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Exploring patterns of variation in amphipod assemblages at multiple spatial scales: natural variability versus coastal aquaculture effect

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ABSTRACT: A 5-factor design survey was carried out to examine the spatial distribution at different scales of amphipod assemblages and sedimentary variables in soft bottoms adjacent to coastal aquaculture installations. Natural variability of sediment variables showed the highest values at the scales of sites (10s of meters) and locality (1 to 10 km), while the greatest component of variation of amphipod assemblages occurred among replicates (on the scale of meters). Regarding the influence of coastal aquaculture, the highest variability of the environmental variables was observed among the different fish farms. On sandy localities, the influence gradient of coastal aquaculture was determined by total free sulphides, whilst, in muddy localities, the main variable was $\delta^{15}N$. This study has important consequences for the establishment of a clear and effective methodology for studying and monitoring the impact of fish farming, highlighting the complicated establishment of a widespread pattern of effects by coastal aquaculture. The necessity to apply a high replication effort at several spatial scales, especially at the scales of meters and 10s of meters, to increase the precision of estimates of assemblage composition should be taken into consideration.

KEY WORDS: Environmental impact · Fish farm · Mediterranean · Soft sediment · Partially hierarchical model · Components of variation · dbRDA

INTRODUCTION

Any human activity that takes place in coastal areas is a potential source of pollution to the marine environment; the impacts of such activities may include eutrophication, oil spills, chemical effluents, brine discharge, metal contamination, thermal pollution, introduction of exotic species etc. (Rapport et al. 1985). Among the different human disturbances, coastal aquaculture may have a negative effect on coastal areas mainly due to high loads of organic matter and nutrients resulting from fish production (Hall et al. 1990, Karakassis et al. 1998). Many studies have been carried out with the aim of identifying and measuring different environmental parameters that could better serve as indicators of environmental disruption of coastal-cage aquaculture (Mirto et al. 2002, Tomassetti & Porrello 2005, Lampadariou et al. 2008, Borja et al. 2009). However, the wide variation in the design and methodology of these studies, the indicators used, as well as the habitat heterogeneity and other environmental factors specific to each case study, have resulted in different conclusions as to the spatial extent and severity of these effects (Kalantzi & Karakassis 2006).

Fish farming interacts with the marine environment at various spatial and temporal scales (Karakassis et al. 2005), ranging from site effects, which affect

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only a particular farm and its immediate environment, to regional impacts, covering spatial scales of many kilometres (Silvert 1992, Gyllenhammar & Håkanson 2005). Therefore, the detection of the causes and ecological consequences of aquaculture requires the determination of scales, which allows quantification of the variation and description of patterns (Levin 1992, Underwood et al. 2000, Terlizzi et al. 2005a). Despite this, there are relatively few studies that have tested significant differences between control and fish farm areas based on rigorous scientific designs (e.g. Maldonado et al. 2005, Vezzulli et al. 2008, Aguado-Giménez et al. 2012), so little information exists on the potentially adverse effects of aquaculture wastes across multiple spatial scales of variation, for example, from meters to hundreds of kilometres.

Additionally, previous studies have demonstrated that changes on the sea bottom due to coastal aquaculture can cause a strong impact on the structure and characteristics of benthic macrofauna (Edgar et al. 2005, Fabi et al. 2009). However, few studies have focused on the effects of fish farming on crustacean assemblages, except for macrocrustaceans on maërl bottoms (Hall-Spencer & Bamber 2007) or peracarid assemblages on soft bottoms (Fernandez-Gonzalez & Sanchez-Jerez 2011). More remarkably, amphipods have not yet been evaluated as a tool for monitoring the effects of aquaculture, in contrast to other taxonomic groups, like polychaetes (Tomassetti & Porrello 2005) or nematods (Mirto et al. 2002). Amphipods are one of the most important groups of invertebrates associated with benthic habitats (Thomas 1993, Sanchez-Jerez et al. 1999, Vázquez-Luis et al. 2009) and play an important role as a trophic resource for fishes and large crustaceans (Bell & Harmelin-Vivien 1983). They are more sensitive to pollution than other macrobenthic assemblages and have accordingly been applied for assessing different types of environmental impacts (Bellan-Santini 1980, Gómez-Gesteira & Dauvin 2000, Guerra-García & García-Gómez 2001, De-la-Ossa-Carretero et al. 2012).

In order to achieve the goal of sound development of the aquaculture industry, the establishment of sampling protocols and environmental monitoring plans based on appropriate spatial and temporal scales, which allow correct interpretation of the differences between disturbed and control areas, is required. Therefore, we investigated the effects of fish farming, along an environmental gradient caused by the presence of several aquaculture installations, using amphipod assemblages. Specifically, our study focused on evaluating these changes considering 2 different periods of intensity of aquaculture production and on analysing the relative importance of spatial scale in the distributional patterns of amphipods and sediment variables from a multivariate point of view. The latter was done by comparing variability components from metres to hundreds of kilometres. Furthermore, the potential relationships between sediment characteristics and amphipods were assessed.

MATERIALS AND METHODS

Study area and sampling procedure

The study area is located in the western Mediterranean Sea, along the coast of Spain (Fig. 1). Three areas (north, central and south) and 2 localities in each area were selected. Within each locality, an influence gradient of coastal aquaculture was tested, encompassing 3 increasing distances from fish cages downstream in the main current direction: farm (just below the cages), intermediate (at the edge of the farm facilities, defined by the delimitation buoys) and control (reference area positioned at least 1 km away from each fish farm, in order to minimize the potential interactions with dispersed farm wastes; Porrello et al. 2005). Three sites were randomly sampled at each distance. Three random replicates were collected from each site for amphipod analysis, and 3 additional replicates were taken for sediment analysis. Samples were collected using a Van Veen grab (0.04 m^2).

The study was carried out between 2009 and 2010, with 2 sampling campaigns: the first one just after summer, coinciding with the most intensive period of feeding and the highest water temperatures, and the other one was at the end of winter, coinciding with the least intensive feeding period and the coldest water temperatures.

To study amphipod assemblages, each sampling replicate was sieved in seawater through a 500 µm mesh and preserved in 4% formalin. In the laboratory, samples were sorted, and the amphipods were separated and preserved in 70% ethanol for subsequent identification and counting. These were identified to species level whenever possible, using the handbook of Mediterranean amphipods (Ruffo 1982), or available published literature, in the case of new species or new records.

For sediment analysis, several sub-samples were taken from each sampling replicate to determine the different variables. Sediment particle size was determined by the wet sieve method (Buchanan 1984),

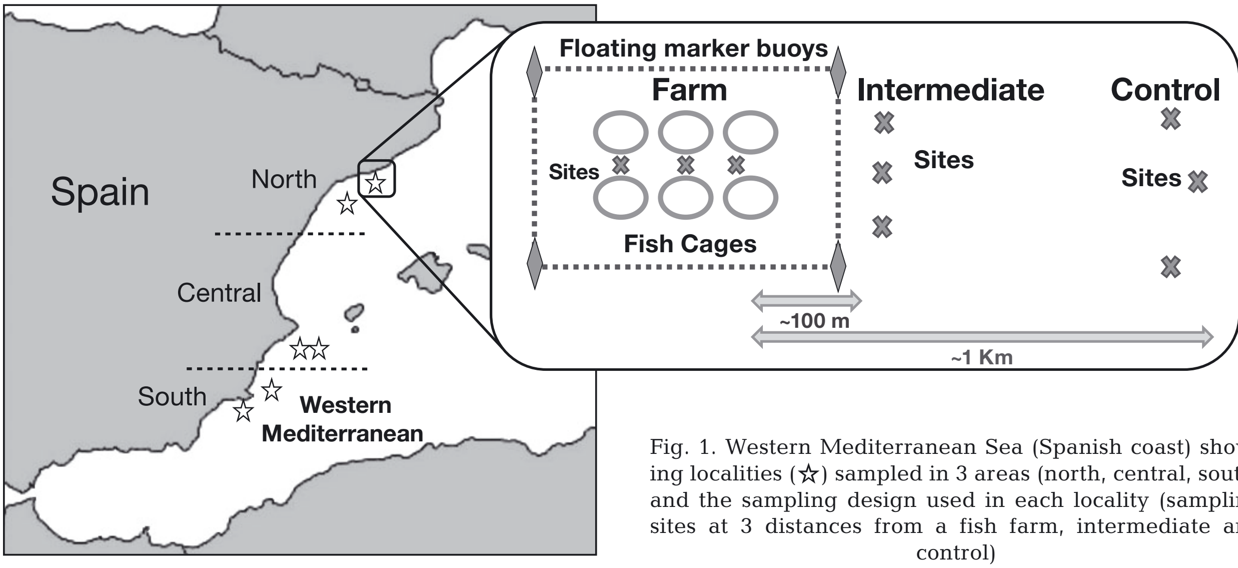

Fig. 1. Western Mediterranean Sea (Spanish coast) showing localities (☆) sampled in 3 areas (north, central, south) and the sampling design used in each locality (sampling sites at 3 distances from a fish farm, intermediate and control)

selecting the finest fraction (<0.063 mm) as a variable for statistical analysis. Total free sulphides (TFS) content was measured in a sulphide antioxidant buffer solution and ascorbic acid, using a silver/sulphide half-cell electrode following the method described by Wildish et al. (1999). Total phosphorus (TP) was determined colorimetrically by the Murphy & Riley method (1962), after persulfate digestion. Total sulphur (TS), total nitrogen (TN) and total organic carbon (TOC) were determined with a CHNS autoanalyser (elemental auto-analyser LECO 932); ^{15}N isotopic composition was measured using an EA-IRMS (Thermo Finnigan) analyser in continuous flow configuration joined to a stable ratio mass spectrometer (Delta Plus). The ^{15}N isotopic composition was expressed as: $\delta^{15}N$ (‰) = $[(R_{sample}/R_{standard}) - 1] \times 10^3$, where $R = {}^{15}N/{}^{14}N$, atmospheric N_2 being the standard and 0.1‰ the analytical precision.

Statistical analysis

The data were analysed according to a 5-factor model, where the main factors were: 'distance' with 3 levels (farm, intermediate and control), 'intensity of fish production' with 2 levels (high and low) and 'area' with 3 levels (north, central and south, separated by 100s of kilometres). All factors were fixed and orthogonal. Spatial scales were represented by 2 additional factors: 'locality', random and nested in 'area', with 2 levels (separated by 1 to 10 km) and 'site', random and nested in 'locality', with 3 levels (separated by 10s of metres). Three replicates were taken (separated by metres).

The SIMPER routine was used to determine which amphipod species better characterised the groups derived from the 3 areas and to calculate the contribution of each species to the dissimilarity among zones (farm, intermediate and control). To investigate the effects on the amphipod assemblage as a whole, the entire experimental design was analysed using permutational multivariate analysis of variance (PERMANOVA), based on the Bray-Curtis dissimilarities of the untransformed data (Anderson 2001a, McArdle & Anderson 2001). The analysis was tested using 4999 random permutations of residuals under a reduced model (Anderson 2001b), with appropriate units as required by the design (Anderson & ter Braak 2003). When the number of possible permutable units was not enough to get a reasonable test by permutation, a p-value was obtained using a Monte Carlo test (Anderson & Robinson 2003).

To estimate spatial variability and, furthermore, to assess the influence of aquaculture over it, the 3 spatial scales were analysed independently for each distance and intensity of fish production using PERMANOVA, with all factors included as random effects, and the same method and number of permutations applied in the full model. The components of pseudo-variation (Anderson et al. 2005) for each spatial scale, independent of the other spatial scales, were extracted from mean square estimates, using a direct multivariate analogue to the usual ANOVA estimators of variance components (Underwood 1997). Equally, when any negative value was present, it was set to zero, eliminated from the analysis and components of pseudo-variation were recalculated, in the same way as with ANOVA (Fletcher & Underwood

2002, Anderson et al. 2008). The variability at each spatial scale is expressed as a proportion of the total variation (sum of all pseudo-variance components) to simplify comparisons among analyses (Anderson et al. 2005).

The relationship between the entire set of environmental variables and the amphipod assemblage was investigated using distance-based redundancy analysis (dbRDA), based on the Bray-Curtis dissimilarities and limited to 3 axes (McArdle & Anderson 2001). Environmental variables were transformed to normalise them to comparable scales and remove skewness (Clarke & Warwick 1994). TP, TS and TN were normalised to enable comparisons of different scales using square-root transformation, and TFS was normalised using $\log(x + 1)$ transformation.

Multivariate statistical analyses were performed using PRIMER-E software (PRIMER software; Clarke & Gorley 2006) with the add-on package PERMANOVA+ (Anderson et al. 2008).

RESULTS

Amphipod assemblages

A total of 65 amphipod species (Appendix 1) and 2842 specimens were recorded. The most representative species in all areas belonged to the genus *Ampelisca*, present in 64.5% of samples. This taxon was also the most abundant, followed by *Pariambus typicus*, *Pseudolirius kroyerii*, *Photis* sp. and *Medicorophium annulatum*. However, some of these species were related to a single area, like *M. annulatum* in the south or *P. kroyerii* in the central area (Table 1). The species most responsible for the dissimilarity among distances (farm, intermediate and control), according to SIMPER routine, are shown in Table 1. The abundance values of most of these species were lower at farms than in control areas, with the exception of *Siphonoecetes dellavallei* and *Aora spinicornis*, which were more abundant below the cages. Nevertheless, a general increase at an intermediate distance is observed (Table 1).

Regarding the total number of species, it was lower in farm areas (mean value 8.05) than in intermediate (mean value 10.75) and control areas (mean value 10.83). In relation to the mean total abundance, it was also lower in farm areas (126.15 ± 21.94 ind. m^{-2}) than in intermediate (316.67 ± 50.93 ind. m^{-2}) and control areas (215.04 ± 22.61 ind. m^{-2}). Equally, in some of the species, a pattern of increase at intermediate distance was found in total abundance; however, high spatial variability was also present (Fig. 2), which probably contributed to the absence of significant differences.

Amphipod assemblage composition was also analysed using the full partially hierarchical model. Results obtained with multivariate PERMANOVA detected significant differences in the main factor 'distance' ($p < 0.01$); additionally, significant interactions between smaller scales, represented by random factors, and main factors were also detected (Table 2). Because of these significant interactions, the effects of main factors were not interpreted (Underwood 1997).

Sediment variables

Mean values of sediment variables at each area and distance are shown in Table 3. Sediments in the central area were mostly mud (fraction <63 µm: 87.79 to 88.68%), while the south and north areas were

Table 1. Summary of SIMPER analysis results showing the percentage contribution to similarities among areas and dissimilarities among distances, as well as mean (±SE) abundances of the most important amphipod species

Taxon	Area similarity			Distance dissimilarity			Abundances		
	North	Central	South	Farm vs. Intermediate	Farm vs. Control	Intermediate vs. Control	Farm	Intermediate	Control
Ampelisca spp.	80.64	63.42	52.91	31.84	39.57	31.78	64.12 ± 11.20	141.90 ± 18.8	105.63 ± 9.6
Pseudolirius kroyerii	–	32.62	–	16.23	5.58	16.62	2.55 ± 0.50	31.02 ± 3.97	4.40 ± 1.18
Photis sp.	5.56	–	6.73	5.43	5.07	6.44	15.74 ± 2.20	0.93 ± 0.26	18.06 ± 2.54
Pariambus typicus	–	–	1.32	4.92	–	4.48	0.93 ± 0.26	52.78 ± 20.20	1.16 ± 0.29
Siphonoecetes dellavallei	–	–	7.34	4.30	2.65	2.61	10.88 ± 2.02	0.23 ± 0.13	0.46 ± 0.19
Medicorophium annulatum	–	–	11.88	3.42	5.33	6.31	0.46 ± 0.19	10.19 ± 2.28	20.37 ± 3.15
Aora spinicornis	3.74	–		3.17	2.97	1.22	8.10 ± 1.75	4.40 ± 0.65	1.39 ± 0.49
Cumulative percentage	89.94	96.04	80.18	69.31	61.17	69.46			

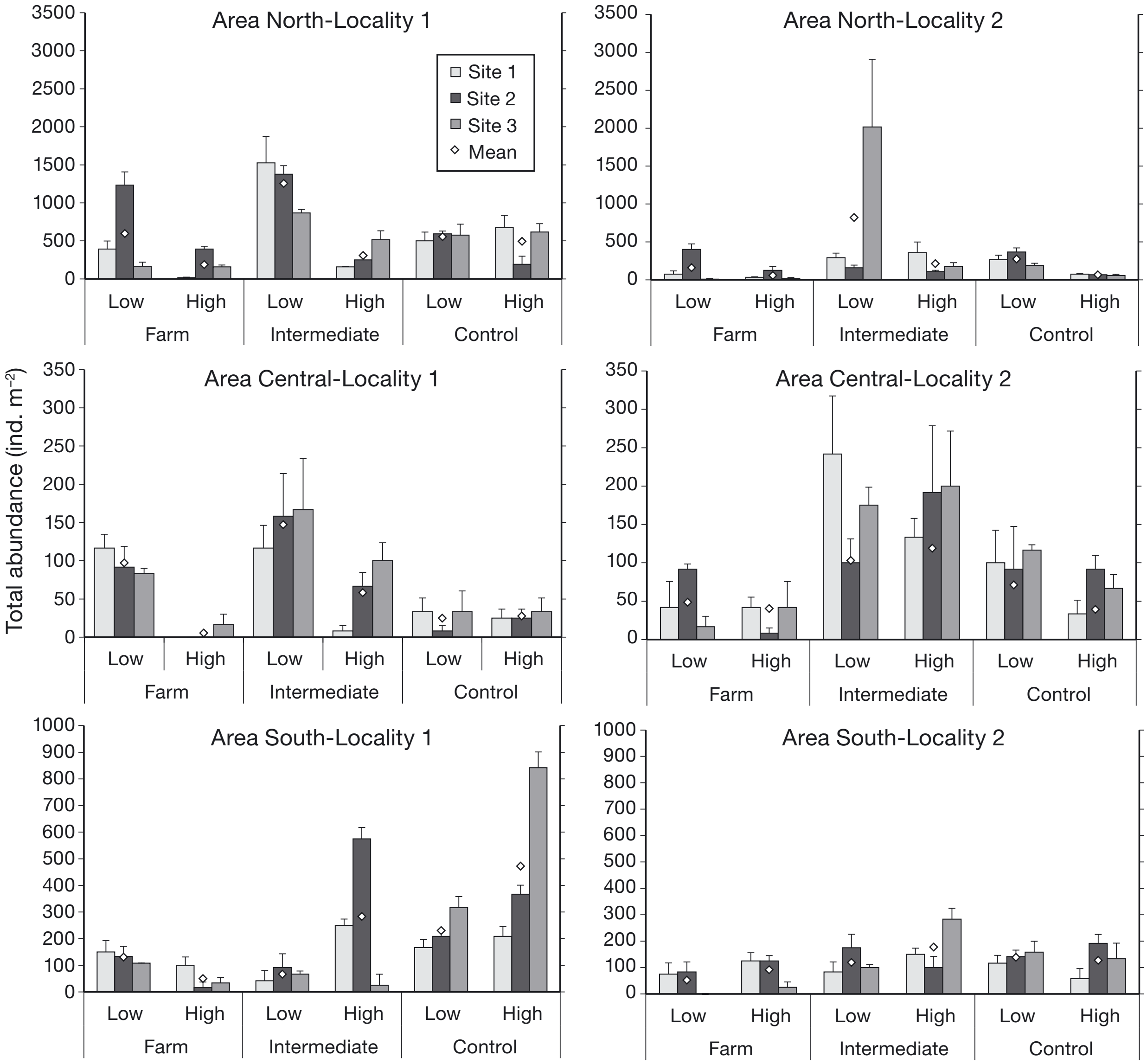

Fig. 2. Mean (+SE) values of amphipod total abundance at each distance (farm, intermediate and control) and period of fish production (low and high), represented separately for each area (north, central and south) and locality

mainly composed of sand or mixed sediments. The percentage of TOC was also higher in the central area than in the north or south area.

Concerning the distance from fish cages, TFS, TS and TP showed increasing values from the furthest zone, control, to the closest zone, farm, in all areas. This trend also appeared with $\delta^{15}N$ contents in the north and central areas; however, the higher values of this parameter in the south area were in the intermediate zone. In the case of TOC, concentrations were variable depending on the area: in the north, the highest value was reached in the farms; in the central, it was reached in the intermediate zone; and in the south, in the control.

Results obtained with multivariate PERMANOVA detected significant differences in the main factor 'distance' ($p < 0.01$). As with amphipod assemblages, significant interactions between smaller scales and main factors were also detected (Table 2), so the effects of main factors were not interpreted.

Spatial patterns of variation of amphipods and environmental variables at multiple scales

When the components of pseudo-variation were extracted for each spatial scale separately, we observed that the highest variability of amphipod as-

Table 2. Results of 5-factor multivariate PERMANOVA based on Bray-Curtis similarity of amphipod assemblages. Main factors were distance (Di), period of fish production (Pe) and area (Ar), fixed and orthogonal. Random factors were: locality (Lo) nested in area, and site (Si) nested in locality. Significant results at the 0.05 level are given in **bold** type; p-values given in *italics* were obtained using the Monte Carlo test

Source	df	Amphipod assemblages		Environmental variables	
		MS	p(perm)	MS	p(perm)
Di	2	23081	**0.0096**	175.73	**0.0064**
Pe	1	9767.7	0.1908	24.08	0.3116
Ar	2	50767	*0.1022*	464.37	***0.0174***
Lo(Ar)	3	28623	**0.0002**	101.27	**0.0002**
Di × Pe	2	4446.9	0.35	13.884	0.545
Di × Ar	4	8539.3	0.1306	46.621	0.3916
Pe × Ar	2	8424	0.1922	15.151	0.6424
Si(Lo[Ar])	12	2340.3	**0.0002**	5.8863	**0.0002**
Di × Lo(Ar)	6	6043.5	**0.0004**	39.799	**0.0002**
Pe × Lo(Ar)	3	5182.8	**0.0032**	22.749	**0.0002**
Di × Pe × Ar	4	4218	0.3936	14.088	0.585
Di × Si(Lo[Ar])	24	2997.3	**0.0002**	4.7825	**0.0002**
Pe × Si(Lo[Ar])	12	2228.4	**0.0002**	3.4942	**0.0002**
Di × Pe × Lo(Ar)	6	3877.4	**0.0024**	15.952	**0.0004**
Di × Pe × Si(Lo[Ar])	24	2106.5	**0.0002**	4.0297	**0.0002**
Residual	216	1267.4		1.2237	
Total	323				

semblages, between 39.25 and 55.25% (Fig. 3a), was reached at the smallest spatial scale (among replicates). At the next scale, the relative importance of site was greater in the farm than in the intermediate or control zone, while from locality to locality, components of variation of the farm decreased in proportion to the values reached by the other 2 distances, intermediate and control. The largest scale, area, contributed very little (<5%) to the total variability, except in the least intensive period, when intermediate and control zones reached 23.16 and 26.35%, respectively. In the most intensive period, components of variability tended to be higher than those calculated for the least intensive one, especially at the site scale.

For environmental variables (Fig. 3b), contrary to the results of amphipod assemblages, components of variation at the small scale were not high. In the low-production period, the greatest component of variation for the intermediate and control zones occurred at site and locality in that order, while in the high-production period, these zones reached the highest values of variability, 52.23 and 59.77%, respectively, at the large scale, showing differences among areas.

The most remarkable result for the farm zone was the large variation that occurred at the scale of locality, among different fish farms, compared with the intermediate and control zones, irrespective of the intensity of fish production (Fig. 3b).

Table 3. Mean (±SE) values of sediment variables in each area (north, central and south) and at each distance (farm, intermediate and control). Environmental variables are: total free sulphides (TFS), total organic carbon (TOC), total nitrogen (TN), total sulphur (TS), total phosphorus (TP), ^{15}N isotopic composition ($\delta^{15}N$) and mud fraction (<63 µm)

Environmental	North			Central			South		
variable	Farm	Intermediate	Control	Farm	Intermediate	Control	Farm	Intermediate	Control
TFS (ppm)	11433.4 ± 1657.4	1317.6 ± 179.6	903.5 ± 62.1	3397.5 ± 238.2	2275.6 ± 159.8	1102.0 ± 104.1	1605.4 ± 150.1	540.2 ± 52.4	246.3 ± 29.2
TOC (%)	7.48 ± 0.47	6.81 ± 0.42	4.98 ± 0.12	6.88 ± 0.07	7.94 ± 0.13	7.53 ± 0.05	3.50 ± 0.45	4.11 ± 0.33	4.78 ± 0.24
TN (%)	0.17 ± 0.02	0.13 ± 0.01	0.30 ± 0.07	0.16 ± 0.01	0.16 ± 0.01	0.14 ± 0.003	0.10 ± 0.01	0.09 ± 0.005	0.09 ± 0.003
TS (%)	0.07 ± 0.01	0.04 ± 0.003	0.03 ± 0.004	0.05 ± 0.005	0.03 ± 0.003	0.03 ± 0.002	0.14 ± 0.03	0.14 ± 0.03	0.03 ± 0.003
TP (%)	0.11 ± 0.01	0.08 ± 0.01	0.03 ± 0.002	0.46 ± 0.07	0.26 ± 0.03	0.23 ± 0.02	0.20 ± 0.01	0.19 ± 0.01	0.17 ± 0.01
$\delta^{15}N$	5.39 ± 0.12	5.08 ± 0.12	4.33 ± 0.09	5.00 ± 0.13	4.21 ± 0.04	4.29 ± 0.04	3.55 ± 0.11	3.69 ± 0.10	3.39 ± 0.08
<63 µm (%)	26.11 ± 1.50	31.31 ± 2.54	60.06 ± 3.16	87.79 ± 3.63	87.99 ± 0.94	88.68 ± 2.62	33.91 ± 4.10	36.72 ± 4.44	40.28 ± 1.32

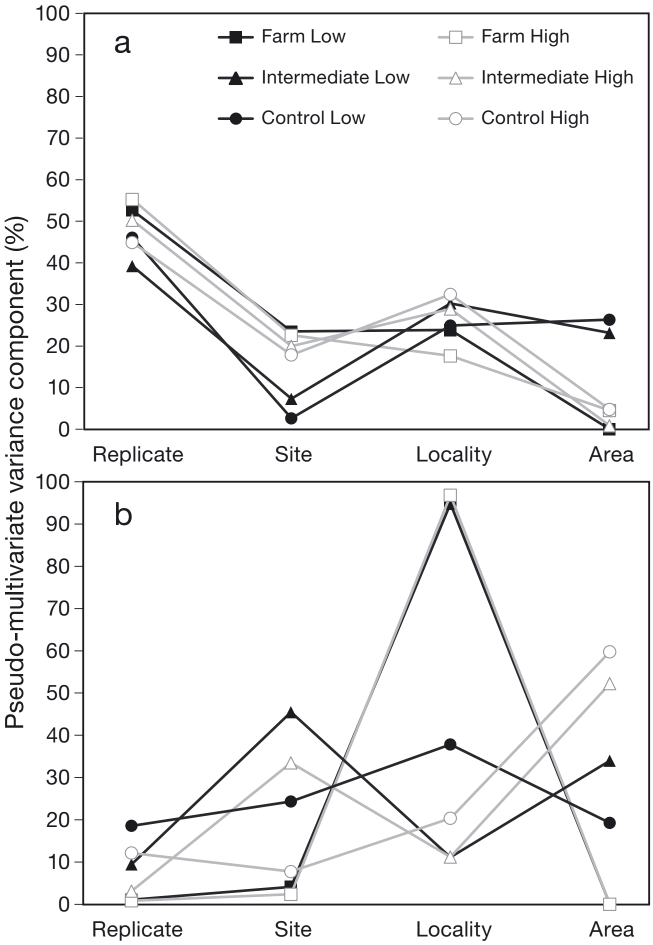

Fig. 3. Proportion of variability accounted for by pseudo-multivariate variation of distance (farm, intermediate, control) and period of fish production (low and high) at each spatial scale (increasing from replicate to area) for the (a) amphipod assemblages and (b) environmental variables

Relationship between amphipod assemblages and environmental variables

Results of the dbRDA routine showed that all of the dbRDA axes together explained 15.9% of the overall variability in the ecological data. In the biplot of the first 2 dbRDA axes (Fig. 4a,b), variation in sediment granulometry, from fine (<63 µm) to coarse grain sizes was strongly correlated with the first axis, while TFS correlated with the second axis. There was no distinct separation of the 3 distances farm, intermediate and control in this biplot (Fig. 4a); however, there was a separation of the localities based on the grain size distribution of the sediment (Fig. 4b). Thus, muddy (<63 µm: >80%), sandy (<63 µm: <50%) and mixed sediment (<63 µm: 50 to 80%) localities were analysed separately using new dbRDAs.

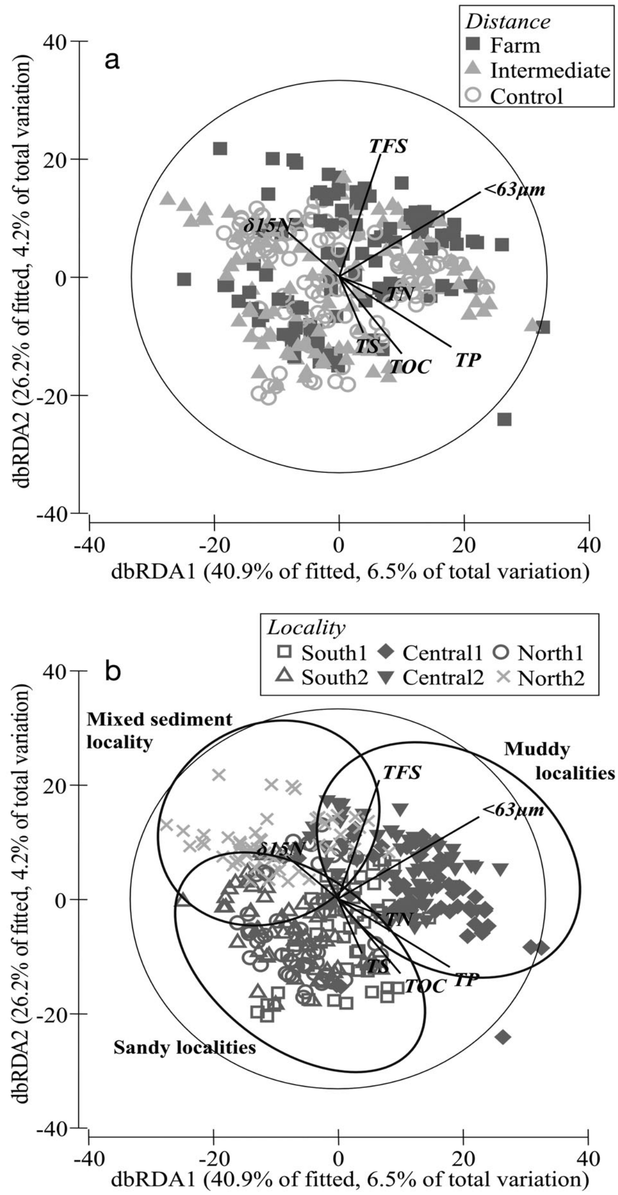

Fig. 4. Distance-based redundancy analysis (dbRDA) of the overall data showing the ordination of (a) distances and (b) localities, in which a separation of sediment granulometry groups was observed (encircled areas). Environmental variables are: total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), total free sulphides (TFS), total sulphur (TS), ^{15}N isotopic composition (δ^{15}N) and mud fraction (<63 µm)

The first 2 axes from the dbRDA of muddy localities (Central 1 and 2; Fig. 5a) explained 90.93% of the variability in the fitted relationship between the ecological and environmental variables and 21.68% of the total variation. So there was more residual variation explained than in the original analysis. The biplot of such axes showed a gradient across 3 increasing distances that can be modelled by TOC, in the first axis, and by δ^{15}N and < 63µm, in the second one.

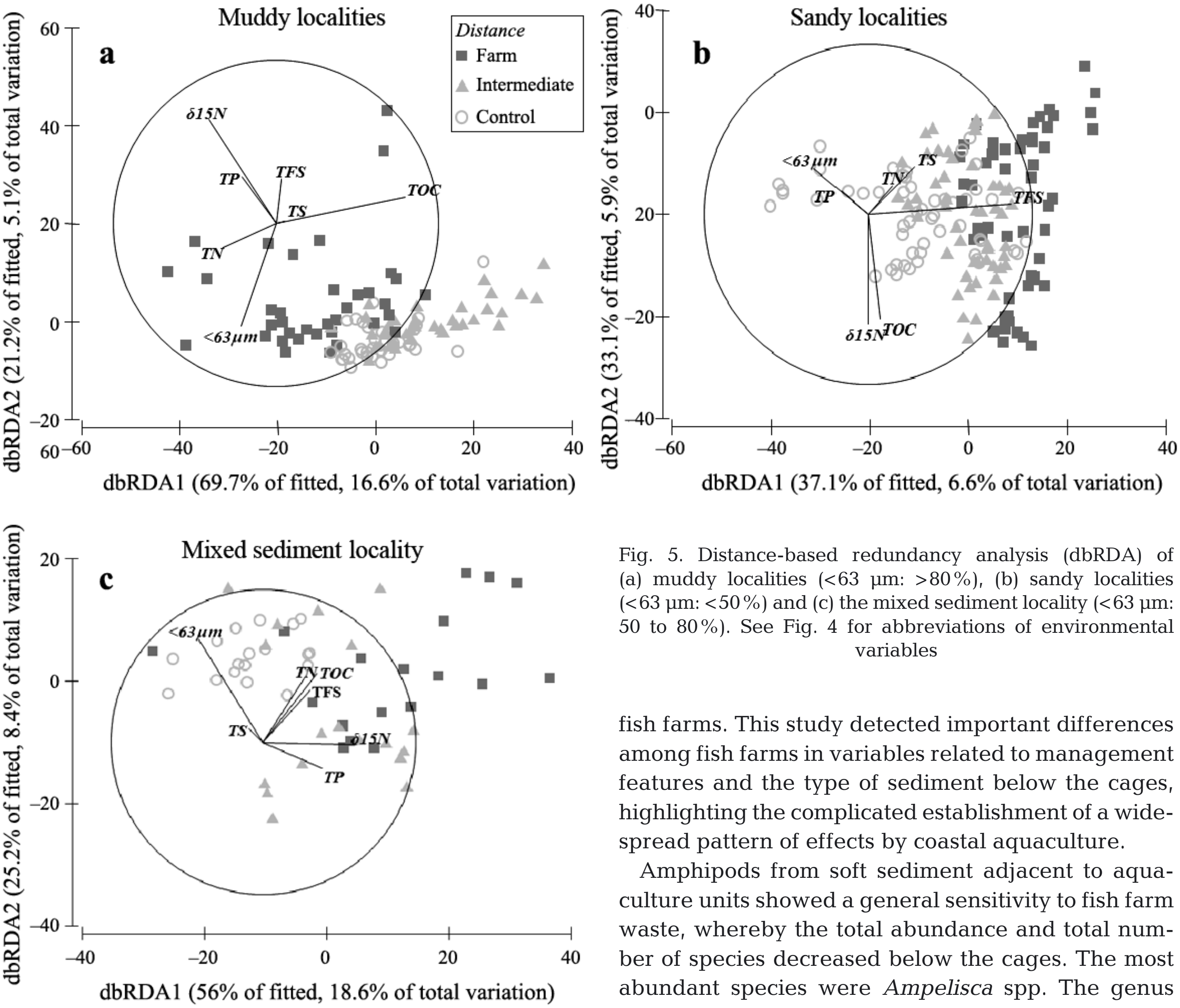

Fig. 5. Distance-based redundancy analysis (dbRDA) of (a) muddy localities (<63 µm: >80%), (b) sandy localities (<63 µm: <50%) and (c) the mixed sediment locality (<63 µm: 50 to 80%). See Fig. 4 for abbreviations of environmental variables

The resulting pattern of dbRDA with sandy samples (South 1 and 2 and North 1; Fig. 5b) indicated that there was a gradient of aquaculture in the assemblage structure of amphipods that is correlated with TFS. The second axis identified the variability among sites based on δ^{15}N and TOC.

The dbRDA analysis of the mixed sediment locality (North 2; Fig. 5c) revealed a separation among farm, intermediate and control zones, which was related to δ^{15}N and <63 µm, in the first and the second axis, respectively.

DISCUSSION

In the marine environment, natural spatial patterns of amphipods at different scales, from metres to hundreds of kilometres, were affected by the presence of fish farms. This study detected important differences among fish farms in variables related to management features and the type of sediment below the cages, highlighting the complicated establishment of a widespread pattern of effects by coastal aquaculture.

Amphipods from soft sediment adjacent to aquaculture units showed a general sensitivity to fish farm waste, whereby the total abundance and total number of species decreased below the cages. The most abundant species were *Ampelisca* spp. The genus *Ampelisca* is a frequent member of soft-bottom communities, and its suitability as a bioindicator to evaluate the quality of coastal marine environments has been widely reported (e.g. Gómez-Gesteira & Dauvin 2000, Nikitik & Robinson 2003). In this study, the abundance of *Ampelisca* spp. was decreased in farm sediments; however, the highest abundances were reached at intermediate distance. This taxon did not completely disappear from farm sites, similar to *Photis* sp., suggesting that domicolous species, which live in tubes, could be less affected by fish farming than those living in direct contact with the sediment (De-la-Ossa-Carretero et al. 2012).

Regarding the free-living caprellids, *Pseudolirius kroyeri* and *Pariambus typicus*, both showed a positive response to a moderate effect of the fish farm, notably increasing their abundances at intermediate distance. However, *P. kroyeri* was mainly related to muddy localities, while *P. typicus* was more abundant in sandy sediments. These species have been classified as sensitive, as well as tolerant, so their sensitivity to pollution is not clear (De-la-Ossa-Carretero et al. 2012, Guerra-Garcia et al. 2012). According to our results, the responses of these species depend on the level of pollution; they are tolerant to moderate levels of fish farming pollution, but sensitive to more severe ones.

In the south area, 2 species from the Corophidea family, *Siphonoecetes dellavallei* and *Medicorophium annulatum*, showed different responses to fish farm waste. The gammarid *S. dellavallei* increased in abundance below the cages, while *M. annulatum* decreased in the farm sediments. Nevertheless, sensitivity of Corophidae species to organic enrichment could be ambiguous as they may be affected by local environmental conditions (De-la-Ossa-Carretero et al. 2012).

The assemblages were characterised by a large number of species, of which most were only represented by a few individuals. Consequently, the best approach in this case was to compare the whole assemblage through multivariate analysis. Significant differences in the main factor 'distance' were detected using amphipod assemblage composition data, but significant interactions at smaller scales were also relevant (i.e. between the scale of locality and the main factor 'distance'). This means that patterns of response in one fish farm could not be extrapolated to another; equally, these changes may be affected by the period of fish production.

Although the interpretation of variance components is not straightforward and should be done with caution (Morrisey et al. 1992, Stark et al. 2003a), it helps us to understand the importance of each spatial scale of sampling and compare these values among different situations, in this case, allowing comparisons along a distance gradient from fish farms.

Amphipods showed the highest degree of variability at the lowest spatial scale in all the cases. Many other studies have recorded a high proportion of variation at the smallest spatial scale for benthic organisms (Morrisey et al. 1992, Stark et al. 2003a, Fraschetti et al. 2005, Chapman et al. 2010), relating it to features of the ecology affecting the organisms, such as the availability of food, behavioural aggregation, predation, competition, or different settlement cues (Anderson et al. 2005, Rotherham et al. 2011 and references therein).

Natural spatial patterns of amphipods at different scales were affected by the presence of fish farms in the marine environment. Amphipod assemblages within fish farming areas were more variable at the site level compared to those of other 2 distances at the same scale. The increased variability at a small scale has been reported to be a general feature of assemblages in stressed environments (Warwick & Clarke 1993, Terlizzi et al. 2002, Stark et al. 2003a). In contrast to this, we found that at a broader scale — among localities — assemblages were more similar among different fish farms than among controls. This suggested that amphipod assemblages in disturbed areas, despite the variability among sites, show a unified response to fish farm wastes and, as other press perturbations, can lead to homogenisation of ecosystems (Glasby & Underwood 1996, Claudet & Fraschetti 2010). Variability changes under disturbance conditions may be due to changes in richness of species, total abundance, or abundance of particular species, resulting in changes of biodiversity or changes in functional diversity (Warwick & Clarke 1993, Chapman et al. 1995, Terlizzi et al. 2005b).

Regarding environmental variables, wide dissimilarity of fish farm variables at the level of locality was observed. This variability among different fish farms was considerably higher than the variability observed for intermediate and control distances at the same scale and, additionally, happened independently of the time of sampling. The effects of aquaculture activities on bottom sediments, such as organic enrichment (derived from an excess of uneaten food and fish egesta), silting, increased oxygen demand, etc., vary from farm to farm and depend on local variables such as hydrographic regime, sediment type and water depth, as well as management variables such as fish production, efficiency of feeding method and feed quality (Tomassetti et al. 2009). Therefore, these differences among fish farms are probably related to a combination of local and management variables.

Based on results of components of variation, it appears that a lack of correlation between spatial patterns of amphipods and biogeochemical properties of sediments exists. In these cases, where the description of patterns is insufficient to understand the fish farm and sediment relationship, manipulative experiments could be useful to solve ecological uncertainties (Clarke & Warwick 1994, Stark et al. 2003b).

Gradients of influence by aquaculture activities were detected when the effects of sediment variability were removed using separate dbRDAs, which suggests that granulometry should always be considered for a correct evaluation of the environmental impact. A different response of the biological and geochemical compartments of the sea bottom according to the sediment type has been reported in several studies (Kalantzi & Karakassis 2006, Papageorgiou et al. 2010).

TFS seems to be an explicative variable in sandy samples. An increase in this parameter indicates that high rates of organic matter loading at these cage sites have generated anoxic sediments (Hargrave et al. 1997). Amphipods from sandy localities were, thus, more sensitive to the toxic effects of sediment anoxia and high sulphide concentrations. However, muddy habitats are usually composed of silty, reduced sediments with high organic loadings (Hyland et al. 2005). Under these conditions of anoxia and organic enrichment, amphipod assemblages responded to an aquaculture gradient correlated with $\delta^{15}N$. The $\delta^{15}N$ values reveal the presence of organic matter derived from fish farming (Holmer et al. 2007), mainly from an excess of uneaten food and fish faeces, both from farmed and wild fish (Fernandez-Jover et al. 2007). Sediments under the cages were enriched in $\delta^{15}N$, but showed lower TOC content compared to control sites. These differences between levels of organic enrichment can be related to the biochemical composition of the organic matter (Dell'Anno et al. 2002). This means that when the biopolymeric fraction of organic carbon — the sum of protein, carbohydrate and lipid carbon — is low, systems are characterised by a larger carbohydrate fraction, while systems with a higher biopolymeric fraction are characterised by the dominance of proteins (Pusceddu et al. 2007 and references therein). So sediments affected by fish farm wastes, which receive large amounts of biopolymeric organic matter, tend to accumulate N-rich compounds (Sarà et al. 2004, Pusceddu et al. 2007).

Finally, the locality with mixed sediment also showed an aquaculture gradient related mainly to $\delta^{15}N$ values and, to a lesser extent, to TOC, TFS and TN. We observed different sediment types at the farm, intermediate and control distances, because the latter exhibited finer granulometry. These differences were probably not caused by the fish farm, but by the inappropriate choice of the control distance at this locality. They not only must be unaffected by aquaculture or other disturbances, but they must have the same habitat features as the disturbed locations, e.g. a similar granulometry (Glasby & Underwood 1998, Stark et al. 2003a).

CONCLUSIONS

Amphipod assemblages were sensitive to fish farm waste, being capable of detecting an environmental gradient caused by the presence of aquaculture installations. Additionally, natural spatial patterns of amphipods at different scales were affected by the presence of these installations in the marine environment. Aquaculture effects changed considerably among the different fish farms studied, where sediment type played a crucial role in the response of the environment to the presence of a fish farm. Also, management features could be very important in defining environmental effects of aquaculture on benthic habitats.

These findings have important consequences for the establishment of a clear and effective methodology for studying and monitoring the impact of fish farming, highlighting the complicated establishment of a widespread pattern of effects due to coastal aquaculture. The necessity of applying high replication effort at several spatial scales, especially at the scales of metres and 10s of metres, to increase the precision of estimates of assemblage composition, should be taken into consideration. Such protocols, integrating all these considerations, are demanded in order to ensure both the required environmental protection and the sustainability of the aquaculture industry.

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Appendix 1. Taxonomic list of the amphipod species collected and presence (•)/absence (–) recorded from different areas of the western Mediterranean (see Fig. 1)

Taxon	North	Central	South	Taxon	North	Central	South
Abludomelita aculeata	–	–	•	*Leucothoe lilljeborgi*	•	•	•
Ampelisca spp.	•	•	•	*Leucothoe oboa*	•	•	•
Amphilochus brunneus	•	–	–	*Maera sodalis*	–	–	•
Aora spinicornis	•	•	•	*Medicorophium aculeatum*	–	•	–
Apherusa vexatrix	–	•	–	*Medicorophium annulatum*	•	–	•
Atylus guttatus	•	–	•	*Medicorophium longisetosum*	•	–	–
Atylus vedlomensis	•	–	•	*Medicorophium runcicorne*	•	•	•
Autonoe karamani	–	–	•	*Megaluropus massiliensis*	–	–	•
Caprella sp.	•	•	•	*Megamphopus cornutus*	•	–	•
Cheirocratus assimilis	–	–	•	*Melita hergensis*	•	–	–
Cheirocratus sundevalli	–	•	•	*Microdeutopus armatus*	–	–	•
Deflexilodes acutipes	•	–	•	*Microdeutopus versiculatus*	–	–	•
Dexamine spinosa	•	–	–	*Microprotopus longimanus*	•	–	–
Elasmopus rapax	•	–	•	*Microprotopus maculatus*	•	–	–
Ericthonius sp.	–	•	–	*Orchomene humilis*	–	–	•
Eusiroides dellavallei	•	–	–	*Orchomenella nana*	•	–	•
Gammarella fucicola	•	–	–	*Othomaera othonis*	–	–	•
Gammaropsis maculata	•	–	•	*Pariambus typicus*	•	–	•
Gammaropsis sophiae	–	–	•	*Parvipalpus linea*	•	–	•
Guernea sp.	•	–	–	*Pericuolodes longimanus*	•	•	•
Harpinia ala	–	–	•	*Perioculodes aequimanus*	•	–	–
Harpinia crenulata	–	–	•	*Photis longicaudata*	•	–	•
Harpinia dellavallei	–	–	•	*Photis longipes*	•	•	•
Harpinia pectinata	•	•	•	*Phthisica marina*	•	•	•
Hippomedon massiliensis	–	–	•	*Pseudolirius kroyerii*	•	•	•
Hippomedon oculatus	•	–	–	*Siphonoecetes dellavallei*	•	•	•
Hyale campotrix	–	–	•	*Stenothoe* sp.	•	•	•
Jassa marmorata	•	•	•	*Synchelidium haplochelos*	•	–	•
Lepidecreum longicorne	•	•	•	*Synchelidium longidigitatum*	•	–	•
Leptocheirus mariae	–	•	•	*Tethylembos viguieri*	–	–	•
Leptocheirus pectinatus	–	•	•	*Urothoe elegans*	•	–	•
Leucothoe incisa	•	•	•	*Westwoodilla rectirostris*	•	•	•

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