Marine Biology (2005) 148: 293–305
DOI 10.1007/s00227-005-0080-8

RESEARCH ARTICLE

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Quantifying effects of pollution on biodiversity: a case study of highly diverse molluscan assemblages in the Mediterranean

Received: 10 April 2005 / Accepted: 23 June 2005 / Published online: 1 September 2005

Abstract Structured sampling designs are important in the assessment of environmental impacts of variable ecological systems. Recent developments have provided a useful framework extending existing univariate techniques into a multivariate context. Measures of taxonomic relatedness have also been introduced, which complement existing measures of diversity of assemblages. In this study, the potential effects of sewage discharge on spatial patterns of highly diverse molluscan assemblages in a Mediterranean rocky subtidal habitat were investigated. Nine 20 cm×20 cm quadrats were taken from each of three sites (80 m–100 m apart) within a putatively impacted location near a sewage outfall (*I*) and at each of two control locations (*Cs*) by destructive sampling by SCUBA divers at a depth of 3 m–4 m. A total of 5507 specimens of 151 species were collected. The average and the variance in total abundance of molluscs were greater, on average, at *I* than at *Cs*. Higher abundances at the sewage outfall were largely driven by greater numbers of juvenile molluscs. The Shannon diversity of molluscs (H') was significantly lower at *I*, but no difference among locations was detected for the total number of species (*S*). In addition, the taxonomic distinctness (Δ^*) of molluscs was greater at *Cs*, although it was more variable at *I*. Multivariate analyses showed that there was a significant difference in the structure of assemblages at *I* compared with *Cs*. The location near the outfall was characterized by greater abundances of several species, including especially the gastropods *Pusillina philippi*, *Bittium latreilli*, and *Bittium reticulatum*. There was also greater variability in the structure of assemblages among sites and among quadrats at control locations compared to those near the outfall. Using a suite of univariate and multivariate measures, including diversity indices, detailed information on taxonomic structure and analyses of variability at different spatial scales, provided useful insights into the effects of sewage impacts on these diverse assemblages. These results also highlighted the importance of analysing measures of variance, as well as mean in considering effects of stress in natural communities.

Communicated by R. Cattaneo-Vietti, Genova

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Introduction

Identifying unambiguously the effects of anthropogenic impact on single response variables (e.g., total number of species or individuals, index of diversity, biomass or abundance of a single species) requires a comparison of the magnitude of change in a putatively impacted location to natural changes in multiple locations not affected by the source of disturbance under examination. BACI and beyond-BACI procedures (Green 1979; Underwood 1992, 1993), based on traditional analysis of variance, provide a framework to address these issues and are currently among the most powerful tools available in environmental impact assessment (Benedetti-Cecchi 2001; Hewitt et al. 2001; Underwood et al. 2003).

It is generally acknowledged that whole assemblages are more sensitive to environmental changes than individual indicator species variables or other univariate indices and that measures of assemblage patterns can provide the most useful information for interpreting the consequences of pollution onto ecological systems (Underwood and Peterson 1988; Clarke 1993). Several

multivariate procedures for visualising patterns or testing hypotheses are available to ecologists (e.g., Legendre and Legendre 1998) to analyse changes in assemblages in response to environmental impacts. Most of them (e.g., ANOSIM, Clarke 1993) have been widely used over the past decade and have contributed to the advance of current scientific environmental impact assessment. A limitation of many of these methods, however, is that they do not allow partitioning of multivariate variability in complex experimental designs, which poses problems for assessing environmental impacts.

In recent years, new statistical tools have been developed for the analysis of multispecies responses in structured ecological experiments (Legendre and Anderson 1999; Anderson 2001; McArdle and Anderson 2001). These methods allow tests of all terms, including nested hierarchies, contrasts and interactions, in structured multifactorial designs. The only additional assumption necessary, over and above those already required for non-parametric procedures such as ANOSIM (Clarke 1993), is the use of an additive model to partition the dissimilarity matrix in accordance with the particular ANOVA design. Such procedures have only recently been adopted for the analysis of multivariate data in complex designs to assess environmental impacts (Terlizzi et al. 2005), providing a promising framework for future impact studies.

Describing assemblages at fine levels of taxonomic resolution is often logistically difficult, however, requiring the counting of all organisms and their taxonomic identification. Accurate taxonomic analysis is often unachievable due to costs in processing samples and, above all, lack of taxonomic expertise (Maurer 2000; Boero 2001). Thus, although tests of hypotheses about the effects of anthropogenic disturbance on multivariate assemblages may be continuously advanced by the development of innovative statistical procedures, the widespread demise of taxonomy (May 1990; Giangrande 2003) may yet prevent an adequate taxonomic definition of the considered variables.

Clearly, the level of taxonomic accuracy at which assemblages are described depends on the objective of the study (Warwick 1993). For many groups of benthic organisms, it has been demonstrated that an environmental impact can be detected even when the analysis is based on a taxonomic level higher than species (Heip et al. 1988; Warwick 1988; Ferraro and Cole 1992). In such contexts, the use of a coarser level of taxonomic discrimination (i.e., *taxonomic sufficiency*, Ellis 1985) can reduce costs associated with detailed taxonomic analyses. This can potentially increase the power of the analysis, by allowing more resources to be allocated to spatial and temporal replication of experiments (Dauvin et al. 2003; Terlizzi et al. 2003a). The decision to use coarse taxonomic resolution should not, however, be made a priori. A close relationship between results of analyses based on coarse levels of resolution with those based on species-level analyses to quantitatively describe patterns of changes in assemblages under disturbance regimes must be demonstrated before coarse taxonomic resolution can be proposed for routine monitoring (Olsgard and Somerfield 2000). This is critical not only in the context of environmental management, but also so that explanatory models about the ecological processes underlying changes can be proposed and tested. Quantitative descriptions of assemblage patterns under disturbance provide the basis for a better understanding of mechanisms structuring assemblages and, also, allow tests of generality of particular ecological concepts developed from other studies (Andrew and Mapstone 1987; Underwood et al. 2000).

The level and accuracy of taxonomic identification can also clarify the response of univariate measures of biodiversity to stress. The detection of changes in biodiversity in relation to environmental impacts has some major drawbacks when biological diversity is expressed simply as the total number of species (Clarke and Warwick 1999). Most of these problems also apply to other diversity indices that variously emphasize the equitability components of diversity. New diversity indices, termed taxonomic distinctness, which address some of these problems have been proposed by Warwick and Clarke (1995). The rationale for the use of these measures is to consider not just the relative abundance of each species in an observation unit but also the distance between each pair of individuals or each pair of species through a Linnean classification tree, i.e., to take into account the taxonomic relatedness among species in a sample. Earlier studies on the response of taxonomic distinctness measures to disturbance events suggested that they might have a greater sensitivity than that seen for species richness or other indices of evenness (Warwick and Clarke 1995). A further major advantage is their lack of dependence on sample size (Clarke and Warwick 1998). Nevertheless, subsequent empirical studies (e.g. Somerfield et al. 1997) did not find consistent patterns of change in taxonomic distinctness with increasing environmental disturbance. Published studies in which measures of taxonomic distinctness have been applied, however, have used data from soft-sediment macro and meiobenthic fauna and bottom dwelling fish (Warwick and Clarke 1998; Hall and Greenstreet 1998; Rogers et al. 1999; Mistri et al. 2000; Warwick and Light 2002) and there is still a need to test the utility of these new indices in a wider set of habitats and on other components of the biota.

Molluscs are widely distributed in marine assemblages and may be extremely abundant in subtidal and intertidal habitats. The group is characterized by a large and consolidated taxonomic knowledge and most studies have been devoted, both on soft and hard substrates, to quantifying patterns of distribution with a very detailed level of taxonomic resolution. These studies covered a wide array of marine environments, ranging from cold and polar areas (Buhl-Mortensen and Høisæter 1993; Cattaneo-Vietti et al. 2000) to temperate (Milazzo et al. 2000; Kelaher et al. 2001; Terlizzi et al. 2003b) and tropical environments (Zuskin et al. 2001; Bouchet et al.

2002). On hard substrates, however, as for the vast majority of vagile invertebrate faunal groups (e.g., polychaetes, amphipods and decapods), little is known about the response of molluscan assemblages to anthropogenic disturbances (Lasiak 1998).

Sewage effluent, often discharged from outfalls into the marine environment, can result in significant effects on coastal marine biota (Pearson and Rosenberg 1978; Smith et al. 1999). On hard substrates, structured sampling designs (Archambault et al. 2001; Roberts et al. 1998) are increasingly used to quantify the effects of sewage on a wide range of biological assemblages, including fish, algae, and sessile organisms (e.g., Smith et al. 1999 and references therein). For molluscs, the effects of sewage pollution have been explored on patterns of growth and spatial distribution of a few species of intertidal limpets (Tablado et al. 1994; Bishop et al. 2002) but there has been no published quantification of changes in molluscan assemblages on rocky reefs exposed to anthropogenic point sources of sewage pollution.

In the present study, the putative impact of a shoreline sewage outfall on the structure of molluscan assemblages associated with shallow subtidal rocky reef was examined at a hierarchy of spatial scales. The purpose was (i) to quantify the effects of sewage pollution on natural patterns of spatial variation or biodiversity in molluscan assemblages and (ii) to examine how different analytical techniques may complement each other in the assessment of environmental impacts.

Methods

Study area and experimental design

The study area (17°55′N, 40°11′E) is located along the south-western coast of Apulia (Ionian Sea, SE, Italy) and is characterised by wave-exposed calcarenitic rocky plateaus extending from the water surface to about 10 m depth on fine sand with a gentle-medium slope (Terlizzi et al. 2002). Sampling was undertaken in November 2002 at the outfall location (hereafter indicated as *I*) and two control or reference locations (C1 and C2, hereafter indicated as *Cs*). Control locations were chosen at random from a set of eight possible locations separated by at least 2.5 km and providing comparable environmental conditions to those occurring at the outfall (in terms of slope, wave exposure, type of substrate). They were also chosen to be located on either side of the outfall.

Results of previous studies in this area showed that the outfall alters the patterns of spatial distributions of sessile macrobenthos (Terlizzi et al. 2002) and rocky reef-associated fish (Guidetti et al. 2003). In general, the location disturbed by sewage is characterised by complex algal assemblages dominated by *Colpomenia sinuosa*, *Gelidium* spp., *Pterocladiella* spp., filamentous brown and green algae. At both control locations, the sessile assemblages are mainly dominated by the algae *Dictyota dichothoma*, *Padina pavonica*, *Acetabularia acetabulum*, and *Cystoseira* spp. Specific information about the type and volumes of discharged waste waters, as well as a more detailed account of spatio-temporal patterns of abundance and species composition of sessile assemblages in relation to the presence of the outfall is reported elsewhere (Terlizzi et al. 2005).

At each of the three locations (*I* and two *Cs*), three sites, separated by 80 m–100 m were randomly chosen. At *I*, one site was located immediately adjacent to the point of discharge and the remaining other two were on its right and left, respectively. For each site, assemblages were sampled at a depth of 3 m–4 m on sloping rocky surfaces. Nine random replicates were collected at each of the three sites within each location, yielding a total of 81 units of observation.

Sampling procedure and taxonomic discrimination

SCUBA divers undertook sampling, where each replicate unit was obtained by scraping the organisms located within a 20 cm×20 cm metal frame off of the rocky surface and into a 0.4 mm mesh bag. To limit the loss of small, cryptic, and motile specimens, an airlift sampler (Benson 1989) was used before and after the scraping of the surface (Chemello 1991).

After collection, samples were fixed in 4% buffered formalin solution. Molluscs were then sorted under magnification and preserved in 70% alcohol before being identified to species level. Nomenclature followed Bedulli et al. (1995a) (Polyplacophora), Bodon et al. (1995) and Bedulli et al. (1995b) (Gastropoda), and Bedulli et al. (1995c) (Bivalvia).

Analyses of data

The total number of specimens (N) as well as the total number of species (S) and the Shannon diversity index (H') was calculated for each observation unit. Following the methods given in Clarke and Warwick (1998, 1999), a species aggregation list of molluscs was made using the taxonomic levels of species, genus, family, order, and class. The list, coupled with the data matrix, was used to calculate the taxonomic distinctness index (Δ^*) (using the DIVERSE routine contained in the PRIMER statistical package, Clarke and Gorley 2001) for each unit of observation. The calculation of Δ^* (see Eq. 2 in Clarke and Warwick 1998) used constant step lengths ($\omega = 1$) between each taxonomic level, namely, $\omega = 1$ for two individuals of the same genus but different species, $\omega = 2$ for species of the same family but different genera, and so on.

Univariate asymmetrical analyses of variance (Underwood 1991, 1994; Glasby 1997) were used to compare N, S, H', and Δ^* at *I* versus *Cs*. The model consisted of two factors: Location (one disturbed and two control locations) and Site (three levels, random, nested in Location), with $n = 9$ replicates. For analysis,

the Location term was partitioned into two portions: the contrast *I* versus *Cs* and the variability among *Cs*. The term Site(Location) (S(L)) was similarly divided into S(*I*) and S(*Cs*). Finally, the residual variation was divided into two parts: the residual variability for observations within *I* (Res *I*) and the residual variability for observations within *Cs* (Res *Cs*).

Denominators for *F* ratios were identified following the logic of the asymmetrical designs (see particularly Underwood 1992; Glasby 1997). However, using the overall residual variation as denominator makes the assumption that the variance among quadrats at *I* and *Cs* is the same, which may not be the case if an impact affects small-scale spatial patterns (Bishop et al. 2002). A two-tailed *F* test on residual terms tested for differences in spatial variances among replicates between *I* and *Cs*. If significant, terms that involved sources of variation specific for *I* or *Cs* were constructed using the natural denominator for that term, not the overall residual. For example, the S(*I*) term was tested over Res *I* rather than the pooled residual. This procedure involves a reduced number of degrees of freedom in the denominator of *F*, causing a decrease in statistical power, but takes into account a possible effect of impact in changing natural patterns of variability at the scale of replicate quadrats (Terlizzi et al. 2005).

The ANOVA is robust to heterogeneous variances (Underwood 1997), particularly when there are many independent estimates of variance, as in our case. All the analyses, thus, were done on untransformed data. Nevertheless, Cochran's *C*-test was done prior to analyses and results with significant Cochran's values (subject to excess type I error), were interpreted using a more conservative significance level of $\alpha = 0.01$ (Underwood 1997). The analyses were done using the GMAV 5 computer program (University of Sydney, Australia).

Permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) based on Bray–Curtis dissimilarities (Bray and Curtis 1957) on untransformed data (81 samples and 151 variables) was used to estimate variation in the multivariate assemblages between *I* and *Cs*. The analyses tested the same hypotheses described for univariate asymmetrical ANOVA (see above) but in a multivariate context (Terlizzi et al. 2005). Each term in the analysis was tested using 4999 random permutations of the appropriate units (Anderson and ter Braak 2003). Where 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 random sample from the asymptotic permutation distribution (Anderson and Robinson 2003). The analyses were done using the computer programs DISTLM.exe and PERMANOVA.exe (Anderson 2004).

Multivariate patterns were visualized by non-metric multi-dimensional scaling (nMDS) ordinations on the basis of a Bray–Curtis dissimilarity matrix on untransformed data. To obtain a plot of centroids, principal coordinates were calculated from the full Bray–Curtis dissimilarity matrix among all pairs of the 81 observations and centroids, as arithmetic averages, were calculated using these principal coordinates. The Euclidean distance between each pair of centroids was then calculated and used as the distance matrix for the nMDS algorithm (Anderson 2001). This was done because arithmetic averages (centroids) of raw data are not the same as centroids defined in the space of a non-Euclidean dissimilarity measure, such as the Bray–Curtis measure. Plots of individual replicates were also produced for each location separately.

The multivariate average dispersion index among replicate units (Warwick and Clarke 1993) was computed (using the PRIMER MVDISP routine) for each of the three sites nested in each location. Such a value indicated, for each site, the level of variability among replicate units (as measured using the Bray–Curtis dissimilarity) and allowed comparisons of small-scale patchiness in assemblage structure between *I* and *Cs*.

The similarity percentages procedure (done with the PRIMER SIMPER routine) was used to identify the percentage contribution that each species made to the observed value of the Bray–Curtis dissimilarity between *I* and *Cs* (Clarke 1993). A cut-off criterion was applied

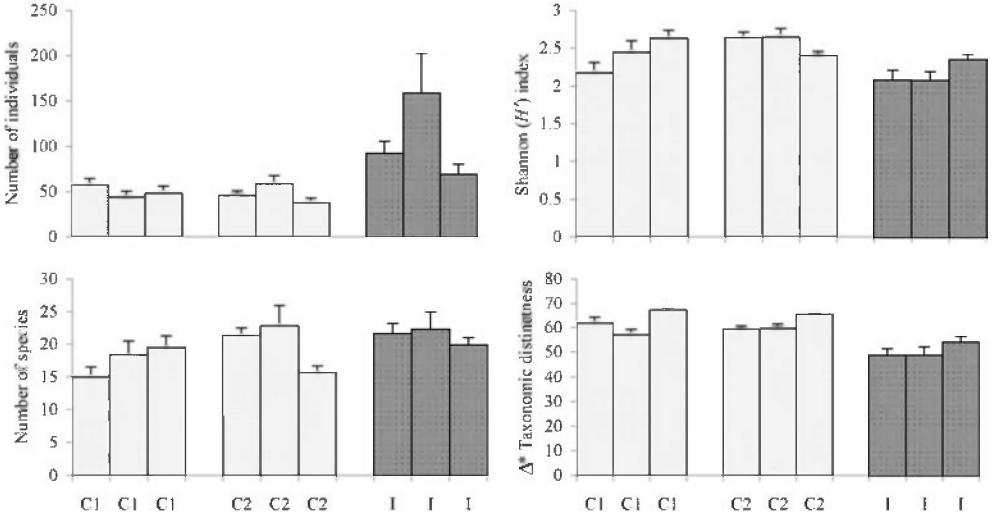

Fig. 1 Mean (± SE, $n = 9$) values of variables analysed at each of three sites at *I* and *Cs* (C1 and C2)

Table 1 Summary of asymmetrical analyses of variance testing for differences between *I* and the average of *Cs*

		Number of individuals				Number of species				Shannon (*H'*) index				Δ* Taxonomic distinctess			
Source	d.f.	MS	*F*	*p*	F_{denom}	MS	*F*	*p*	F_{denom}	MS	F	*p*	F_{denom}	MS	*F*	*p*	F_{denom}
Location = L	2	30870.457				86.161				1.101				1675.661			
Cs	1	101.407	0.147	0.721	S(*Cs*)	71.185	0.819	0.417	S(*Cs*)	0.288	0.915	0.393	S(*Cs*)	6.066	0.023	0.886	S(*Cs*)
I versus *Cs*	1	61639.506	8.865	0.025	S(L)	1.623	0.049	0.832	S(L)	1.914	6.738	0.041	S(L)	3345.257	15.658	0.007	S(L)
Site(L) = S(L)	6	6952.753	2.759	0.018	Residual	62.309	1.895	0.093	Residual	0.284	2.639	0.023	Residual	213.650	3.523	0.004	Residual
S(*Cs*)	4	690.630	1.631	0.182	Res *Cs*	86.963	2.644	0.040	Residual	0.314	2.923	0.027	Residual	258.772	6.886	0.000	Res *Cs*
S(*I*)	2	19477.000	2.901	0.074	Res *I*	13.000	0.395	0.675	Residual	0.223	2.072	0.133	Residual	123.406	1.156	0.332	Res *I*
Residual	72	2520.133				32.889				0.108				60.645			
Res *Cs*	48	423.366				32.444				0.110				37.580			
Res *I*	24	6713.667				33.778				0.103				106.775			
Cochran's		C = 0.769				C = 0.297				C = 0.218				C = 0.277			
C-test		($p < 0.01$)				($p < 0.05$)				(ns)				($p < 0.05$)			
Two tailed		15.858				1.041 (ns)				0.943				2.841			
F-test		($p < 0.001$)								(ns)				($p < 0.01$)			

In each case, the term used for the denominator mean square is given in the column F_{denom}. Data with heterogeneous variance (indicated by a significant Cochran's *C*-test) were interpreted with a more conservative level of $alfa = 0.01$. Two-tail *F*-tests compared spatial variances at the scale of replicate quadrats between *I* and *Cs*

to allow identification of a subset of species whose cumulative percentage contribution reached 90% of the dissimilarity value.

Results

A total of 5507 specimens were collected, and characterized as 151 species of Polyplacophora (223 individuals, 9 species), Gastropoda (4185 individuals, 114 species), and Bivalvia (1099 individuals, 28 species). A detailed taxonomic list of the species identified is given in Appendix A.

The analysis of mean numbers of individuals revealed no significant difference between *Cs*. The use of a conservative significance level ($\alpha = 0.01$) prevented the detection of significant differences between *I* and *Cs*, even if an indication of a greater number of individuals at *I* was suggested by the graph (Fig. 1). The two-tailed *F*-test indicated a large and significant difference between *I* and *Cs*, with larger variability among replicate quadrats at *I* (Table 1).

There was no evidence of impact on mollusc diversity when expressed as number of species. No significant difference in spatial variance in the number of species among replicate quadrats was detected by the two-tailed *F*-test (Table 1, Fig. 1). However, mean values of the Shannon diversity index (*H'*) differed among sites within control locations [S (*Cs*)] but not among sites within the disturbed location [S (*I*)] (Table 1). This was interpreted as a possible effect of the outfall in modifying patterns of spatial variability at the scale of sites. Also, the term *I* versus *Cs* was significant, indicating significant spatial differences between *I* and the average of *Cs* in Shannon diversity. Comparison of variance components at the scale of replicate quadrats indicated no significant differences in the variability of *H'* among quadrats between *I* and *Cs* (Table 1, Fig. 1).

The analysis of mean taxonomic distinctness (Δ*) indicated a significant main effect of *I* versus *Cs*. Also, there was evidence of an effect of the outfall in modifying spatial patterns at the scale of sites (indicated by the statistical significance of the term S (*Cs*) and by the non-significance of the term S (*I*), Table 1). In addition to patterns in mean values of Δ*, comparison of variance components of Res (*Cs*) and Res (*I*) revealed significantly more residual variation at *I* compared to *Cs* at the scale of quadrats (Table 1, Fig. 1).

Asymmetrical PERMANOVA provided evidence for statistically significant differences in assemblages between *I* and the average of *Cs* (as indicated by the significance of the term *I* versus *Cs*, Table 2). There was no significant difference detected between *Cs*, although assemblages differed significantly among sites at *I* and at *Cs*. Spatial differences in the structure of the assemblages were well portrayed by the nMDS plot of the nine site centroids (Fig. 2), which indicated a clear separation between *I* and *Cs*. The variability among sites also appeared to be less at *I* than at *Cs* (Fig. 2).

Table 2 Asymmetrical PERMANOVA based on the Bray–Curtis dissimilarities of the untransformed multivariate data (151 species)

Source	d.f.	SS	MS	F	*p*	F_{denom}	Permutable units
Location = L	2	40858.681	20429.341	2.851	0.000	S(L)	9 S(L) cells
Cs	1	10507.181	10507.181	1.178	0.291	S(*Cs*)	6 S(*Cs*) cells
I versus *Cs*	1	30351.501	30351.501	4.236	0.007	S(L)	9 S(L) cells
Site(L) = S(L)	6	42991.631	7165.272	4.104	0.000	Residual	81 raw data units
S(*Cs*)	4	35691.242	8922.811	4.703	0.002	Res *Cs*	54 raw data units
S(*I*)	2	7300.388	3650.194	2.531	0.001	Res *I*	27 raw data units
Residual	72	125691.373	1745.714				
Res *Cs*	48	91074.267	1897.381				
Res *I*	24	34617.107	1442.379				

Each test was based on 4999 random permutations of the appropriate units. In each case, the term used as a denominator for the calculation of pseudo- *F* is shown in the column F_{denom}. *p*-values given in italics were obtained using 4999 Monte Carlo samples from the asymptotic permutation distribution

This pattern was supported by the relative sizes of the multivariate pseudo variance components for sites obtained using mean squares from the PERMANOVA (Table 2), which were estimated at 245.3 for *I* and 780.6 for *Cs* (e.g., Searle et al. 1992). Within each location, significant differences in multivariate assemblage structure among sites were also apparent (Fig. 3, Table 2).

Multivariate patterns of small-scale variability among replicate units differed markedly between *I* and *Cs* (Fig. 4). More particularly, the assemblages at *I* were characterised by the lowest values of the multivariate dispersion index, suggesting an effect of decreasing variability in the structure of assemblages at the scale of replicate quadrats at the outfall (as measured by the Bray–Curtis measure).

There were 40 taxa identified by SIMPER as contributing the most (90%) to the average Bray–Curtis dissimilarity between *I* and the average of *Cs* (Table 3). Of these, 27 (out of the 114 in the original data set) were gastropod species, 10 (out of 28) were bivalves and 3 (out of 9), polyplacophoran. Among gastropods, *Pusillina philippi*, *Bittium reticulatum*, *Bittium latreilli*, and *Columbella rustica* made a strong cumulative contribution towards differences between *I* and *Cs*, accounting for more than 50% of the observed value of dissimilarity. Among bivalves, *Gastrochaena dubia* and *Mytilaster minimus* also made important contributions to discriminate between *I* and *Cs* (Table 3). Within the species identified by SIMPER, with the exception of the polyplacophoran, *Lepidochitona monterosatoi*, there were no exclusive species at *I* or *Cs*. Some species were more abundant at *Cs* than *I*. For example, the average number of individuals per quadrat of *C. rustica* and *G. dubia* was 5.07 and 3.31 at *Cs* and 2.41 and 1.85 at *I*, respectively (Table 3). This pattern was reversed for *P. philippi*, *B. reticulatum*, and *B. latreilli*, whose values of

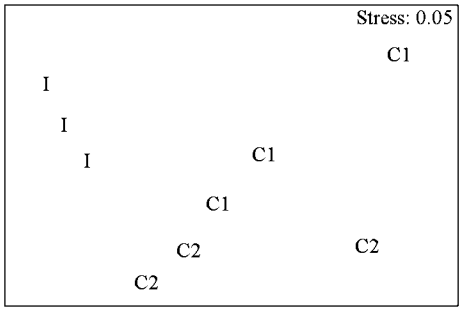

Fig. 2 Non-metric multidimensional scaling ordination (nMDS plot) on the basis of the Bray–Curtis dissimilarity measure of centroids of the $n = 9$ observations for each site. Centroids were calculated using principal coordinates (see text for details)

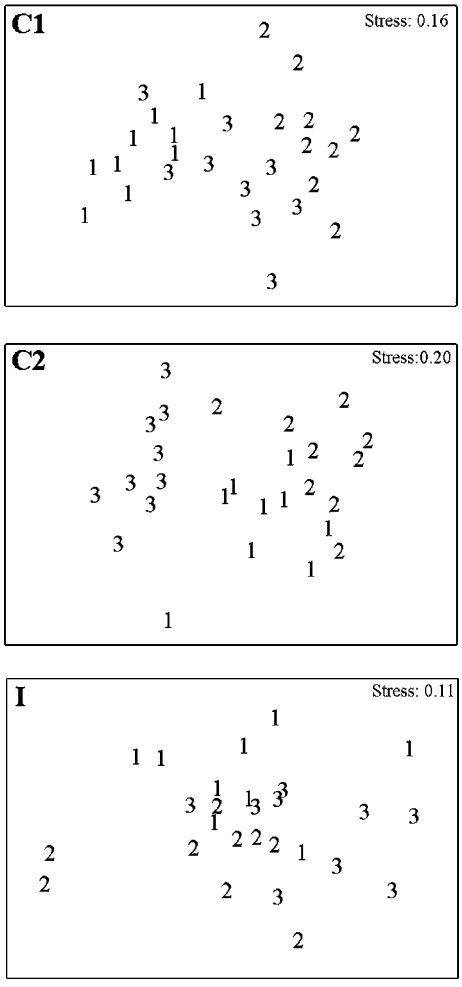

Fig. 3 Non-metric multidimensional scaling ordinations of replicate observations at each of three random sites (labelled simply 1, 2 or 3) within each location: C1, C2, and *I*. For the impact location, the site labelled 2 was the site closest to the outfall

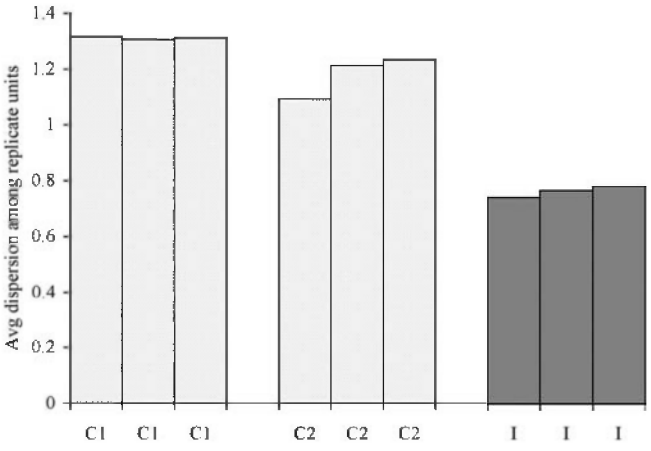

Fig. 4 Average multivariate dispersion index among replicate units for each site at the outfall (*I*) and the two controls (C1 and C2)

average abundance were far higher at *I* than *Cs* (3.87, 2.35, and 4.13 at *Cs* versus 36.67, 22.56, and 13.96 at *I*, respectively). The contribution of these three species was clearly illustrated by plotting *k*-dominance curves (Lambshead et al. 1983) for species abundance at *I* and *Cs* (Fig. 5). Although no statistically significant differences among the curves were detected by ANOSIM (global $R = 0.144$, $p = 0.23$), the curves showing the distribution of the number of individuals among species at *Cs* markedly differed from curve at *I*, which was characterised by few numerically dominant species. There was a comparable shape of the curves at C1 and C2 indicating similar patterns of distribution of individuals among species at control locations.

Discussion

This study detected significant differences between molluscan assemblages at a location exposed to sewage discharge and those at control locations. Multivariate analyses indicated that differences between *I* and *Cs*

Table 3 Average abundance of species contributing the most to the Bray–Curtis dissimilarity (data untransformed) value (75.09) between *I* and the average of *Cs*

Species	Group *Cs* Av.Abund	Group *I* Av.Abund	Contrib%	Cum.%
P. philippi	3.87	36.67	26.11	26.11
B. reticulatum	2.35	22.56	13.65	39.76
B. latreilli	4.13	13.96	8.27	48.03
C. rustica	5.07	2.41	4.44	52.47
G. dubia	3.31	1.85	4.04	56.51
M. minimus	3.52	2.59	3.50	60.01
Chiton olivaceus	0.83	2.04	1.86	61.87
Cardita calyculata	1.31	1.74	1.71	63.59
S. lactea	1.33	0.96	1.62	65.20
Aplysia parvula	1.67	0.22	1.60	66.80
M. galloprovincialis	0.98	1.89	1.52	68.32
Conus mediterraneus	0.87	1.19	1.48	69.80
Clanculus cruciatus	0.43	1.52	1.39	71.19
Alvania cimex	0.94	0.93	1.32	72.52
Acanthochitona fascicularis	0.94	0.70	1.27	73.79
Lithophaga lithophaga	0.89	0.59	1.21	75.00
Muricopsis cristata	0.61	1.00	1.16	76.16
Hiatella rugosa	1.09	0.37	1.16	77.32
Diodora gibberula	1.06	0.59	1.14	78.46
Nassarius incrassatus	0.70	1.04	1.08	79.54
Alvania pagodula	0.04	1.30	0.89	80.43
Barleia unifasciata	0.83	0.04	0.84	81.26
Cerithium vulgatum	0.52	0.52	0.77	82.03
D. cristatum	0.85	0.04	0.68	82.70
Cerithiopsis tubercularis	0.54	0.33	0.65	83.36
Pollia dorbignyi	0.41	0.41	0.65	84.00
Hexaplex trunculus	0.28	0.44	0.56	84.56
Eatonina fulgida	0.50	0.11	0.50	85.06
Gibbula turbinoides	0.20	0.33	0.49	85.55
Rissoina bruguieri	0.30	0.41	0.49	86.04
Crisilla semistriata	0.30	0.30	0.48	86.53
Arca noae	0.30	0.33	0.48	87.01
Cerithium rupestre	0.43	0.11	0.47	87.48
L. monterosatoi	0.43	0.00	0.44	87.91
Ocinebrina edwardsii	0.22	0.30	0.43	88.35
Sinezona cingulata	0.37	0.26	0.42	88.77
Vermetus triquetrus	0.26	0.19	0.42	89.18
Galeomma turtoni	0.31	0.19	0.41	89.59
Barbatia barbata	0.24	0.15	0.38	89.97
Lima hians	0.15	0.30	0.35	90.33

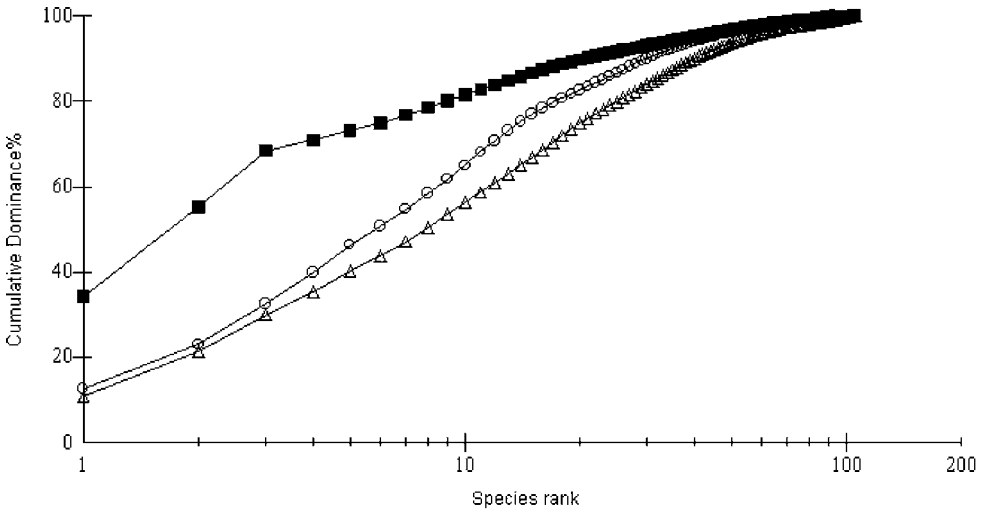

Fig. 5 *k*-dominance curves for species abundances at *I* and *Cs* (*x*-axis logged). *I* black squares; *Cs* white circles and triangles for C1 and C2, respectively

were significantly larger than the variation among control locations. Several species characterised these differences with a few of them (i.e. *P. philippi*, *B. latreilli*, and *B. reticulatum*) widely dominating the assemblage at the disturbed location.

The ANOVA indicated no significant differences between *I* and *Cs* for either the mean number of individuals (*N*) or the mean number of species (*S*), whereas an overall difference between *I* and *Cs* was found in the mean values of the other two univariate diversity measures (i.e. H' and Δ^*). Two-tailed *F*-tests indicated that for *N* and Δ^*, spatial variation at the scale of quadrats was significantly larger at *I* compared to *Cs*.

There are many potential mechanisms, which may be responsible for changes in assemblages exposed to sewage pollution. Clearly, as our data are descriptive, it is not possible to infer much regarding specific processes that may lead to different structures in assemblages between *I* and *Cs*. In addition, the lack of temporal replication in the study we describe limits the basis for any general inferences concerning impacts. Potential causes for the differences observed can be hypothesised but unravelling possible, not mutually exclusive, explanations about the processes producing changes in molluscan assemblages at the outfall would require detailed experimental manipulations. Furthermore, the absence of fundamental knowledge concerning the biology and ecology of the vast majority of these molluscan species precludes the ability to generate meaningful hypotheses regarding mechanisms that may underlie the patterns uncovered here. Thus, we offer only some general tentative ideas.

One possible model is linked to sedimentation. A discharge of primary or secondary treated sewage can increase the amount of suspended solids in the water column and modify the rate of sedimentation. This in turn, possibly interacting with other environmental factors often covarying with sediments (e.g., hydrodynamics, salinity, organic enrichment, and associated oxygen demands), can act to modify the chemical and physical features of the environment, influencing patterns of abundance and species distribution within assemblages (Airoldi 2003). Burial by sediments in subtidal oyster reefs, for instance, is known to cause mortality in oysters and other filter-feeding sessile invertebrates (Lenihan 1999). This could explain the observed lower average abundances of sessile bivalves of the species *G. dubia*, *M. minimus*, and *Striarca lactea* and of the vermetid gastropod *Dendropoma cristatum* at *I* compared to *Cs*. Changes in the characteristics of the surface of the substratum by increasing sedimentation could also explain the relatively higher abundances at *I* of deposit-feeding gastropod species, namely *B. latreilli* and *B. reticulatum*. Given the sensitivity of molluscs to the accumulation of heavy metals (Reish et al. 1999), inorganic contamination of sediments is another possible mechanism worth considering.

Sewage plumes can also increase water turbidity and this, possibly interacting with processes of sedimentation (Irving and Connell 2002), can have an influence on the heterogeneity of benthic algae (i.e., the relative abundance of different structural components), resulting in changes in habitat complexity for small invertebrate vagile organisms (Kennelly 1989; Gee and Warwick 1994; Schreider et al. 2003). Previous accounts on the effects of the same outfall on sessile assemblages (Terlizzi et al. 2002) reported strong differences in algal substrate cover and species composition. More particularly, the algal assemblages at the outfall were strongly dominated by red algae of the family of Gelidiaceae and by filamentous brown algae of the genera *Ectocarpus* and *Sphacelaria*. Similar patterns of extensive mats of macroalgae associated with organic polluted waters have been described elsewhere (Lopez-Gappa et al. 1990; Chryssovergis and Panayotidis 1995). The possible change in algal complexity that is supposed to regulate

the structure of molluscan assemblages (Russo 1997; Chemello and Milazzo 2002) may explain differences between *I* and *Cs* found in this study.

Another possible model concerns the supply of recruits. The importance of some species (i.e., *P. philippi*, *B. latreilli*, *B. reticulatum*, and *Mytilus galloprovincialis*) in differentiating the assemblage at *I* versus *Cs* was largely driven by the higher abundances, at *I*, of juvenile forms. The reasons for increases in the number of recruits of these species at *I* is clearly unknown, but the complex suite of changes in chemical, physical, and biological attributes of the habitat as a consequence of sewage discharge can modify the demographic structure of populations (Caley et al. 1996; Fraschetti et al. 2003). Note, in passing, that a methodological aspect of the finding that very small juvenile species largely influenced multivariate differences between *I* and *Cs* is that special care (i.e. patience and time) must be taken in processing and sorting samples, to ensure a proper quantification of the presence and relative abundances of species.

An intriguing outcome of univariate analyses was that the detection of effects of the sewage effluent on mollusc diversity depended on the way diversity was expressed. Asymmetrical ANOVA detected no significant differences in mean number of species (*S*) at *I* compared to *Cs* nor were there differences between *I* and *Cs* in spatial patterns of variation at the scale of sites and replicate quadrats. The analysis of the Shannon index (H') revealed a possible effect of the outfall in modifying patterns of spatial variability at the scale of sites and an overall difference between *I* and *Cs*, with the lowest mean H' values found at *I*. When considering taxonomic distinctness (Δ^*) the analysis indicated a significant effect of the outfall in modifying natural spatial patterns of mollusc diversity at all of the considered scales (i.e., location, site, replicate quadrats), with a sharp decrease in mean Δ^* values at *I*.

As defined by its formulation (Clarke and Warwick 1998), Δ^* is a measure purely of taxonomic relatedness that is independent of species diversity or sample size. The relevant attribute of Δ^* is that it contains information about the interrelationships between species that are entirely neglected in the calculation of standard diversity indices. In this way, Δ^* can complement the information obtained by other kinds of diversity measures and contribute to quantify and characterise the effects of disturbance on assemblage biodiversity.

There are two possible mechanisms to explain changes in taxonomic distinctness (Somerfield et al. 1997). The first is that, in disturbed assemblages, less closely related species are replaced by more closely related species. The second is that the disturbance acts to remove species from an assemblage selectively, from particular higher taxa. In this case, changes in H' should track those in Δ^*. Our results, therefore, supported this latter mechanism, given that the reduction of mean values of H' at *I* reflected similar changes in the mean values of Δ^*. The complementary, independent information measured by H' and Δ^* bring new insights to measuring changes in diversity in disturbed assemblages. Integrating these different measures of diversity should, however, be undertaken with caution, because measures other than Δ^*, such as *S* or H', are dependent on sample size and the proportion of the total number of species sampled with a given number of samples can vary from location to location.

Univariate analysis suggested a general increase in mean numbers of individuals close to the outfall. Similarly, spatial variation at the scale of replicate quadrats was higher at *I* than *Cs*. Such a pattern of a positive correlation between the mean and the variance is a common feature of counts of species abundances (Taylor 1961; Hurlbert 1990). Taxonomic distinctness, however, showed larger spatial variation at *I*, although the mean values of Δ^* were generally reduced at *I* compared to *Cs*. That is, the variance of Δ^* decreased with increases in its mean. This suggests that when assemblages have higher average distinctness (having a greater range of species across higher levels of taxonomic resolution), the more stable this taxonomic structure is. In contrast, assemblages represented by only one or a few families (or other higher taxa), appear to have a much more variable pattern of taxonomic relatedness among species.

The observed decrease in variance with increase in mean of Δ^* may be a purely statistical phenomenon. For example, as an assemblage obtains a greater number of distinct species across a range of families, perhaps the historical constraints on the possibilities for variation in taxonomic structure within a phylum force a reduction in the variance of Δ^*. On the other hand, the increase in variance of Δ^* observed at the impact location may be an ecological symptom of stress. Clearly, more work with a greater range of phyla in different circumstances is required to achieve a better understanding of the behaviour of the variance of Δ^* in ecological and environmental studies (Clarke, personal communication). In any case, these findings support the view that careful studies of environmental impacts should consider potential effects on variances in addition to potential mean effects on single response variables (Bishop et al. 2002; Benedetti-Cecchi 2003).

In addition to univariate patterns, differences in small-scale patchiness between *I* and *Cs* also emerged by multivariate analysis, with the assemblages at *I* being characterised by the lowest values of multivariate dispersion among replicates. A change in variability has been proposed as a diagnostic feature of disturbed marine assemblages (Caswell and Cohen 1991; Warwick and Clarke 1993; Fraschetti et al. 2001). There are several reasons why variability among replicates may increase or decrease under disturbance conditions. These include changes in the total number of species, changes in phylogenetic relatedness among species, changes in the variance-to-mean ratio for particular species, or changes in taxonomic composition of the assemblages (Warwick and Clarke 1993; Chapman et al. 1995). Integrating univariate and multivariate approaches can

help to distinguish among these possibilities and, in general, to explain mechanisms causing changes in marine systems.

Acknowledgements Christian Vaglio and Dario Negro greatly assisted in fieldwork. Anna Lucia Delos provided invaluable assistance with the processing of samples and sorting operations. Financial support extended by PEET (Partnerships for Enhancing Expertise in Taxonomy) and MURST (COFIN and FIRB projects). The authors acknowledge the support by the MARBEF Network of Excellence "Marine Biodiversity and Ecosystem Functioning," which is funded in the Community's Sixth Framework Programme (contract no. GOCE-CT-2003-505446)

Appendix A

Taxonomic list of the species collected

Polyplacophora

Lepidopleurida
Lepidopleuridae
Lepidopleurus (*Lepidopleurus*) *cajetanus* (Poli, 1791)
Lepidopleurus (*Leptochiton*) *scabridus* (Jeffreys, 1880)
Ischnochitonidae
Ischnochiton (*Ischnochiton*) *rissoi* (Payraudeau, 1826)
Callochiton septemvalvis (Montagu, 1803)
Lepidochitona monterosatoi Kaas and Van Belle, 1981
Chitonidae
Chiton (*Rhyssoplax*) *olivaceus* Spengler, 1797
Chiton (*Rhyssoplax*) *phaseolinus* Monterosato, 1879
Acanthochitonidae
Acanthochitona crinita crinita (Pennant, 1777)
Acanthochitona fascicularis (Linné, 1767)

Gastropoda

Docoglossa
Patellidae
Patella ulyssiponensis Gmelin, 1791
Vetigastropoda
Fissurellidae
Emarginula octaviana Coen, 1939
Diodora gibberula (Lamarck, 1822)
Scissurellidae
Scissurella costata D'Orbigny, 1824
Sinezona cingulata (Costa O.G., 1861)
Haliotidae
Haliotis tuberculata tuberculata Linné, 1758
Turbinidae
Tricolia tenuis (Michaud, 1829)
Trochidae
Clanculus (*Clanculopsis*) *cruciatus* (Linné, 1758)
Gibbula (*Colliculus*) *racketti* (Payraudeau, 1826)
Gibbula (*Colliculus*) *turbinoides* (Deshayes, 1835)
Gibbula (*Steromphala*) *divaricata* (Linné, 1758)
Gibbula (*Tumulus*) *umbilicaris umbilicaris* (Linné, 1758)
Jujubinus exasperatus (Pennant, 1777)
Jujubinus striatus striatus (Linné, 1758)
Calliostoma laugeri laugeri (Payraudeau, 1826)
Neritopsina
Neritidae
Smaragdia viridis (Linné, 1758)
Caenogastropoda
Cingulopsidae

Gastropoda

Eatonina (*Coriandria*) *cossurae* (Calcara, 1841)
Eatonina (*Coriandria*) *fulgida* (Adams J., 1797)
Rissoidae
Rissoa guerinii Récluz, 1843
Rissoa similis Scacchi, 1836
Rissoa variabilis (von Muehlfeldt, 1824)
Rissoa violacea violacea (Récluz, 1843)
Alvania cancellata (Da Costa, 1778)
Alvania cimex (Linné, 1758)
Alvania lactea (Michaud, 1832)
Alvania lineata Risso, 1826
Alvania pagodula (Bucquoy, Dautzenberg and Dollfus, 1884)
Alvania parvula (Jeffreys, 1884)
Alvania subcrenulata (Bucquoy, Dautzenberg and Dollfus, 1884)
Crisilla semistriata (Montagu, 1808)
Manzonia crassa (Kanmacher, 1798)
Pusillina philippi (Aradas and Maggiore, 1844)
Setia amabilis (Locard, 1886)
Setia turriculata Monterosato, 1884
Rissoina bruguieri (Payraudeau, 1826)
Barleeidae
Barleia unifasciata (Montagu, 1803)
Anabathridae
Nodulus contortus (Jeffreys, 1856)
Caecidae
Caecum trachea (Montagu, 1803)
Cerithiidae
Cerithium rupestre Risso, 1826
Cerithium vulgatum Bruguière, 1792
Bittium latreilli (Payraudeau, 1826)
Bittium reticulatum (Da Costa, 1778)
Vermetidae
Vermetus triquetrus Bivona Ant., 1832
Vermetus (Thylacodus) granulatus (Gravenhorst, 1831)
Dendropoma cristatum (Biondi, 1857)
Serpulorbis arenaria (Linné, 1767)
Skeneopsidae
Skeneopsis planorbis (Fabricius, 1780)
Fossaridae
Fossarus ambiguus (Linné, 1758)
Cerithiopsidae
Cerithiopsis fayalensis Watson, 1886
Cerithiopsis minima (Brusina, 1865)
Cerithiopsis nana Jeffreys, 1867
Cerithiopsis tubercularis (Montagu, 1803)
Dizoniopsis coppolae (Aradas, 1870)
Triphoridae
Cheirodonta pallescens (Jeffreys, 1867)
Monophorus perversus (Linné, 1758)
Marshallora adversa (Montagu, 1803)
Metaxia metaxa (Delle Chiaje, 1828)
Epitoniidae
Gyroscala lamellosa(Lamarck, 1822)
Eulimidae
Vitreolina philippi(Rayneval and Ponzi, 1854)
Neogastropoda
Muricidae
Hexaplex trunculus (Linné, 1758)
Muricopsis cristata (Brocchi, 1814)
Ocinebrina aciculata (Lamarck, 1822)
Ocinebrina edwardsii (Payraudeau, 1826)
Stramonita haemastoma (Linné, 1766)
Coralliophilidae
Coralliophila meyendorffii (Calcara, 1845)
Buccinidae
Buccinulum corneum (Linné, 1758)
Pisania striata (Gmelin, 1791)
Chauvetia turritellata (Deshayes, 1835)

Gastropoda

Engina leucozona (Philippi, 1843)
Pollia dorbignyi (Payraudeau, 1826)
Pollia scacchiana (Philippi, 1844)
Columbellidae
Columbella rustica (Linné, 1758)
Mitrella scripta (Linné, 1758)
Nassariidae
Nassarius (*Hima*) *incrassatus* (Stroem, 1768)
Nassarius (*Telasco*) *cuvierii* (Payraudeau, 1826)
Fasciolariidae
Fasciolaria lignaria (Linné, 1758)
Cystiscidae
Gibberula miliaria (Linné, 1758)
Gibberula philippi (Monterosato, 1878)
Gibberula recondita Monterosato, 1844
Mitridae
Mitra cornicula (Linné, 1758)
Costellariidae
Vexillum (*Pusia*) *ebenus* (Lamarck, 1811)
Vexillum (*Pusia*) *savignyi* (Payraudeau, 1826)
Vexillum (*Pusia*) *tricolor* (Gmelin, 1791)
Conidae
Mangelia taeniata (Deshayes, 1835)
Mangelia vauquelini (Payraudeau, 1826)
Raphitoma echinata (Brocchi, 1814)
Raphitoma horrida (Monterosato, 1884)
Raphitoma laviae (Philippi, 1844)
Raphitoma linearis (Montagu, 1803)
Raphitoma lineolata (Bucquoy, Dautzenberg and Dollfus, 1883)
Leufroyia concinna (Scacchi, 1836)
Conus mediterraneus Hwass in Bruguière, 1792
Heterostropha
Rissoellidae
Rissoella diaphana (Alder, 1848)
Omalogyridae
Ammonicera fischeriana (Monterosato, 1869)
Pyramidellidae
Eulimella laevis (Blainville, 1827)
Odostomia lukisii Jeffreys, 1859
Odostomia plicata (Montagu, 1803)
Odostomia (*Megastomia*) *conoidea* (Brocchi, 1814)
Chrysallida emaciata (Brusina, 1866)
Chrysallida incerta (Milaschewitch, 1916)
Chrysallida juliae (De Folin, 1872)
Folinella excavata (Philippi, 1836)
Odostomella doliolum (Philippi, 1844)
Ondina warreni (Thompson, 1845)
Cephalaspidea
Philinidae
Philine catena (Montagu, 1803)
Aglajidae
Chelidonura italica Sordi, 1980
Bullidae
Bulla striata Bruguière, 1792
Runcinidae
Runcina ferruginea Kress, 1977
Anaspidea
Aplysiidae
Aplysia (*Pruvotaplysia*) *parvula* Guilding in Moerch, 1863
Aplysia (*Pruvotaplysia*) *punctata* (Cuvier, 1803)
Notaspidea
Pleurobranchidae
Berthella aurantiaca (Risso, 1818)
Nudibranchia
Tritoniidae
Tritonia manicata Deshayes, 1853
Doridida
Doridida gen. sp.

Gastropoda

Basommatophora
Siphonariidae
Williamia gussoni (Costa, 1829)

Bivalvia

Arcida
Arcidae
Arca noae Linné, 1758
Barbatia barbata (Linné, 1758)
Noetidae
Striarca lactea (Linné, 1758)
Mytilida
Mytilidae
Mytilus galloprovincialis Lamark, 1819
Mytilaster minimus (Poli, 1795)
Modiolus barbatus (Linné, 1758)
Gregariella petagnae (Scacchi, 1832)
Musculus costulatus (Risso, 1826)
Lithophaga lithophaga (Linné, 1758)
Limida
Limidae
Lima (*Limaria*) *hians* (Gmelin, 1791)
Lima (*Limaria*) *tuberculata* (Olivi, 1792)
Ostreida
Spondylidae
Spondylus gaederopus Linné, 1758
Anomiidae
Anomia ephippium Linné, 1758
Venerida
Lucinidae
Ctena decussata (Costa, 1829)
Lucinella divaricata (Linné, 1758)
Galeommatidae
Galeomma turtoni Turton, 1825
Lasaeidae
Lasaea rubra (Montagu, 1803)
Montacutidae
Mysella bidentata (Montagu, 1803)
Carditidae
Cardita calyculata (Linné, 1758)
Glans trapezia (Linné, 1767)
Chamida
Chamidae
Chama gryphoides Linné, 1758
Cardiidae
Parvicardium scriptum (Bucquoy, Dautzenberg and Dollfus, 1892)
Plagiocardium (*Papillocardium*) *papillosum* (Poli, 1795)
Veneridae
Tapes (Ruditapes) *decussatus* (Linné, 1758)
Irus irus (Linné, 1758)
Petricolidae
Petricola (*Lajonkairia*) *lajonkairii* (Payraudeau, 1826)
Myida
Gastrochaenidae
Gastrochaena dubia (Pennant, 1777)
Hiatellidae
Hiatella rugosa (Linné, 1767)

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