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Charalampos Dimitriadis<sup>a</sup> & Drosos Koutsoubas<sup>a</sup>

<sup>a</sup> Department of Marine Sciences, Faculty of Environment, University of the Aegean, Lesvos Island, Greece

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## Community properties of benthic molluscs as indicators of environmental stress induced by organic enrichment

Charalampos Dimitriadis and Drosos Koutsoubas\*

*Department of Marine Sciences, Faculty of Environment, University of the Aegean, Lesvos Island (Greece)*

This study investigates the multivariate spatio-temporal population patterns of molluscan taxocoenosis along an organic load gradient caused by the presence of a fish farm unit in the semi-enclosed micro-tidal Gera bay in the island of Lesvos (northeast Aegean), by exploring three different properties (species abundance, trophic web structure and geographic ranges) as indicators for detecting community disturbance. Results have shown that community properties related to ecosystem functioning (i.e. food web analysis) and macro-ecological aspects (i.e. zoogeographical orientation community features) have proved to be more sensitive in detecting the effects of organic load perturbation than species abundance, thus indicating the use of such properties in environmental assessment approaches in the future.

**Keywords:** benthic community; molluscs; aquaculture; organic load gradient; environmental assessment; NE Aegean

### Introduction

Biological communities are characterized by: (1) the structural features of species populations (described by measures such as abundance, biomass, diversity indices and species multivariate spatio-temporal patterns); (2) features that are related to the functioning of biological communities and ecosystems (e.g. food web, functional diversity), and (3) community macro-ecological features (e.g. species' geographical ranges). Anthropogenic stress regimes, especially organic load enrichment of coastal areas, can cause serious alterations to species community patterns (Pearson and Rosenberg 1978) that are more pronounced in benthic communities (Karakassis et al. 2000). Thus, disturbance is a key factor controlling the spatial and temporal composition of marine soft sediment communities (Probert 1984; Hall 1994) resulting in heterogeneous community patches within and between habitats (Dernie et al. 2003).

Community analysis of structure and dynamics for the purposes of an environmental assessment, based on abundance/biomass or presence/absence data for species has been a widely explored issue (e.g. Field et al. 1982; Heip 1992; Clarke and Green 1998) and has become the typical approach of benthic community analysis.

Disturbance regimes could also have an impact on the functional features of the communities and especially on the structure of the trophic webs. Single-level food webs can provide environmental assessment in such a way that direct or indirect effects of perturbation and disturbance can be detected under the hypothesis that

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\*Corresponding author. Email: drosos@aegean.gr

different trophic levels have different responses to environmental stress or gradient (Culp et al. 2005). Single level food webs can be explored with the use of functional feeding groups, that is, classifications of species into groups that share similar requirements in resource use and also play similar functional roles in a community (Chapin et al. 1996; Díaz and Cabido 1997). Feeding guilds can be used as functional feeding groups (Merritt and Cummins 1996), and their total abundance measures can produce multivariate community patterns in terms of community structure and dynamics along an organic gradient for an environmental assessment approach. Although the functional feeding groups approach for the needs of a multivariate food web analysis has been performed in terrestrial ecosystems, it has not been widely used in aquatic ecosystems except for some cases concerning riverine environments and benthic insect communities (Culp et al. 2005).

Across the geographic range of species, complex zoogeographical patterns occur due to the variation of environmental variables and disturbance regimes (Gaston 2003). Consideration of these patterns poses the question of how species abundance changes within this range (e.g. Roy et al. 1995; Gaston and Blackburn 1996; Hecnar 1999). Within a local community the way geographical range is represented across a local disturbance gradient and the possibility of using zoogeographical patterns for the needs of an environmental assessment is an interesting issue. This approach has already been investigated in marine benthic communities distributed in extreme and unpredictable environments such as estuaries, lagoons and hydrothermal vents (Tiganus 1988; Thiermann et al. 1997; Arvanitidis et al. 1999). The results of these studies have revealed that organisms with wider geographic ranges, such as cosmopolitan species, are dominant in stressed environments. This has been attributed to the fact that those species, having increased dispersal ability and resistance to environmental variations, are more likely successfully to colonize various habitats in contrast to species with narrower geographic range (Gaston 2003).

Benthic molluscs are an important component of total macrofauna and play a significant role in ecosystem structure and biodiversity maintenance (Zenetos 1996b; Koutsoubas et al. 2000a). However, a biotope approach based on the molluscan fauna distributed along the continental shelf of the Greek seas has been used in very few cases (e.g. Zenetos et al. 1991; Zenetos 1997; Koutsoubas et al. 2000a). These studies have revealed that a molluscan inventory could be used successfully to address the environmental status of the total macrobenthic assemblages (Koutsoubas et al. 2000a; Arvanitidis et al. 2005).

The present study is part of a larger project investigating the potential effects of aquaculture activity in the benthic and pelagic domain in the semi-enclosed coastal area of Gera Bay in the island of Lesbos (northeast Aegean Sea). A series of studies investigating relationships between aquaculture and the marine environment has been conducted over the last decade in the Greek seas (e.g. Karakassis et al. 1999, 2000; Karakassis and Hatziyanni 2000; Machias et al. 2005; Pitta et al. 2005). However, when examining the effects of organic load produced by the sea farm cages on the benthos domain the majority of studies, not only in the Greek seas but also in other European areas, focus on total macrofauna (e.g. Karakassis et al. 2000; Heilskov and Holmer 2001). The present contribution aims to investigate mollusc multivariate, spatio-temporal, population patterns along an organic load gradient caused by the presence of a fish farm, by exploring three different community

properties: (1) the structural (abundance patterns) property, (2) the functional (trophic level patterns) property and (3) the macro-ecological (geographic ranges) property, in an effort to reveal whether these properties are capable of detecting community disturbance.

**Methods**

**Study area**

Gera Gulf, in the entrance of which the study area (Palioloutro) is located (Figure 1), is a semi-enclosed gulf in the island of Lesvos (north Aegean, eastern Mediterranean), characterized by low mean depth (10 m) and poor water exchange with the open Aegean Sea (Arhonditsis et al. 2002). Over the last 15 years a fish farm (mainly seabass and seabream) has operated in Palioloutro with a mean annual production of 150 tons. Released nutrients and organic outputs from the fish farm

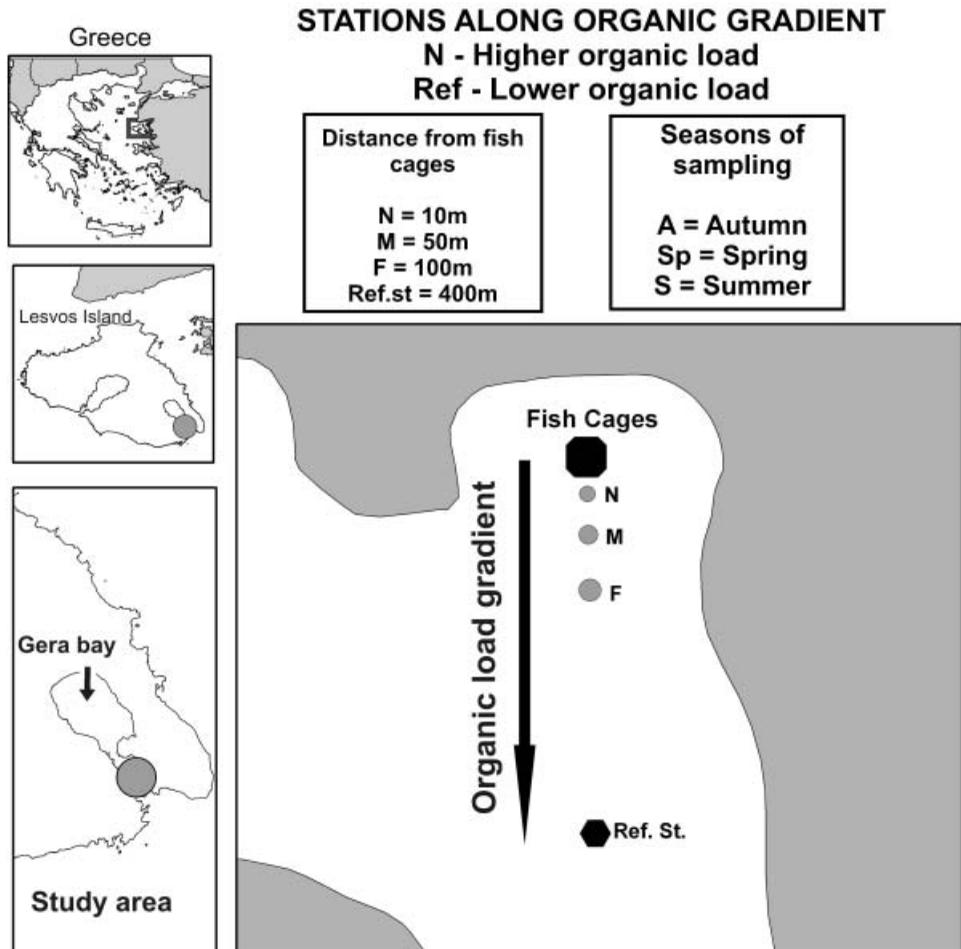


Figure 1. A map of the island of Lesvos indicating Gera Gulf, the study area (Palioloutro) where the fish farm unit is located and the sampling sites.

into the surrounding area have contributed to the increased values of Chl- $\alpha$  concentrations recorded in the proximity of the cages, while with increasing distance from the cages there is a gradual decrease. Similarly, organic material concentrations in the sediment ranged from 0.8–2.4%, gradually increasing with decreasing distance from the fish cages. Finally, the sediment profile is characterized by fine particles up to intermediate distance from the cages (i.e. 100 m) and more coarse material further away (Dimitriadis et al. 2004).

### ***Sampling design and processing***

Benthic quantitative macrofauna samples were collected during 2001–2002 by means of a macrofauna Van Veen grab (sampling surface 0.1 m<sup>2</sup>) on a seasonal basis from four stations along the established organic load gradient (Figure 1). Three replicate grab samples were taken at each station, which were washed through a 0.5 mm sieve, fixed in 10% formalin and kept separately with the addition of the vital stain Rose Bengal. Macrofauna were sorted into major taxonomic groups, identified to species level, counted and weighed. For the species identification a series of key books and related literature were used (e.g. Koutsoubas et al. 1997; Delamotte and Vardala-Theodorou 2001, and references therein).

### ***Data analysis***

Molluscs were classified according to their feeding guilds and their zoogeographical categories, thus forming species groups (functional feeding groups, FFG and zoogeographical groups, ZCG, respectively). Classification of molluscs according to their feeding guilds was based on information provided by Graham (1955), Russo et al. (1985) and Bernasconi and Stanley (1994). Across the sampled area molluscan taxocoenosis has been considered to form a single trophic level as a module or sub-network embedded within the larger ecosystem web (Holt 1997). The formed single trophic level structure of FFG can be described from the “internet” model having major and minor “hubs” within the network architecture (Jordan and Scheuring 2002). In this concept, predators (i.e. carnivores group) have only one link to the other FFG (i.e. detritus feeders, suspension feeders, deposit feeders, herbivores, parasitic) that in turn have direct connections to ecosystem resources (e.g. sediment organic material, suspended compounds). Assignment of mollusc species to the different zoogeographical categories (i.e. Atlanto-Mediterranean species, Mediterranean Endemics, Boreal) was based on Koutsoubas (1992), Zenetos (1996a) and Koutsoubas et al. (1997, 2000a, 2000b).

Molluscan taxocoenosis were analyzed by means of univariate and multivariate statistical methods. Univariate analysis involved the measure of abundance and biomass (per species and mean per sampling site), species richness, diversity indices:  $J'$ , Evenness;  $H'$ , Shannon-Wiener index,  $d'$ , Margalef species richness and Simpson's  $1-\lambda'$  index as well as the new taxonomic biodiversity indices ( $\Delta^+$ ,  $s\Delta^+$ ,  $\Lambda^+$ ) based on taxonomic distinctness (Warwick and Clarke 1995, 1998; Clarke and Warwick 1998) plotted on 95% probability funnels. Multivariate community patterns were investigated using similarity matrices of Bray-Curtis index on species abundance and FFG and ZCG abundance, by means of the PRIMER-5 software (Clarke and Warwick 1994). The contribution of each species to the average Bray-Curtis dissimilarity between and within groups of species was identified using SIMPER analysis. Statistical significance

of all groups' similarities was tested with the use of ANOSIM analysis (Clarke and Warwick 1994). Significance of diversity value differences between and within seasons was detected with Mann-Witney test (Zar 1984).

## Results

A total of 2247 individuals belonging to 91 gastropod, bivalve and scaphopod mollusc species was identified from the study area during the three sampling periods (Table 1). Special reference should be made to two of the mollusc species found, namely the gastropods *Epitonium aculeatum* (Allan, 1818) and *Atys brocchi* (Michelotti, 1847), which are reported for the first time as elements of the molluscan fauna of the north Aegean Sea. Feeding guilds and zoogeographical categories are presented in Table 1. Sediment (74.5%) and suspension feeders (15%) were the most abundant feeding guilds, followed by the carnivores (6.8%), and the other guilds (i.e. detritus, herbivores and parasitic feeders, 4.7%). Considering zoogeographical categories, the Atlanto-Mediterranean species were the most abundant (64.2%) followed by Boreal (25%) and Mediterranean Endemic species (10.3%).

Lower species numbers were detected in the immediate vicinity of the fish cages (e.g. three species in station NS – higher organic load) and higher ones far from the cages (lower organic load) for all sampling seasons (e.g. 31 species in station SpF). On the other hand, the highest values of species abundance were noticed at the intermediate organic load stations (e.g. 113 individuals/0.1 m<sup>2</sup> in station AM) and at the reference station (e.g. 116 individuals/0.1 m<sup>2</sup> in station ARef) for the majority of the sampling periods. Mean dry biomass for all stations was calculated at 0.97gr/0.1 m<sup>2</sup> and ranged from 2.25 to 0.17 gr/0.1 m<sup>2</sup> at stations SF and SpM, respectively. The bivalve *Mysella bidentata* occupied 39% of the total abundance in the autumn sampling period, whereas in the summer sampling period 30% of the total abundance was occupied by the bivalves *Tellina distorta* and *Loripes lacteus*. Lower dominance levels (20%) were observed in the spring sampling period (mainly occupied by the bivalve *Corbula gibba*). Diversity indices ( $d'$ ,  $J'$ ,  $H'$ ,  $1-\lambda'$ ,  $\Delta^+$ ,  $s\Delta^+$ ,  $\Lambda^+$ ) showed no significant differences between seasons (Mann-Witney test results) but there were significant differences ( $p < 0.05$ ) within each season distinguishing the area close to the fish cages from the rest of the study area. Although all molluscan diversity indices values increased with decreasing values of organic load, this was more pronounced in the taxonomic indices  $s\Delta^+$  and  $\Lambda^+$ .

Taxonomic diversity values of  $\Delta^+$  and  $\Lambda^+$  indices plotted on 95% probability funnels (Figure 2) revealed that the entire study area was within the local “natural” taxonomic diversity levels as a result of the random selection from the total collected species pool (Clarke and Warwick 1998). High and close to average values of  $\Delta^+$  and low values of  $\Lambda^+$  found at the stations of high organic load SN and AN, should be attributed to the presence of few species at each station (only three and seven correspondingly).

### *Multi-species abundance analysis*

Multivariate analysis (nMDS and cluster analysis superimposition) of the sampling sites based on the total species list abundances revealed a tendency of separation (ANOSIM test global  $R = 0.29$ ,  $p < 2\%$ ) of the study area into three main groups (Figure 3).

Table 1. Ecological and zoogeographical attributes of mollusc species found at the study area.

Species list	Stations	FG	ZC
<b>GASTROPODA</b>			
<i>Acteon tornatilis</i> (L, 1758)	AM, SpN, SpRef	Carn	B
<i>Alvania cimex</i> (Linnaeus, 1758)	SpN	Det	E
<i>Atya brochi</i> (Michelotti, 1847)	SpM	Carn	AM
<i>Bela brachystoma</i> (Philippi, 1844)	SpM	Carn	E
<i>Bela nedula</i> (Montagu, 1803)	SpRef	Carn	B
<i>Bulla striata</i> (Bruguere, 1789)	SRef	Carn	A-M
<i>Bolinus brandaris</i> (L, 1758)	AF,	Carn	A-M
<i>Bittium lacteum</i> (Philippi, 1836)	SpN	Det	B
<i>Bittium latreillei</i> (Payraudeau, 1826)	SpM	Det	B
<i>Bittium reticulatum</i> (Da Costa, 1778)	SpM	Det	B
<i>Calyptra chinensis</i> (L, 1758)	ARef	Det	A-M
<i>Crenilabium exile</i> (Forbes in Jeffreys, 1870)	AM	Carn	A-M
<i>Cylichna cylindracea</i> (Pennant, 1777)	SRef	Carn	B
<i>Cylichna umbilicata</i> (Montagu, 1803)	SM, SF, AM, AF, ARef, SF	Carn	B
<i>Eulima glabra</i> (Da Costa, 1778)	SpF, SpRef	Par	A-M
<i>Epitonium aculeatum</i> (Allan, 1818)	SpF	Det	B
<i>Euspira guillemini</i> (Payraudeau, 1826)	SpF	Carn	E
<i>Euspira nitida</i> (Donovan, 1804)	SF, SpF	Carn	B
<i>Fusinus syracusanus</i> (Linnaeus, 1758)	SpF	Carn	E
<i>Gibbula ardens</i> (Von Salis, 1793)	SpM	Herb	A-M
<i>Hyalia vitrea</i> (Montagu, 1803)	SN, SF, AF, SpN, SpRef	Det	B
<i>Jujubinus montagui</i> (Wood, 1828)	SpM	Herb	A-M
<i>Jujubinus striatus</i> (L, 1758)	ARef, SpN	Herb	A-M
<i>Mangelia attenuata</i> (Montagu, 1803)	SF, SpF	Carn	B
<i>Mangelia unifasciata</i> (Deshayes, 1835)	SpF	Carn	A-M
<i>Mangelia vauquelini</i> (Payraudeau, 1826)	AN	Carn	A-M
<i>Melanella polita</i> (Linnaeus, 1758)	SpRef	Par	B
<i>Murex brandaris</i> (Linnaeus, 1758)	SF	Carn	B
<i>Nassarius reticulatus</i> (Linnaeus, 1758)	SF, SRef, SpN, SpM, SpF	Carn	A-M
<i>Nassarius incrassatus</i> (Stroem, 1768)	AM, AF, ARef	Carn	B
<i>Nassarius pygmaeus</i> (Lamarck, 1822)	SpF	Carn	B
<i>Ocenebrina edwardsii</i> (Payraudeau, 1826)	ARef	Carn	A-M
<i>Odostomia conoidea</i> (Brocchi, 1814)	SRef, SpN, SpF, SpRef	Par	B
<i>Odostomia rissoides</i> (Hanley, 1844)	AF, ARef	Par	B
<i>Peringiella</i> sp.	AF	Det	–
<i>Philina catena</i> (Montagu, 1803)	SpN	Carn	B
<i>Pussilina parva</i> (Da Costa, 1778)	SpN	Det	B
<i>Raphitoma echinata</i> (Brocchi, 1814)	SpN	Carn	E
<i>Retusa semisulcata</i> (Philippi, 1836)	SpF	Carn	A-M
<i>Ringicula conformis</i> (Monterosato, 1877)	ARef, SpRef	Carn	B
<i>Trophon muricatus</i> (Montagu, 1803)	SpF	Carn	E
<i>Turbonilla rufa</i> (Philippi, 1836)	SF	Par	E
<i>Turbonilla lactea</i> (Linnaeus, 1758)	ARef	Par	B
<i>Turbonilla striatula</i> (Linnaeus, 1758)	SpN, SpF, SpRef	Par	E
<i>Vitreolina incurva</i> (B.D.D., 1884)	AF	Par	E
<b>BIVALVIA</b>			
<i>Abra prismatica</i> (Montagu, 1808)	AN, AM, AF, ARef, SpF	Dep	B
<i>Abra longicallus</i> (Scacchi, 1834)	SF, SRef, SpF, SpRef	Dep	A-M

Table 1. (Continued)

Species list	Stations	FG	ZC
<i>Abra tenuis</i> (Montagu, 1803)	SpF	Dep	B
<i>Acanthocardia tuberculata</i> (Linnaeus, 1758)	SRef, AM	Susp	A-M
<i>Anodontia fragilis</i> (Philippi, 1836)	AM, AF, ARef,	Dep	A-M
<i>Axinulus croulinensis</i> (Jeffreys, 1876)	SF	Dep	A-M
<i>Azorinus chamasolen</i> (Da Costa, 1778)	SpF, SpRef	Dep	B
<i>Clausinella fasciata</i> (Da Costa, 1778)	Aref	Det	E
<i>Corbula gibba</i> (Olivi, 1972)	SM, SF, SRef, AN, AM, AF, ARef, SpN, SpM, SpF, SpRef	Susp	A-M
<i>Ctena decussata</i> (O.G. Costa, 1829)	ARef, SpRef	Dep	A-M
<i>Cuspidaria cuspidata</i> (Olivi, 1792)	SpRef	Susp	A-M
<i>Donax variegatus</i> (Gmelin in L., 1791)	SRef	Dep	A-M
<i>Dosinia lupinus</i> (Linnaeus, 1758)	SF, SRef,	Susp	E
<i>Gastrochaena dubia</i> (Pennant, 1777)	SpRef	Susp	A-M
<i>Gouldia minima</i> (Montagu, 1803)	SpM, SpF	Dep	A-M
<i>Hiatella arctica</i> (Linnaeus, 1767)	SF, SRef, AM, ARef, SpF	Susp	A-M
<i>Loripes lacteus</i> (Linnaeus, 1758)	SM, SF, SRef, AN, AM, AF, ARef, SpN, SpM, SpF, SpRef	Dep	B
<i>Lucinella divaricata</i> (Linnaeus, 1758)	SM, SF, SpN, SpM, SpRef	Dep	B
<i>Macoma cumana</i> (O.G. Costa, 1829)	SpRef	Dep	A-M
<i>Modiolus adriaticus</i> (Lamarck, 1819)	SF	Susp	E
<i>Myrtea spinifera</i> (Montagu, 1803)	AN, AF	Dep	A-M
<i>Musculus discors</i> (Linnaeus, 1767)	SF	Susp	B
<i>Musculus subpictus</i> (Linnaeus, 1767)	AM	Susp	AM
<i>Mysella bidentata</i> (Montagu, 1803)	SM, AM, AF, ARef, SpN, SpF, SpRef	Dep	A-M
<i>Neolepton</i> sp.	AM, AF, ARef	Dep	–
<i>Nucula aegeensis</i> (Forbes, 1844)	AN, AM, AF, ARef	Dep	E
<i>Nucula nitidosa</i> (Wincworth, 1930)	SM, SF, SRef, SpM	Dep	B
<i>Nucula nucleus</i> (Linnaeus, 1758)	SM, SpRef	Dep	B
<i>Nuculana pella</i> (Linnaeus, 1767)	SF, SRef, AM, AF, ARef, SpF, SpRef	Dep	E
<i>Parvicardium exigum</i> (Gmelin in L., 1791)	SRef	Susp	A-M
<i>Parvicardium ovale</i> (G.D. Sowerby, 1841)	SRef, SpM, SpRef	Susp	A-M
<i>Parvicardium papillosum</i> (Poli, 1795)	SpF	Susp	A-M
<i>Phaxas adriaticus</i> (Coen, 1933)	SRef, AF	Dep	E
<i>Phaxas pellucidus</i> (Pennant, 1777)	SpRef	Dep	B
<i>Psammobia fervensis</i> (Gmelin in L., 1791)	SRef, SpRef	Dep	A-M
<i>Psammobia costulata</i> Turton, 1822	AM	Dep	A-M
<i>Tellina distorta</i> Poli, 1791	SM, SF, SRef, AM, AF, ARef, SpN, SpM, SpF, SpRef	Dep	A-M
<i>Tellina donacina</i> Linnaeus, 1758	SF, AN, AM, AF, ARef, SpRef	Dep	A-M
<i>Tellina tenuis</i> Poli, 1791	SF, SRef	Dep	A-M
<i>Tellymia ferruginosa</i> (Montagu, 1808)	SM	Dep	A-M
<i>Thyasira flexuosa</i> (Montagu, 1803)	SN, SF, SRef, AM, AF, ARef, SpRef	Dep	A-M
<i>Venericardia antiquata</i> (B.D.D., 1892)	SF, SRef, SpF	Susp	E
<b>SCAPHOPODA</b>			
<i>Dentalium inaequicostatum</i> (Dautzenberg, 1891)	SM, SRef, SpN, SpF, SpRef	Carn	E

Table 1. (Continued)

Species list	Stations	FG	ZC
<i>Dentalium vulgare</i> Da Costa, 1778	SN, SM, SF, SRef, AM, AF, ARef, SpF, SpRef	Dep	B
<i>Fustiaria rubescens</i> (Deshayes, 1825)	SM, SF, SRef, AM, AF, SpF, SpRef	Dep	E
<i>Cadulus politus</i> (Wood, 1842)	SRef	Det	E

Note: FG, feeding guilds; Carn, carnivores; Det, detritus feeders; Dep, deposit feeders; Par, parasitic feeders; Herb, herbivores; Susp, suspension feeders; ZC, zoogeographical category; E, Mediterranean endemic; A-M, Atlanto-Mediterranean species; B, boreal species.

The first group includes the stations of high and intermediate organic load (i.e. stations close to fish cages) for autumn and spring sampling periods. The second group consists of the station with high organic load for the summer sampling period and the third group is formed by the remaining stations. SIMPER analysis detected high dissimilarity, exceeding 68% in species composition, between sampling seasons produced mainly by the bivalve species *Mysella bidentata*, *Thyasira flexuosa*, *Nucula aegeensis* and *Tellina distorta*. On a spatial scale high dissimilarity in species composition (>80%) was observed between the stations of high and intermediate organic load mainly due to the presence/absence of the bivalve species *Mysella bidentata*, *Tellina distorta* and *Corbula gibba*. Low dissimilarity (<49 %) of species composition was observed between and within low organic load stations. These results reveal that dissimilarity between and within stations decreased with decreasing organic load.

### Trophic level analysis

Multivariate FFG spatio-temporal analysis indicated an elevated degree of discrimination (ANOSIM test global R=0.65, p<1%) of the area into five groups

Table 2. Diversity indices values for the total study area.

	S	N	d'	J'	H'(log <sub>2</sub> )	1-λ'	sD <sup>+</sup>	D <sup>+</sup>	L <sup>+</sup>
SN	3	1.33	6.95	0.94	1.50	2.50	250	83.33	0
SM	12	38	3.02	0.87	3.12	0.87	839.39	69.94	313.29
SF	25	103.66	5.17	0.72	3.35	0.87	1781.94	71.27	225.95
SRef	25	87.33	5.36	0.71	3.31	0.85	1743.05	69.72	245.29
AN	7	3.66	4.61	0.94	2.66	1.13	466.66	66.66	158.73
AM	21	113.66	4.22	0.54	2.38	0.61	1473.33	70.15	204.73
AF	22	94	4.62	0.79	3.54	0.90	1646.97	71.60	216.03
ARef	24	116.66	4.83	0.67	3.07	0.80	1733.33	72.22	192.56
SpN	19	31	5.24	0.85	3.63	0.91	1396.29	73.48	164.62
SpM	14	12	5.23	0.92	3.50	0.97	1015.38	72.52	197.64
SpF	31	76.33	6.92	0.74	3.67	0.88	2265.55	73.08	221.08
SpRef	30	80.99	6.59	0.76	3.77	0.91	2162.06	72.06	210.27

Note: S, species number; N, abundance; d', species richness; J', Pielou's evenness; H'(log<sub>2</sub> base), Shannon diversity; 1-λ', Simpson's index; sD<sup>+</sup>, Total taxonomic distinctness; D<sup>+</sup>, Average taxonomic distinctness; L<sup>+</sup>, Variation of taxonomic distinctness.

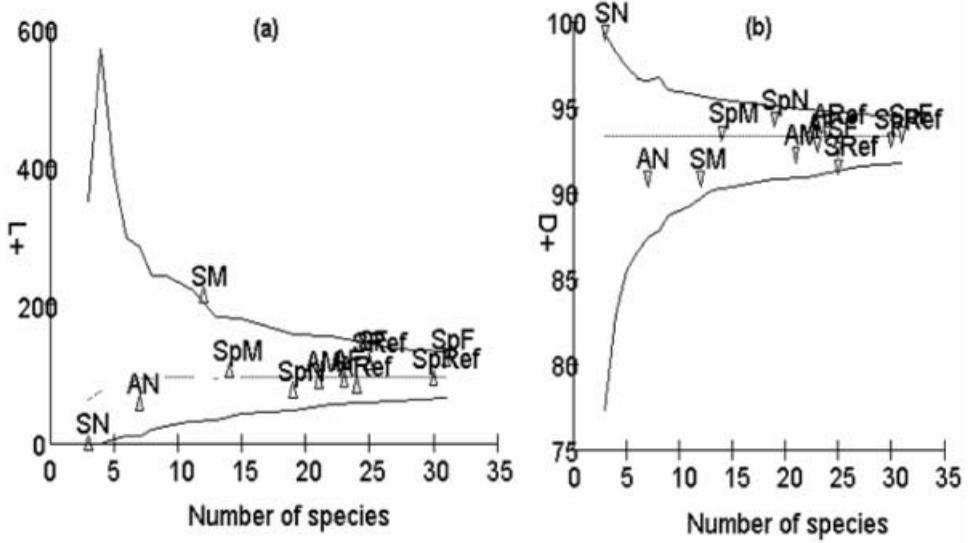


Figure 2. Probability funnels of diversity indices  $\Delta^+$  (a) and  $\Lambda^+$  (b) for all sampling stations and seasons in the study area.

(nMDS superimposition) (Figure 4). The first group includes the station of high organic load in summer. The second and the third groups consist of the stations of high and intermediate organic load in autumn and spring, respectively, the fourth includes the station of high load in spring and the fifth includes the remaining stations. Discrimination based on the FFG spatio-temporal analysis showed not only a higher degree of statistical significance grouping from the analysis based on species abundance ( $R=0.65$  vs  $R=0.29$ ), but in addition a different number of groups (5 vs 3).

Superimposition of FFG abundance on the total area grouping map (Figure 4) revealed a decline in FFG abundance with increasing organic load. Deposit feeders seemed to colonize intermediate and high organic load areas more effectively, displacing the rest of the feeding guilds. However, the critical factor for the interpretation of this pattern was the number of the links of the trophic net that were

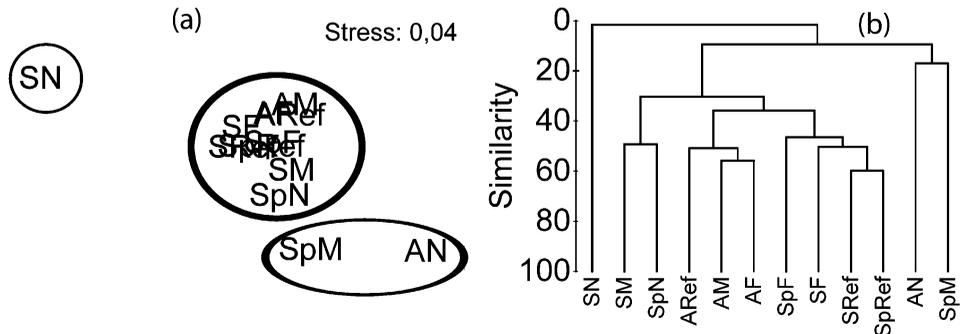


Figure 3. MDS plot (a) and cluster analysis (b) of species abundances highlighting three main groups of areas.

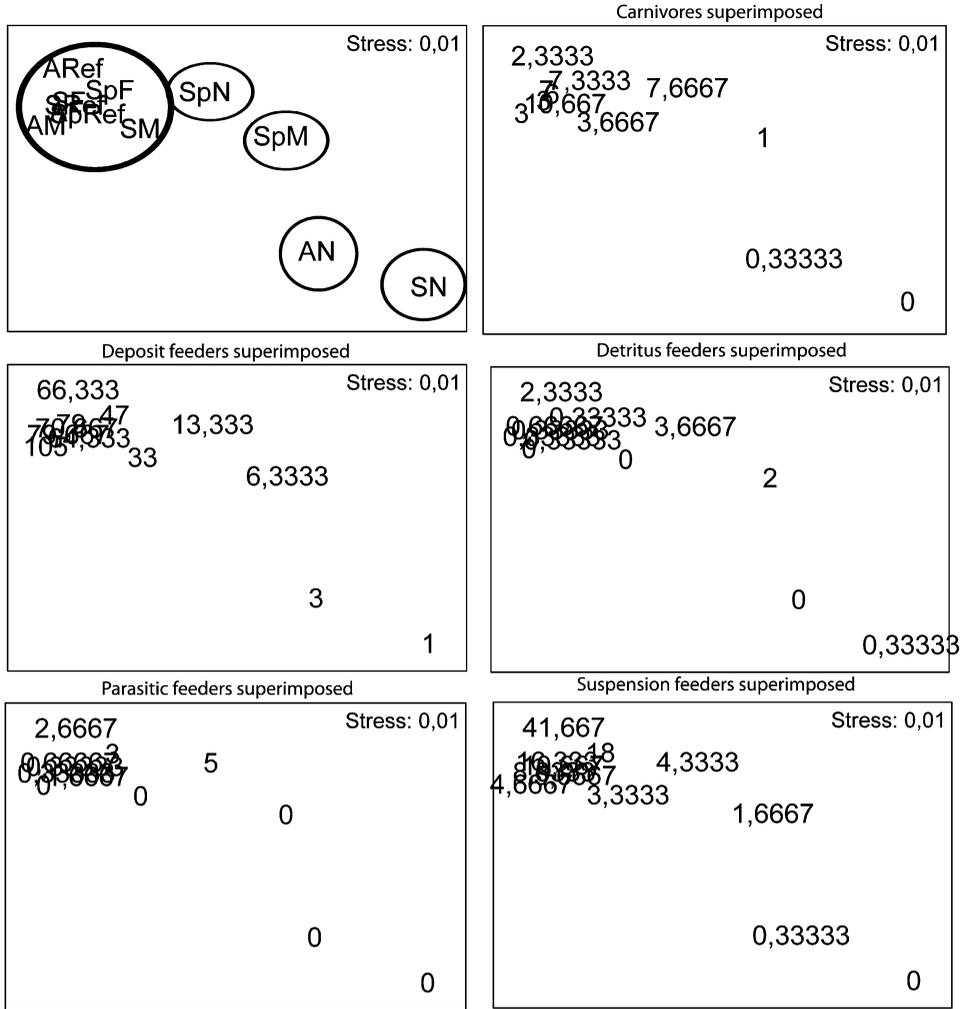


Figure 4. MDS plots of Functional Feeding Groups (FFG) where mean abundance of each group is superimposed.

present within each group of areas. In group 1 the trophic net collapses at station SN, and in the second group only two links were present at station AN, a fact that could be attributed to the direct effect of high organic load on the mollusc species. The high organic load station SpN (group 3), loses one trophic link, and in Group 4, which includes station SpN of high organic load, all possible links were present but all FFG have low abundance values. Group 5 had the maximum possible links and was characterized by high abundance values for deposit and suspension feeders. All possible links were also present in group 5 of the majority of the sampling stations, with the exception of stations AM and SM (intermediate organic load) where detritus and parasitic feeder links were absent. Organic load tends crucially to determine the structure of the trophic net, since the net collapses with high organic presence or misses links at intermediate levels. On the contrary, low organic level areas showed greater trophic complexity and hence more highly structured communities.

**Zoogeographical ranges analysis**

Multivariate analysis of ZCG as a quantification of geographic ranges within a local community produced significant discrimination of the study area (ANOSIM test global  $R=0.60$ ,  $p<1\%$ ) into five groups (Figure 5). The first group includes the station of high organic load in summer, the second group is formed by the station of high organic load for the sampling period of autumn, the third group consists of the station of intermediate load for the summer period, the fourth group includes the stations of intermediate and high organic load during summer and spring periods respectively, and finally the fifth group is formed by the remaining stations.

Superimposition of ZCG abundance on the multi-dimensional scaling plot (Figure 5) shows the replacement of Mediterranean endemic species with the wider distributed Atlanto-Mediterranean and Boreal species. The change in the zoogeographical affinities pattern of the community to the area of high organic load is interpreted as an indication of disturbance effect. Despite the fact that the independent variables incorporated in the multivariate analysis of the ZCG were few, therefore posing a significant lockstep which might induce constraints in the reliability of the method, statistical significance was achieved ( $p<2\%$ ) and area-grouping strength was also confirmed by low stress value of nMDS method (stress=0.01) superimposition.

**Discussion**

It has been proposed that distribution patterns of single taxonomic groups such as amphipods (Gómez-Gesteira and Dauvin 2000) and polychaetes (Samuelson 2001)

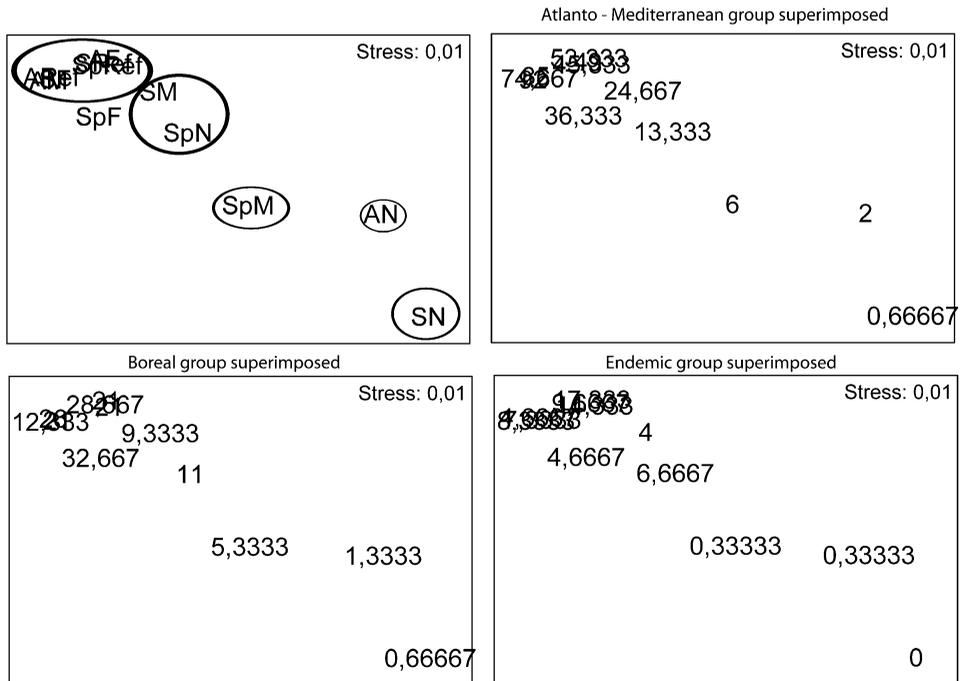


Figure 5. MDS plots of Zoogeographical Categories Groups (ZCG) where mean abundance of each group is superimposed.

could be used as indicators of environmental stress and pollution (Arvanitidis et al. 2005). Benthic molluscs have also been used as descriptors of soft bottom communities (Russo et al. 1985; Zenetos 1996b), but there is little information concerning their use as an appropriate taxonomic group for the needs of an environmental assessment of community response to natural or anthropogenic-induced stress regimes (Koulouri et al. 2006). In this study, it has been shown that molluscs, as a group, can be used as a descriptor of spatio-temporal community pattern alteration caused by an established organic load gradient. Molluscan multi-species abundance distribution along this gradient has been proved to be rather a weak property in the effort clearly to distinguish the high and intermediate organic load areas. However, among diversity indices used in this study, taxonomic indices patterns were more sensitive in revealing molluscan diversity pattern in relation to organic load than others (e.g.  $d'$ ,  $J'$ ,  $H'$ ,  $1-\lambda'$ ) and were capable of separating the high from the intermediate organic load areas. The usefulness of the taxonomic indices in detecting disturbance patterns in coastal areas has been indicated in a series of recent studies (e.g. Clarke and Warwick 1998; Arvanitidis et al. 2005).

Warwick and Clarke (1998) have noted that changes of total species abundances or total species richness are not necessarily sensitive indicators of disturbance gradients, and probably properties related to community function should be considered under a disturbance regime. Trophic level analysis in the study area indicated a clear separation of the high organic load areas from the intermediate areas and indicated the crucial effect of increased organic load on molluscan trophic net functioning. Molluscan population resilience, defined as the rate at which populations recover their densities, depends on species birth and death rates, as well as on connections to other species and in particular on the length of the food chain in which the species occur (Pimm 1991). In the study area the single trophic net at high organic load sites either collapses (i.e. disappearance of predators) during summer or is present with reduced trophic links during autumn. It is assumed that the persistence of different populations within the single trophic web, under the established organic load gradient in the area, is responsible for the discrimination of the different community patches along the gradient. The discrimination of the study area according to trophic level structure and dynamics revealed a higher degree of statistical significance grouping and a higher degree of separation strength than the species abundance analysis, and hence higher sensitivity as a disturbance assessment approach. It is worth mentioning that the trophic analysis results were similar to

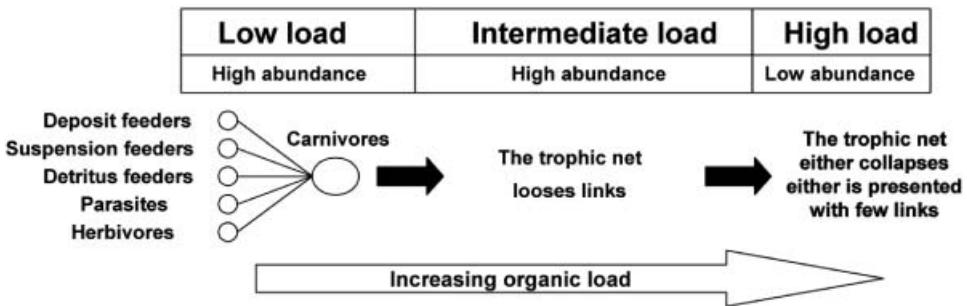


Figure 6. Effect of increasing organic load on trophic net structure.

those obtained from the taxonomic relatedness indices. Certain mollusc families responsible for the observed pattern of taxonomic relatedness were also responsible for the pattern obtained from the FFG analysis, therefore revealing a considerable imprint of phylogeny within functional feeding groups. This phylogenetic imprint has also been recorded for other major taxonomic groups (e.g. insects) distributed in terrestrial ecosystems, in which trophic analysis has been applied (e.g. Leishman and Westoby 1992; Díaz and Cabido 1997).

According to Price et al. (1988) there is evidence that species with a wide geographical range tend to displace species with a narrower range. Therefore, species with wider native geographical ranges are more likely to colonize an area successfully, probably because of their greater dispersal abilities and tolerance of a wide range of habitats and environmental conditions (Duncan et al. 2001). At community level, the species with larger populations tend to be the more zoogeographically widespread for a given taxonomic assemblage (Brown and Briggs 1991; Gaston et al. 1997), while locally rare species have restricted distribution. This pattern was confirmed from the zoogeographical structure and dynamics approach in the study area. Indeed, in sites with higher organic loads, endemic Mediterranean species were replaced by populations of species with higher resilience, such as the Boreal and the Atlanto-Mediterranean species. A similar phenomenon has been recorded from the naturally-stressed coastal environment in Gialova lagoon in relation to Polychaetes. In this particular case study there was a dominance of cosmopolitan polychaete species in the lagoon (Arvanitidis et al. 1999). However, in molluscs the truly cosmopolitan species are considered to be those with a pelagic life (e.g. species belonging to the Gastropod order Thecosomata), and the percentage of benthic cosmopolitan molluscs in the Greek seas is negligible (Koutsoubas 1992). Therefore, in the present case, instead of the missing cosmopolitan species, Boreal and Atlanto-Mediterranean species have been considered the most possible widespread zoogeographical categories. Another interesting result of this study is the fact that along the organic load gradient there was evidence that the species population size decreased faster than the species zoogeographic range size with increasing organic load. Therefore, with the exception of the maximum organic load site, all the zoogeographical groups were present, even if population size had diminished. It has been possible to assess different organic load effects using zoogeographical distribution patterns within the local molluscan taxocoenosis (areas of high and intermediate organic load are clearly separate from the rest). However, much attention should be paid to the fact that large ranges of some species might probably be an artifact of poor or inadequate taxonomy hiding the existence of cryptic species (homonymy) (Murray and Dickman 2000; Shaw 2001). Another matter of concern is that multiple species may have been considered as one, especially when they lack complex morphological characteristics that are used for identification, a case often met in marine invertebrate taxa (Gaston 2003).

## **Conclusions**

Results from this study have revealed that the molluscan taxocoenosis can be used in environmental assessment of coastal marine environments subjected to an established organic load gradient. Molluscan community properties related to ecosystem functioning and macro-ecological aspects, such as food web analysis and

zoogeographical orientation community properties, have proved to be accurate in detecting the effects of organic load perturbation. The use of such properties in environmental assessment should be taken into consideration, along with species abundance, in an ecosystem approach effort to detect precisely the potential disturbance in the marine environment.

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