014

the farm, normally examined by means of many different types of index which use abundances of entire species assemblages. The use of these indexes, such as Shannon–Weaver diversity Index (Aguado-Giménez et al., 2007) or AMBI (Borja et al., 2009; Forchino et al., 2011), are extremely expensive to conduct because the necessity of taxonomic expertise for identifying fauna to species level. Therefore, informative and cheap indicators should be validated to facilitate long-term aquaculture monitoring, where a high number of replicates should be processed.

Some studies have focussed on the use of meiofauna, instead of the traditionally-used macrofauna, as indicator of changes on benthic communities from anthropogenic activities (Coull and Chandler, 1992). Meiofauna serve as sensitive indicators to environmental pollution as a result of their small size, interstitial existence, naturally-occurring high abundances, direct benthic recruitment, short generation times and asynchronous reproduction (Schratzberger et al., 2000; Sutherland et al., 2007).

Most of the studies using the Nematode:Copepod index (hereafter Ne/Co index) have been developed on locations with a relatively high primary production. However, the response of this index on oligotrophic conditions has not been well tested. The Canarian archipelago is a very oligotrophic area because the high distance from the Sahara upwelling and lack of other sources of primary production (Barton et al., 1998).

Therefore, the general objective of this study was to assess, by a long term monitoring program of 6 years at two fish farms located on the Canary Islands (Spain), the effects of organic enrichment associated with aquaculture activities working on oligotrophic conditions on benthic meiofaunal assemblages. A set of environmental sediment variables (granulometry and organic matter content) and meiofaunal samples were collected at two fish farms and at two reference locations, twice per year during 6 years (2003–2008), to examine the relationships among hydrodynamics, sediment features, and meiofauna assemblage structure.

The main objective of this study was to determine whether the Ne/Co index, which uses the abundance of the main meiofaunal groups, as the index of nematodes to copepods, critically reviewed as an indicator of organic pollution from salmon aquaculture (Sutherland et al., 2007), can be applied in oligotrophic sediments. This index is based on the ratio of a more tolerant taxon (nematodes) to a more sensitive taxon (copepods) and can be inexpensive and easily calculated because of the lack of need of taxonomist expertise.

2. Materials and methods

2.1. Study area

2.1.1. Los Gigantes

Los Gigantes Bay, is located on the west coast of Tenerife (Canary Islands, NE Atlantic Ocean) (coordinates: 28°15′42″N/16°50′40″W). This bay has two fish farms, where gilt-head seabream (*Sparus aurata*) (450 t/year) and seabass (*Dicentratus labrax*) (350 t/year) are produced. The study zone is characterized by the presence of extended sandy substrate (fine and medium sands), with a low content of silt/clay. Seagrass meadows were not found in the study area, only a small meadow of *Cymodocea nodosa* located in a sheltered inlet northwards from fish cages.

2.1.2. Igueste de San Andrés

The bay of Igueste de San Andrés (hereafter Igueste) is located on the northeast coast of Tenerife (Canary Islands, NE Atlantic Ocean) (coordinates: 28°30′59″N/16°09′55″W). Species cultured in studied fish cages were gilt-head seabream (*S. aurata*) (500 t/year) and sea bass (*D. labrax*) (250 t/year). As well as Los Gigantes, the zone is characterized by the presence of extensive sandy areas (fine and medium sands), with a low content of silt/clay. Small patches of the seagrasses *C. nodosa* and *Halophila decipiens* were observed throughout the study period.

2.2. Monitoring program

The monitoring program was carried out over 6 years, from June 2003 to December 2008 along 11 sampling campaigns (June 2003 (1st), October 2003 (2nd)), February 2004 (3rd), August 2004 (4th), March 2005 (5th), July 2005 (6th), December 2006 (7th), June 2007 (8th), November 2007 (9th), April 2008 (10th) and December 2008 (11th) in each location. In Los Gigantes, five sites were sampled, three located beneath cage farms (impact group, depth 30 m) and two located northwards and not affected by dominant currents (control, 300 m–>1 km from cages, depth 25–35 m). In Igueste, five sites were sampled, three located beneath cage farms (impact, depth 25 m) and two locations in another bay (Antequera bay) northwards (control, >1 km from cages, depth 15–25 m) (Fig. 1).

Sediment cores (3.6 cm inner diameter, area: 10 cm^2) were pushed into the sediment to a depth of 30 cm by diver. In each sampling site, three replicates were collected for faunistic analysis and one for analysis of abiotic factors (organic matter and granulometry). The whole core was analyzed for both analysis (meiofauna and abiotic variables), since it was observed differences in grain size between superficial and deep (20–30 cm) sediments in the sandy seabeds of the studied areas (Riera, unpubl. data). To homogenized the sediment it was deposited in trays. To assess granulometric composition of the analyzed sediment, 100 g sediment from each sampling location was oven dried at 105 °C, passed through a graded series (2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.125 mm and 0.063 mm) of sieves, and then weighed (Buchanan, 1984). These sieves characterized seven different sedimentary types (gravels, very coarse sands, coarse sands, medium sands, fine sands, very fine sands and silt/clay). The method of Walkley and Black (1934) was used to determine the organic matter content in the sediment, by dichromate oxidation.

For faunal analysis, samples were preserved in 10% seawater formaldehyde solution and sieved through a 0.5 and a 0.063 mm mesh sieve (Somerfield and Warwick, 1996). The fraction remaining on the 0.063 mm mesh sieve was separated into different taxonomical groups under a binocular microscope and preserved in 70% ethanol.

Current velocity of each area were measured by Acoustic Doppler Current Profilers (ADCP, Argonaut-XR, SonTek). ADCPs recorded current velocity every 30 min average in 10 bins covering the whole water column except the near-bed and near-surface layers.

In Igueste, the current meter was deployed over 30 days (April–May 2009) (coordinates. 28°51′17″N/16°16′14″W) at 30 m depth. In Los Gigantes, the current meter was set up during 21 days (June 2009) (28°26′14″N/16°84′54″W) at 35 m depth.

2.3. Data analysis

Ne/Co index was analyzed using multifactorial Analysis of Variance (ANOVA). Our experimental design presents four factors: ‘‘Impact/Control’’ (fixed, with two levels), ‘‘location’’ (fixed and orthogonal, with two levels: Igueste and Los Gigantes), ‘‘season’’ (fixed and orthogonal, with two levels: cold and warm) and ‘‘time’’ (random and nested with season, six times were selected at each season), and three replicate samples were taken each sampling time. Prior to carrying out the ANOVA, data were tested for heterogeneity of variance using Cochran's *C*-test. Because variance remained heterogeneous after data transformation by means of $\sqrt{x+1}$ and $\log(x+1)$, untransformed data were analyzed, as ANOVA is a robust statistical test and is relatively unaffected by heterogeneity of

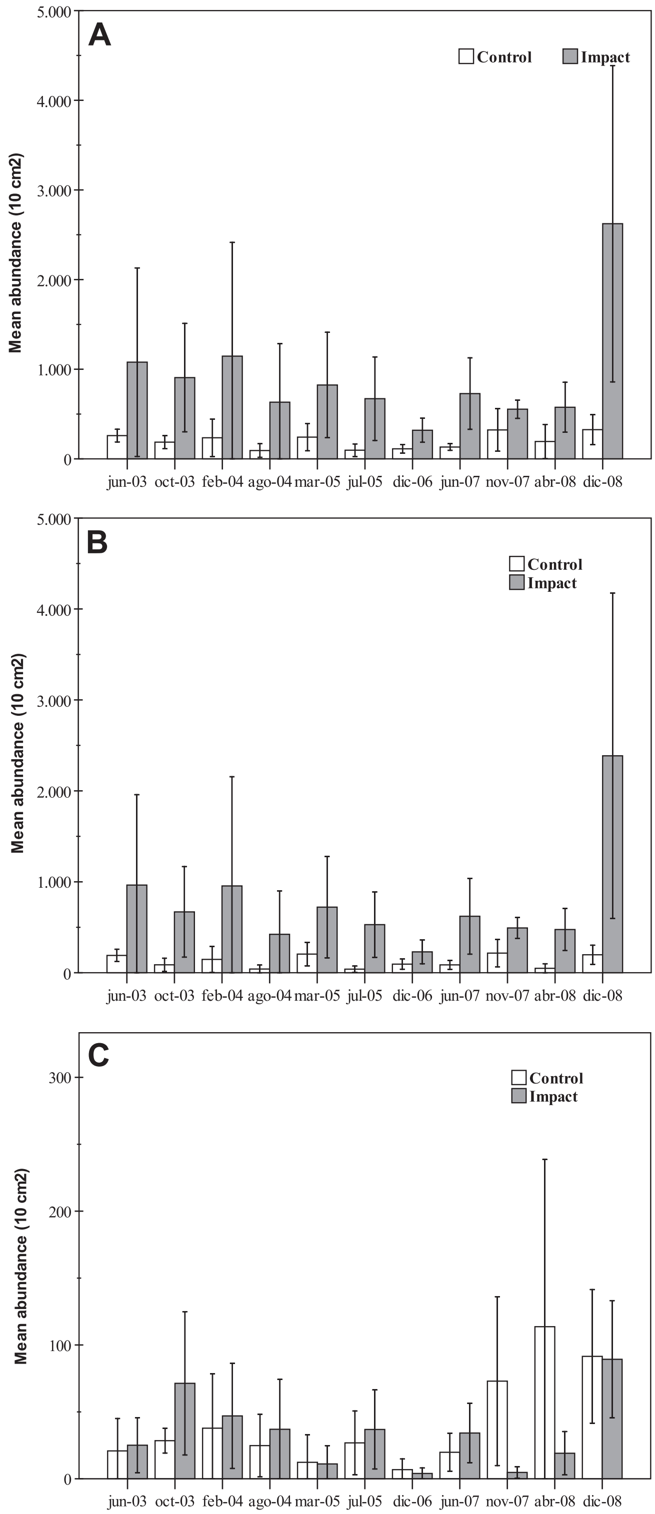

Fig. 1. Mean abundances (±SE) in Los Gigantes throughout the study period. (A) Overall meiofauna. (B) Nematodes. (C) Copepods.

variances, particularly in balanced experiments (Underwood, 1997). In such cases special care was taken in the results interpretation. Thus, to reduce type I error, the significance level was reduced to $p < 0.01$. When ANOVA indicated a significant difference for a given factor, the source of difference was identified using Student–Newman–Keul (SNK) test (Underwood, 1981, 1997).

The temporal trends of 6 years of Ne/Co index monitoring was fitted by Generalized Additive Models (GAM). This term include any GLM estimated by quadratically penalized (possibly quasi-) likelihood maximization. To compare the temporal trends of Ne/Co index between Impact and Control locations we used the MGCV package routines of R (http://www.r-project.org) (Wood, 2011). To model the relationship between dependent variables and predictors, the Gaussian variance and the identity link functions was used. The GAMs of Igueste and Los Gigantes were independently tested using analysis of deviance included in MGCV package, under the performed hypothesis that significant differences should be found between Impact and Control locations. Wald tests of the significance of each parametric and smooth term was performed.

3. Results

3.1. Environmental conditions

3.1.1. Los Gigantes

Granulometric fractions showed differences in gravels and coarse sands, well represented in impacted locations (20.22 ± 2.87% and 18.88 ± 2.10%, respectively). In control locations, medium and fine sands were dominant (29.33 ± 5.15% and 26.07 ± 4.25%, respectively). Silt/clay content was low in all sampling locations (<5%) (Table 1). Organic matter content remained constant among the two sampling groups (Impact-Control), with low percentages (<0.4%) (Table 1). Thus, no elevated organic carbon was observed in studied sediments during the study period (June 2003–December 2008). The highest current speed was recorded in surface waters (13.5–24.6 m s^{-1}) compared to seabed currents (6.3–6.5 m s^{-1}) (Table 2).

3.1.2. Igueste

Fine sands dominated control locations throughout the study period (50.36 ± 34.32%), although impacted locations were characterized by high percentages of very fine sands (53.43 ± 13.45%). Medium sands were better represented in control locations (25.34 ± 12.98%) compared to impacted sites (11.45 ± 8.79%). However, silt/clay was more abundant in impacted sites (12.80 ± 6.78%) compared to control locations (1.50 ± 1.23%) (Table 1). Organic matter content were similar in both sampling groups (Control and Impact), with mean values of 0.38 ± 0.34% and 0.45 ± 0.34%, respectively (Table 1). Surface currents were higher throughout the deployment period (34.8–49.4 m s^{-1}) than seabed currents (7.9–8.8 m s^{-1}) (Table 2).

Table 1
Percentage (±SE) of organic matter content and sedimentary types in sediments of sampling locations throughout the study period.

		Control	Impact
LOS GIGANTES	Organic matter (%)	0.33 ± 0.06	0.39 ± 0.05
	Gravels (%)	2.84 ± 1.01	20.22 ± 2.87
	Very coarse sands (%)	10.11 ± 4.31	13.10 ± 1.23
	Coarse sands (%)	14.99 ± 3.33	18.88 ± 2.10
	Medium sands (%)	29.33 ± 5.15	18.58 ± 1.34
	Fine sands (%)	26.07 ± 4.25	16.89 ± 2.58
	Very fine sands (%)	14.67 ± 4.19	8.32 ± 1.39
	Silt/clay (%)	1.83 ± 0.64	3.77 ± 1.26
Igueste de San Andrés	Organic matter (%)	0.38 ± 0.34	0.45 ± 0.34
	Gravels (%)	0.03 ± 0.02	0.08 ± 0.06
	Very coarse sands (%)	0.78 ± 0.50	2.45 ± 1.87
	Coarse sands (%)	2.34 ± 1.12	6.70 ± 3.78
	Medium sands (%)	25.34 ± 12.98	11.45 ± 8.79
	Fine sands (%)	50.36 ± 34.32	21.23 ± 14.56
	Very fine sands (%)	18.45 ± 13.42	53.43 ± 13.45
	Silt/clay (%)	1.50 ± 1.23	12.80 ± 6.78

Please cite this article in press as: Riera, R., et al. Long-term monitoring of fish farms: Application of Nematode/Copepod index to oligotrophic conditions. Mar. Pollut. Bull. (2012), doi:10.1016/j.marpolbul.2012.01.014

Table 2
Current speed (cm s^{-1}) from the two study areas.

		Seabed layer	Layer 2	Layer 3	Layer 4	Layer 5	Layer 6	Layer 7	Layer 8	Layer 9	Surface layer
LOS GIGANTES	Mean	6.3	6.5	6.7	6.7	6.8	6.9	7.3	7.6	8.6	13.5
	Minimum	0.1	0.0	0.1	0.1	0.0	0.2	0.1	0.1	0.3	0.1
	Maximum	25.2	26.0	26.2	33.7	43.0	32.4	34.9	48.7	58.6	63.7
IGUESTE	Mean	7.9	8.8	9.2	9.4	9.5	9.8	10.4	13.0	20.4	34.8
	Minimum	0.1	0.1	0.1	0.0	0.1	0.1	0.3	0.6	1.0	1.7
	Maximum	21.0	24.8	25.9	26.9	29.6	29.3	31.9	48.4	65.4	87.4

Table 3
Results of multi- and univariate ANOVA testing for differences in meiofaunal abundances, considering "Location" (Loc: fixed and orthogonal, with two levels: Igueste and Los Gigantes), "Season" (fixed and orthogonal) with two levels (cold and warm) and "Time" (random and nested with season, six times were selected at each season), *n* = 3. *p*-values in bold denote significant values ($p < 0.01$).

Factor	Organic matter			Fine sediments	
	df	MS	*p*	MS	*p*
Loc	1	29.437	0.3790	5037090	**0.0224**
Imp/Con	1	141.463	**0.0000**	8365341	**0.0010**
Season	1	0.9490	0.6144	0.9050	0.9160
Loc × Season	1	212.268	**0.0272**	201594	0.6200
Loc × Imp/Con	1	35.019	**0.0059**	2975445	**0.0286**
Imp/Con × Season	1	0.6450	0.1922	2474304	0.0433
Loc × Imp/Con × Season	1	0.7635	0.1580	694990	0.2619
Time (Loc × Season)	16	35.951	**0.0000**	788263	**0.0009**
Imp/Con × Time (Loc × Season)	16	0.3480	0.9284	513823	**0.0211**
Residual	40	0.6865		232028	

Fine sediments varied significantly throughout the study period (2003–2008) between seasons and localities (Igueste and Los Gigantes) (Time (Location × Season); $p = 0.0009$, Table 3). Moreover, significant differences were found considering both interactions (Impact/Control × Time (Location × Season)); $p = 0.0211$, Table 3). If a single factor is considered, Locality ($p = 0.0224$, Table 3) and Impact/Control ($p = 0.001$, Table 3) showed significant differences in the content of fine sediments.

No elevated organic carbon was found in sediments beneath fish cages throughout the study period (April 2003-December 2008), however, significant differences were found between impact and control locations, consistent at both study areas (Location × Impact/Control; $p = 0.0059$, Table 3). Moreover, highly significant differences were found in organic matter content throughout the study period at both sampling areas (Igueste and Los Gigantes) and between seasons (Time (Location × Season); ($p = 0.0000$, Table 3).

3.2. Meiofauna

3.2.1. Los Gigantes

Impacted locations ranged from 319.22 ± 44.71 (December 2006) to 2623.22 ± 588.08 ind. 10 cm^{-2} (December 2008). In control locations, meiofauna abundances ranged from 93 ± 30.91 (August 2004) to 325.67 ± 68.27 ind. 10 cm^{-2} (December 2008) (Fig. 1A). Nematode abundances showed a similar trend in control and impacted locations, ranging from 39.67 ± 13.99 (July 2005) to 215.67 ± 61.23 ind. 10 cm^{-2} (November 2007) in control locations. Impacted locations ranged from 230.11 ± 43.57 (December 2006) to 2385.67 ± 596.38 ind. 10 cm^{-2} (December 2008) (Fig. 1B). Copepods showed low abundances throughout the study period, ranging from 6.83 ± 3.27 (December 2006) to 113.67 ± 51.03 ind. 10 cm^{-2} (April 2008) in control locations. Impacted locations ranged from 4 ± 1.36 (December 2006) to 89.33 ± 14.59 ind. 10 cm^{-2} (December 2008) (Fig. 1C).

3.2.2. Igueste

In impacted locations, meiofaunal abundance ranged from 174 ± 79.77 (December 2006) to 440.44 ± 67.73 ind. 10 cm^{-2} (December 2008). Control stations ranged from 121.56 ± 84.17 (December 2004) to 360.17 ± 258.31 ind. 10 cm^{-2} (December 2004) (Fig. 2A). Nematode abundances ranged from 88 ± 56.28 (December 2004) to 301.33 ± 278.12 ind. 10 cm^{-2} (April 2008) in control locations. In impacted sites, nematode abundances ranged from 118.56 ± 57.84 (December 2007) to 360.11 ± 85.36 ind. 10 cm^{-2} (April 2005) (Fig. 2B). Copepods showed low abundances throughout the study period, ranging from 4.5 ± 3.82 (December 2007) to 51.83 ± 36.32 ind. 10 cm^{-2} (May 2008) in control locations. In impacted sites, copepod abundances ranged from 4.33 ± 1.22 (December 2006) to 113 ± 35.32 ind. 10 cm^{-2} (April 2008) (Fig. 2C).

3.3. Nematode/Copepod index

Ne/Co index showed significant differences in the interaction Impact × Location (Table 4, $p < 0.01$Ne/Co index). Los Gigantes showed significant difference, with a mean value of 17.26 and 81.49, for Control and Impact, respectively (Fig. 3). However, SNK test showed that Igueste did not have differences on Ne/Co index, with similar values with a mean value of 17.56 and 36.97 for Control and Impact locations, respectively (Fig 4).

Significant differences in this index were found between localities considering impact and control sites (Locality × Impact/Control; $p = 0.0115$, Table 4). These differences were due to significant variations between localities ($p = 0.0380$, Table 4) and Impact and control sites ($p = 0.0113$, Table 4).

The analysis of the temporal trends also showed differences between Igueste and Los Gigantes. The results of the analysis of deviance of the GAMs provided a statistical confirmation that temporal trends of Impact and Control location were similar in Igueste, without statistical differences (p value = 0.8196, Table 5). Los Gigantes,

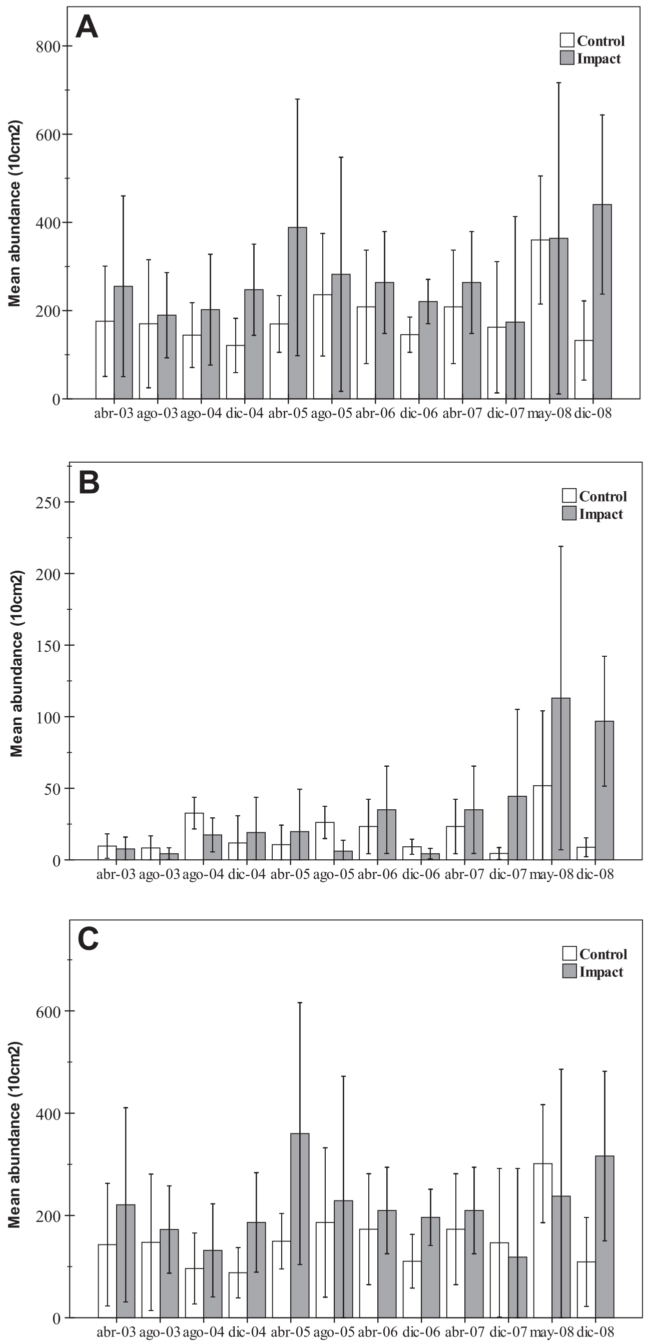

Fig. 2. Mean abundances (±SE) in Igueste de San Andrés throughout the study period. (A) Overall meiofauna. (B) Copepods. (C) Nematodes.

however, showed marginal statistical differences, with a p value of 0.067, indicating that the temporal pattern of Ne/Co index over 6 years was different between Impact and Control locations (Table 5).

4. Discussion

In the present study, the organic enrichment was reported in sediments beneath fish cages, was very slight and thus, no differences were found between affected and non-affected sediments from cages. The presence of continuous coastal currents in the Canary Islands contributed to dissipate the main stressors from fish cages (uneaten pellets and faeces) in sediments, with no signs of eutrophication and/or hypoxia-anoxia. However, changes on meiofaunal abundances, specially nematodes, were observed in affected sediments, and these variations in abundances were registered by the Ne/Co index. Moreover, differences in this index were found between study areas (Los Gigantes and Igueste); that can be partially explained by differences on current speed at both locations. Another important aspect to take into consideration is the consumption of aquaculture wastes by wild fish and invertebrates is significant in the studied fish cages (pers. obs.). This phenomenon has been previously observed in the Canary Islands (Tuya et al., 2005), as well as, other geographic regions (Arechavala-Lopez et al., 2010; Dempster et al., 2009, 2010).

Table 4
Results of multi- and univariate ANOVA testing for differences in Nematode/Copepod index, considering "Location" (Loc: fixed and orthogonal, with two levels: Igueste and Los Gigantes), "Season" (fixed and orthogonal) with two levels (cold and warm) and "Time" (random and nested with season, six times were selected at each season), $n = 3$. p-values in bold denote significant values ($p < 0.01$).

Factor	Ne/Co index		
	df	MS	P
Loc	1	342921879	**0.0380**
Imp/Con	1	370130784	**0.0113**
Season	1	167447051	0.1362
Loc × Season	1	144771029	0.1644
Loc × Imp/Con	1	367687017	**0.0115**
Imp/Con × Season	1	97658206	0.1673
Loc × Imp/Con × Season	1	105190322	0.1525
Time (Loc × Season)	20	69475866	0.1651
Imp/Con × Time (Loc × Season)	20	47556370	0.5546
Residual	96	51274230	

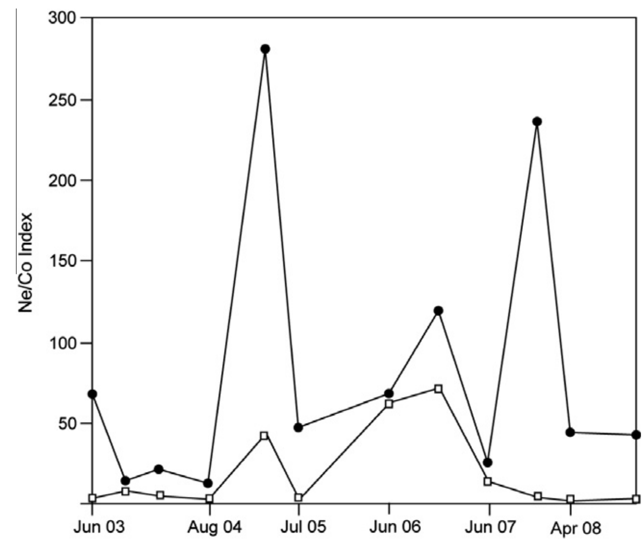

Fig. 3. Variations of Nematode/Copepod index throughout the study period at Los Gigantes. Black dots, impact sites; white squares, control sites.

4.1. Organic matter enrichment

Marine fish farms discharge their wastes directly into the water body environment, and the particulate material sediments (feed wastage, excretion and faecal productions) quickly affecting to benthic communities. However, water currents and eddies disperse these particles, and their "footprint" on the seabed depends on water depth and turbulence. In small amounts this organic matter provides food for benthic animals and demersal fish, but when it accumulates on the seabed, it can block the supply of oxygen to burrowing animals and can drive an increase in oxygen consumption by micro-organisms (Tett, 2007). At the present

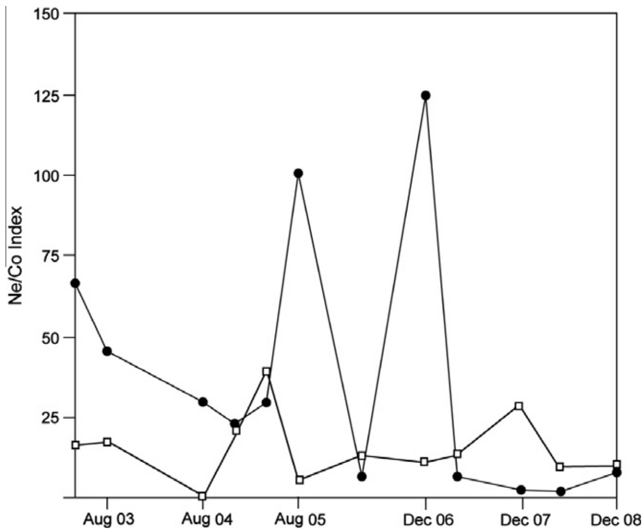

Fig. 4. Variations of Nematode/Copepod index throughout the study period at Igueste. Black dots, impact sites; white squares, control sites.

study, a significant increase of organic matter was not detected, which may indicate that in these locations with strong water flow and sites at >25 m depth generally could hold a higher production for fish farm activities, since waste, without relevant changes on sediment chemistry and community structure (Kutti et al., 2007).

However, meiofauna assemblage showed some changes, in spite of the lack of organic matter enrichment. Fish cages effluents typically alter meiofauna abundances, diversity, biomass and taxonomic composition (Duplisea and Hargrave, 1996; Mirto et al., 2000, 2002, 2010; Mazzola et al., 1999, 2000; La Rosa et al., 2001; Sutherland et al., 2007; Grego et al., 2009), however, these changes are often not consistent, as meiofauna could vary beneath of fish farms (decrease or increase) depending on the site characteristics and farm size. Previous studies reported a decrease of meiofauna abundances in sediments beneath fish cages (Mirto et al., 2002; Sutherland et al., 2007); however, other studies showed the opposite trend, with an increase of meiofauna abundances in sediments affected by biodeposition from fish cages (Mirto et al., 2010; Riera et al., 2011). This positive response of meiofauna abundance to fish farm loadings could be related to the limited organic enrichment in sediments beneath fish cages (Mirto et al., 2010), at distances within 20–35 m from the edge of the cage (Karakassis et al., 1998; Grego et al., 2009). Vezzulli et al. (2003) observed differences in a long-term study (15 y) of meiofauna assemblages, with a decrease of abundances in short-term basis and after several years resulting an increase of meiofaunal abundances in sediments beneath cages.

Previous studies conducted in the Canary Islands showed an increase of meiofauna abundance in sites located immediately beneath of fish cages, with a steady increase of harpacticoid copepods after 6 years of sampling campaigns (Riera et al., 2011). Apparently, meiofauna abundance is affected positively due to the presence of aquaculture farms in the Canarian archipelago, mainly due to an increase of organic load in the environment (Mazzola et al., 1999; Mirto et al., 2000; Grego et al., 2009), or even to changes in grain size beneath cages that favoured the increase of nematodes and harpacticoid copepods (Riera et al., 2011).

4.2. Nematode/Copepod index

These minor changes were exhibit by the Ne/Co index. In the present study, Ne/Co index in impacted sandy sediments varied depending on oceanographic conditions, being <50 (ca. 38) in seabeds with continuous intermediate currents (Igueste) and >50 (ca. 82) in seabeds with currents of lower intensity (Los Gigantes). Raffaelli and Mason (1981) suggested a Ne/Co index of 100 as a pollution threshold, however, Warwick (1981) recommended other values, for sand (Ne/Co > 10) and mud (Ne/Co > 40) seabeds. Sutherland et al. (2007) observed that a sharp cut-off for copepod tolerance occurs at Ne/Co > 50.

More experiments are necessary to fix the threshold of the Ne/Co index for oligotrophic conditions, because of the complex answer of meiofauna to sediment changes. Raffaelli (1987) concluded that observed variations in the Ne/Co index were based on (1) the different habitat requirements of nematodes and copepods, (2) the ability of each to exhibit monotonic response; and (3) degree of organic enrichment examined along a gradient. These sediment variables (grain size and organic matter) represent the different stresses that meiofauna respond to in terms of chemical, organic, and physical properties of the benthos. Moreover, the extent to which organic enrichment influences meiofauna depends on (i) the proportion of sensitive *vs* tolerant species, (ii) the proportion of meso- *vs* epi-endobenthic taxa, (iii) trophic dynamics and (iv) the duration and severity of hypoxic-anoxic conditions (Mirto et al., 2002; Sutherland et al., 2007).

As pointed out by Sutherland et al. (2007), more environmental monitoring studies on off-shore cages are necessary to use the Ne/Co index as a reliable index for monitoring studies. In the present study, we observed that this index could be used to differentiate assemblages from sediments beneath cages and from non-affected areas (control). Other meiofaunal indices have been tested as benthic indicators of aquaculture activities, such as, MEIODIV (MEIOfaunal DIVersity), based on Shannon–Wiener diversity (H′) of harpacticoid copepods (mostly the second meiofaunal dominant group) and MEIOSED (MEIOfauna SEDiment test), consisting parallel samples from a location, one sample is transferred to a control site and the other under cages. However, both indices are not currently validated and important constraints, such as high taxonomic expertise (MEIODIV) and technical caveats to perform superficial sediment disturbance (MEIOSED) (ECASA project, http://www.ecasatoolbox.org.uk).

Additionally, several indices such as AMBI or BENTIX has been applied to evaluate the impact of fish farming and monitoring the environmental using macrofauna assemblage structure. This kind of index, using the diversity of fauna and other ecological information, while more sensitive and reliable, are expensive and time-consuming, requiring professional expertise (Crawford, 2003). In the Canarian archipelago, preliminary studies on macrofauna assemblages from environmental assessment of fish cages showed no clear effects of this activity on macrofauna (Monterroso et al., 2004), however, meiofauna assemblages are subjected to significant changes throughout several years of farm operation

Table 5
Analysis of deviance table for different gamma-based generalized linear models fitted to the meiofaunal abundance of both sites (Los Gigantes and Igueste). Models are fitted sequencially and the columns give the residual degrees of freedom of each model, the residual deviance, the resulting change in deviance and the *p*-value with an *F*-test is used to test for significance.

Study area	df	Residual df	Residual deviance	Residual	Change in deviance	*F*	*p*
LOS GIGANTES	11	7138.8	4898.9	6.8507	2239.9	3.6111	0.06751
IGUESTE	11	1174.9	6.4092	10	1168.5	0.0549	0.8196

Please cite this article in press as: Riera, R., et al. Long-term monitoring of fish farms: Application of Nematode/Copepod index to oligotrophic conditions. Mar. Pollut. Bull. (2012), doi:10.1016/j.marpolbul.2012.01.014

(Riera et al., 2011). Consequently, the use of index such as Ne/Co, based on simple measurements of meiofauna abundances entails a better cost-benefit ratio in several aspects compared to index using macrofauna at species level (i.e. easy sampling methodology, smaller volume of samples and no taxonomic expertise).

In the Canary Islands, meiofauna assemblages are characterized by low abundances (150–700 ind 10 cm^{-2}) in non-affected subtidal seabeds, and apparently there are no signs of effect from aquaculture activities, such as, *Beggiatoa*-layer, organic-enriched sediments, etc. Thus, this faunal fraction together with Ne/Co index is a benthic compartment that has to be taken into account for future environmental monitoring studies in oligotrophic areas, like the Canarian archipelago. Anyway, the use of benthic indicators of aquaculture activities in oligotrophic areas is low, thus, future studies are necessary to check their reliability and applicability.

Acknowledgements

Authors are grateful to the remaining staff of the company CIMA SL for their help in one way or another during the sampling campaigns. To the aquaculture companies CEDRA, SUDOESTE, ACUIGIGANTES and GOLDEN OCEAN for financial support throughout the study period and sampling facilities during field campaigns.

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