Journal of Applied Ecology 2004
41, 114–123

Measurements of sublethal effects on individual organisms indicate community-level impacts of pollution

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Summary

1. Due to the cost and complexity of sampling biological communities, surrogate biological measures or concentrations of toxic contaminants are often used to indicate environmental impacts. Such indicators can be powerful tools, but their effectiveness requires evaluation. Mussels are widely used as bioindicators of environmental contamination. For example, physiological measurements on live mussels form the basis of scope for growth (SFG), an integrated indicator of environmental stress. However, the effectiveness of SFG as an indicator of community level effects has rarely been tested in the field.

2. In this study of six sites on the west coast of the UK, the diversity of macrofaunal communities associated with mussels was reduced at sites with low SFG (< 10 J g^{-1} h^{-1}) compared with those with high SFG (> 15 J g^{-1} h^{-1}). At smaller scales, variation in community structure was related to biomass of mussels, mass of coarse sediments and the fractal dimension of the surface of the mussel bed.

3. We measured hydrocarbon contamination as a first step in identifying contaminants that might underpin the relationship between SFG and diversity. Unresolved complex mixtures (UCM) are often the most abundant hydrocarbon contaminants in the environment, but have, until recently, been largely ignored. We found generally good accordance between UCM concentration in mussel tissues, SFG and diversity, but other pollutants are also likely to be involved.

4. *Synthesis and applications.* Our findings illustrate the complexity of relating changes in diversity to synoptic or sublethal measures of environmental stress in the field. However, the results represent a significant step towards a biotic indicator of environmental quality that integrates impacts across a range of levels of biological organization (from intra-individual to community). Such indicators may be of particular value in the implementation of the European Union Water Framework Directive in Europe and similar environmental legislation elsewhere.

Key-words: ecological quality, indicator, marine biodiversity, mussels *Mytilus* spp., scope for growth (SFG), unresolved complex mixtures (UCM).

Journal of Applied Ecology (2004) **41**, 114–123

Introduction

Human activities are thought to be causing a reduction in global biodiversity, but the relationship between localized anthropogenic impacts and marine biodiversity is poorly understood. There is therefore a need for accurate and practical techniques for assessing the impacts of human activities on biodiversity. Most environmental monitoring is concerned ultimately with effects of environmental contamination on populations and communities of organisms. Due to the high cost and complexity of sampling biological communities, however, surrogate biological measures or concentrations of toxic contaminants are often the only variables measured. They are then assumed to indicate

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population and community-level impacts. Such indicators can be powerful tools, but, because contamination does not necessarily cause impacts at all or any levels of biological organization (Paine *et al.* 1996), their effectiveness needs to be evaluated against measured impacts on populations and communities in the field.

Mussels *Mytilus* spp. are relatively resistant to many pollutants that they accumulate in their tissues, thereby increasing concentrations to levels more easily detected than those in the environment (Widdows & Donkin 1992). Because of this, they are widely used as 'sentinel' organisms to indicate levels of contamination and the potential for community-level effects (Goldberg 1975; O'Connor 2002). Mussels are widespread in coastal environments and provide habitat for diverse assemblages of invertebrates and algae (Seed 1996), many of which are more susceptible to pollution than the mussels themselves. Mussel-dominated assemblages therefore provide an ideal opportunity for assessing directly the effectiveness of surrogate measures of community-level effects.

Scope for growth (SFG) is used as an integrated measure of sublethal stresses on mussels (Widdows, Nasci & Fossato 1997). It is an index of energy status derived from laboratory-based physiological measurements on live mussels collected from field locations (Widdows 1985). Mussels that are not stressed by environmental conditions exhibit high SFG; those that live in stressful conditions, with high levels of contamination, exhibit low SFG. Bayne (1985) argued that effects on SFG should translate into population and community level effects (Anderlini 1992), but this has rarely been tested directly. A range of contaminants can affect SFG, either separately or interactively (Widdows & Donkin 1992). It is therefore of value to look at specific contaminants at field locations to try to determine what may be causing changes in SFG and to attempt to link that to community-level effects.

The presence of unresolved complex mixtures (UCM) of hydrocarbons in tissues indicates pollution by petroleum. Most research into the environmental effects of these oil residues has concentrated on those hydrocarbons that are resolvable by high-performance liquid chromatography (HPLC) and gas chromatography (GC), and identifiable by GC–mass spectrometry (GC-MS), even though UCM clearly dominate gas chromatograms. In fact, as a group the UCM hydrocarbons are more abundant than the resolved hydrocarbons, although this is seldom realized due to the normalization of most chromatograms on the resolved peaks (Rowland *et al.* 2001). Until recent studies demonstrated toxicity to marine organisms, the unresolved components had been largely ignored (Thomas, Donkin & Rowland 1995; Rowland *et al.* 2001; Donkin, Smith & Rowland 2003). The impacts of chronic hydrocarbon contamination on intertidal communities have received very little attention (Crowe *et al.* 2000).

In this study, we aimed to test the relationship between SFG of mussels and the structure of associated communities at a range of locations on the west coast of the UK. Aspects of the habitat provided by the mussels (mussel population structure, topographic complexity, characteristics of interstitial sediment) were measured in order to assess their possible influence on associated organisms. If these variables strongly influence community structure, the potential exists for confounding of any relationship between community structure and pollution. If mussel populations are themselves affected by pollution, it is possible that any community-level effects are indirect consequences of changes to the structure of the habitat provided by the mussels rather than direct effects of pollution on the organisms themselves. Contamination by UCM of hydrocarbons was also measured as a first step in identifying contaminants that might underpin any relationships found. The potential influence of additional contaminants, measured by Widdows *et al.* (2002), was also considered.

Methods

SAMPLING BIOTA

In August 1999, samples of mussels and their associated fauna were collected from rocky shores at three polluted locations in the UK (low SFG, < 10 J g^{-1} h^{-1}), Llandudno (53°19.8′ N, 3°49.6′ W), New Brighton (53°26.6′ N, 3°2.2′ W) and Heysham (54°2.6′ N, 2°54.2′ W), and three relatively unpolluted control locations (high SFG, > 15 J g^{-1} h^{-1}), Llanbedrog (52°51.3′ N, 4°28.1′ W), Kirkholm (54°58.2′ N, 5°3.4′ W) and Loch Striven (55°53.6′ N, 5°2.6′ W). SFG data were taken from Widdows *et al.* (2002) and the categories used here correspond, respectively, to the 'moderate to high stress' and 'low stress' categories defined in that paper. Llanbedrog was not sampled in that study, but we used a measurement from a nearby site at Abersoch to indicate lack of contamination at Llanbedrog. We considered this reasonable given the proximity of the sites (about 20 km) and the generally low degree of contaminating activity in the area (J. Widdows, personal communication). There is also evidence that SFG has a reasonable degree of temporal stability, suggesting that the 3-year gap between SFG measurement and biotic sampling should not invalidate the findings. Evidence was not available for all sites, but additional data for New Brighton showed that SFG had declined from 8·7 to 3·42 J g^{-1} h^{-1} by 1998 and was 3·97 J g^{-1} h^{-1} in 1999, and Blackpool (between New Brighton and Heysham) had also deteriorated from 7·68 to 3·76 J g^{-1} h^{-1} between 1996 and 1998 (J. P. Shaw, A. T. Large, P. Donkin, S. V. Evans, F. J. Staff, D. R. Livingstone, J. K. Chipman & L. D. Peters, unpublished data). Such minor changes are typical of sites chronically polluted by urban and industrial sources (J. Widdows, personal communication). The only site of high SFG for which several years of data were available was Port Quin in Cornwall. It again showed only moderate variation, with values of

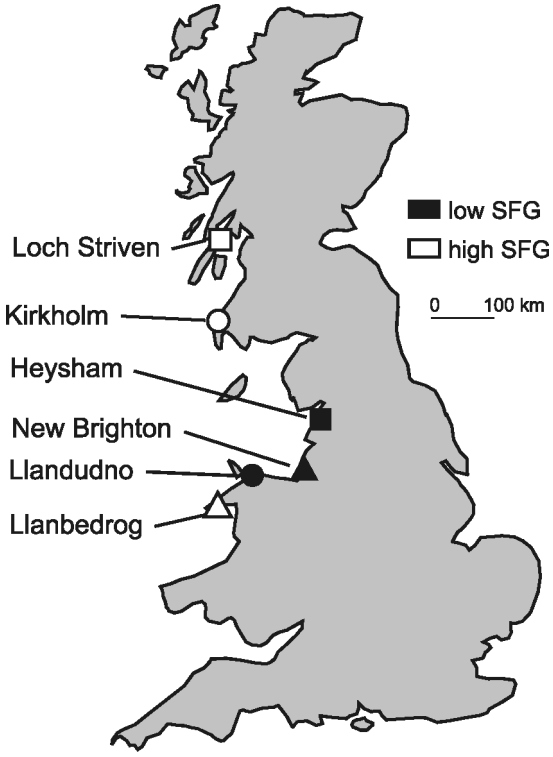

Fig. 1. Map of study locations.

16·33 J g^{-1} h^{-1} and 18·80 J g^{-1} h^{-1} for 1996 and 1997 (Widdows *et al.* 2002) and 20·66 J g^{-1} h^{-1} in 1998 (J. P. Shaw, A. T. Large, P. Donkin, S. V. Evans, F. J. Staff, D. R. Livingstone, J. K. Chipman & L. D. Peters, unpublished data). It was thus unlikely that any of our sites would have moved between the low SFG category (< 10 J g^{-1} h^{-1}) and the high SFG category (> 15 J g^{-1} h^{-1}) during the 3 years from 1996 to 1999.

The control locations were selected to the north and south of the polluted sites (Fig. 1) to avoid possible confounding due to differences in regional species pools (Caley & Schluter 1997). In fact, all the sites were within what is thought to be a single biogeographical domain (S. Hawkins, personal communication). To reduce extraneous variation, the locations were chosen so that they were similarly sheltered from wave action and similarly saline. To check this, salinity was measured in two replicate samples of the water covering the mussels in the incoming tide. Salinity at all sites was within the range 30–33 PSU.

Using notes and grid references provided by J. Widdows, it was possible to find the exact locations sampled for SFG in 1996 (except for Llanbedrog, as discussed above). With the exception of Kirkholm, rock surfaces were quite steeply sloping. Widdows *et al.* (2002) took samples from an area spanning at least 50 m of shore so that they represented conditions at the site as a whole. In the current study, samples of mussels were collected from four haphazardly placed plots per site, also spanning the extent of the site. At each plot, a 15 × 15-cm metal corer, 5 cm high and open on one side, was hammered through the mussels onto the rock surface. Formalin was squirted around the inner edge of the corer to prevent excessive escape of mobile fauna and the entire contents of the core were scraped into a plastic bag. Samples were preserved in formalin and subsequently processed in the laboratory using a fixed protocol of washing and sieving established using pilot trials. Associated macrofauna (retained on a 0·5-mm sieve) were identified to species where possible. All animals retained on a 2-mm sieve were sorted by eye. The 0·5–2-mm fraction often contained large amounts of shell and sand material. This fraction was therefore split and only one half sorted for each plot (using a binocular microscope). To combine data from the two fractions, abundances of animals in the 2-mm fraction were divided by two.

SAMPLING HABITAT CHARACTERISTICS

The topographic complexity of the surface of each plot was measured using a carpenter's profiling tool. The tool comprised 300 1-mm wide sliding plastic laths held against each other in a frame. Measurements were made by pressing the tool onto the surface of the mussels, ensuring that the tips of all of the laths were pressed into contact with the mussels. Two replicate measurements were taken for each plot (one from each diagonal) before the mussels were scraped from the rock. The outlines of the resulting impressions of the surface of each plot were traced onto paper in the field and later scanned into a computer and digitized using a program written by T. Carter (Anglia Polytechnic University, Cambridge, UK). Indices of topographic complexity for each plot were calculated using a program written by M. T. Burrows (Dunstaffnage Marine Laboratory, Oban, UK). The chain method (Beck 1998) was used to provide indices of complexity at a range of step lengths from 2 to 32. This technique can be tailored to indicate availability of topographic structures of different sizes as designated by the step lengths. For example, complexity at step length 4 relates to pits and troughs 4-mm wide (Beck 1998). Biota of different sizes may respond to habitat features of different sizes. Adjusted area is a derivative of the chain measure and involves calculation of the increase in area of substratum attributable to structures of different sizes (M. Burrows, personal communication). Fractal dimension integrates complexity across a range of step lengths into a single value. It relates well to habitat features that affect densities and sizes of rocky shore biota, such as gastropods (Beck 1998).

To assess the population structure of the mussels at each site, all mussels > 5 mm in each sample were measured using digital callipers. Mussels < 5 mm were counted. Total biomass of mussels for each plot was estimated by constructing a length–biomass relationship for each site using measurements from 50 mussels spanning the size range. The biomass of individual mussels of known length was measured by cleaning epibiota from their shells, drying them at 80 °C for 48 h and weighing them.

Dry mass of sediments of each of the following grain sizes was measured for each plot: < 63 μm, 63 μm–1 mm, 1–6·7 mm, > 6·7 mm. The masses of coarser size classes were measured by weighing the different fractions after sieving and drying them overnight at 60 °C. The mass of particles < 63 μm was estimated by carrying out initial sieving within a large bucket containing approximately 8 l of water (sieve stack: 2 mm, 0·5 mm, 63 μm). After sieving, the bucket was topped up to exactly 10 l and stirred to homogenize suspended particles. Two 500-ml subsamples were taken and these were filtered on Whatman's no. 1 filter paper using Buchner funnels. The mean mass of sediment in the subsamples was multiplied by 20 to give the total mass in the sample. If the two subsamples differed by > 0·05 g, the water was stirred again and a further two subsamples were taken.

Organic content was measured in the 63–500 μm fraction of each sample. This was considered to have the potential to exert influence over community structure by affecting food availability for some taxa (Radziejewska 1986). A 10-g subsample was taken from this sediment fraction for each plot. These samples were fired in a muffle furnace overnight at 500 °C and the loss of mass calculated.

ANALYSIS OF CONTAMINATION WITH UCM

Samples of mussels for hydrocarbon analysis were collected at the same time as the biotic samples. Two samples of 10 mussels of 30–40-mm shell length were collected at each location. Samples were stored at –20 °C prior to analysis. The samples were analysed using a method derived from Rowland *et al.* (2001). The soft tissue of each mussel was extracted as wet homogenate over ice to maximize recovery of the lower molecular weight compounds. Authentic reference compounds (deuterated tetracosane and phenanthrene dissolved in acetone) were spiked into 10 g of the wet mussel homogenate, mixed thoroughly and left at 4 °C for 4 h to allow partitioning. The 10 g of spiked wet mussel tissue was then acidified to pH 1 and 15 ml *n*-pentane : 2-propanol (1 : 4, v/v) added and sonicated for 40 min. A further 120 ml of *n*-pentane and 117 ml of pre-extracted water were added, and the mixture shaken and centrifuged (2000 r.p.m., 1118 **g**) for 40 min. The upper (*n*-pentane) layer was transferred to a stoppered conical flask and the lower aqueous layer was decanted and used for repeat extractions (×2). The resulting *n*-pentane layers were dried (anhydrous sodium sulphate; 18 h) and concentrated to 1 ml by controlled evaporation to give a total organic extract.

A procedural blank was carried out in parallel by spiking internal standards into an acidified *n*-pentane : propan-2-ol mixture (1 : 4), followed by extraction and sample concentration in the same manner as the samples.

The total organic extract was then fractionated by sequential elution by solvents of increasing polarity into 'aliphatic' and 'aromatic' fractions using a glass column (*n*-pentane slurry of activated silica under deactivated aluminium oxide). The two fractions were again concentrated to 1 ml. All samples and procedural blanks were analysed by GC and GC-MS (for details of solvents and GC-MS conditions used see Rowland *et al.* 2001). For each sample wet : dry conversion factors were calculated from 2 × 0·5 g tissue dried at 40 °C for 48 h.

The percentage recovery of authentic compound spiked into mussel tissue was calculated from external standard calibration graphs. Quantification of the total resolved and total unresolved components in each fraction was made using an average response factor of the internal standards into each fraction. The area of the unresolved hydrocarbons was calculated by subtraction of the total area of resolved peaks from the total area of the resolved + unresolved peaks.

STATISTICAL ANALYSES

On a site-by-site basis, variations in diversity at sites with high and low SFG were assessed using one-factor analysis of variance (factor = SFG with two levels: high vs. low). Separate analyses were done for species richness and Shannon–Wiener diversity. For each analysis, a single datum was derived from the pooled biotic data derived from the four samples taken at each site (i.e. $n = 3$ sites). Cochran's test was used to test for heterogeneity of variance. Given the well-documented influence of sediment characteristics over infaunal communities (Gray 1981; Little 2000), relationships between diversity and sediment characteristics were assessed by regression. To investigate whether any relationship with pollution may have arisen indirectly via effects of pollutants on mussel population structure, measures of mussel population structure were regressed against SFG. In addition, relationships were tested on a site-by-site basis between diversity and mean number and biomass of mussels in the plots and mean topographic complexity of their surfaces.

Non-metric multidimensional scaling (nMDS) based on Bray Curtis similarities was used to explore the relationship between SFG and community structure on a plot-by-plot basis (Clarke 1993). Computation was done using PRIMER 5 for Windows (Clarke & Gorley 2001). Nested non-parametric multivariate analysis of variance (NP-MANOVA; Anderson 2001) was used to test the hypothesis that assemblages at sites with low SFG were significantly different from those at locations with high SFG. PRIMER's similarity of percentages (SIMPER) routine was used to assess the contributions of different taxa to observed differences (Clarke 1993).

To assess relationships on a plot-by-plot basis between habitat variables and multivariate community structure, PRIMER's BIOENV routine was used (Clarke & Ainsworth 1993). This routine determines which combinations of habitat variables provide the best correlations between matrices of habitat data and biotic data. The degree of correlation between matrices is indicated by a weighted coefficient ρ_w that is analogous

to the Spearman rank coefficient ρ but for which there is no valid test of significance (Clarke & Ainsworth 1993). The variables used in the initial matrix of habitat data were fractal dimension, the chain index of topographic complexity (step values 4, 8 16, 32), adjusted area (step values 4, 8 16, 32), dry mass of sediments of particle sizes < 63 μm, 63 μm–1 mm, 1–6·7 mm, > 6·7 mm, total mass of sediment, percentage organic content of sediment, total number of mussels, total biomass of mussels, and numbers of mussels of size classes < 5 mm, 5–10 mm, 10–20 mm, 20–40 mm, > 40 mm. Of these, chain indices and adjusted areas of step length 4 and 8 were correlated with fractal dimension (r = 0·98, 0·9, 0·98 and 0·90, respectively) and were omitted from the matrix used in the final analysis; similarly chain and adjusted area indices of step length 32 were correlated with chain index of step length 16 (r = 0·93 and 0·94, respectively) and mass of < 63 μm sediment was correlated with mass of 63 μm–1 mm sediment (r = 0·82) and were omitted.

Results

RELATIONSHIP BETWEEN SFG AND COMMUNITY STRUCTURE

Of 57 taxa recognized (Table 1), 44 were distinct species, two were identified to genera and the remainder were aggregated at higher taxonomic levels (e.g. Class Oligochaeta, Phylum Nematoda). There were 18 taxa of worms, 18 molluscs, 13 crustacea, seven other arthropod taxa and an anthozoan.

Species richness ($F_{1,4}$ = 17·16, P < 0·015; Cochran's C = 0·66, NS) and Shannon–Wiener diversity ($F_{1,4}$ = 16·27, P < 0·016; Cochran's C = 0·78, NS) were reduced significantly at sites with low SFG compared with control sites with high SFG (Fig. 2). At this scale, no regressions between diversity and other variables (topographic complexity, biomass and number of mussels, characteristics of interstitial sediment) were significant (Table 2a), indicating that the relationship between SFG and diversity was not confounded by environmental factors. There were no significant relationships between SFG and measures of mussel population structure (Table 2b).

On a plot-by-plot basis, nMDS showed a contrast between high diversity control sites and lower diversity polluted sites, whose communities had converged towards a similar state (Fig. 3). Nested NP-MANOVA revealed significant differences between sites and groups of sites of differing SFG (Table 3).

SIMPER analysis (Table 4) indicated that barnacles *Elminius modestus*, oligochaetes and juvenile *Carcinus maenas* tended to be more abundant at polluted locations, while barnacles *Semibalanus balanoides*, cirratulid polychaetes, spionid polychaetes *Pygospio elegans*, gastropods *Littorina littorea*, *Littorina saxatilis*, isopods *Jaera forsmanii* and amphipods *Chaetogammarus marinus* tended to be more abundant at control locations.

Table 1. List of taxa used in multivariate analyses. Diversity indices did not include different size classes of the same taxa. There were very few individuals of each unidentified taxon

Class/phylum	Taxon
Gastropoda	*Bittium reticulatum*
	Cingula trifasciata
	Gibbula cinerarea
	Littorina littorea < 5 mm
	L. littorea > 5 mm
	L. mariae < 5 mm
	L. mariae > 5 mm
	L. obtusata < 5 mm
	L. obtusata > 5 mm
	L. rudis
	L. saxatilis < 5 mm
	L. saxatilis > 5 mm
	Nucella lapillus < 10 mm
	N. lapillus > 10 mm
	Omalogyra sp.
	Patella vulgata
	P. depressa
	Onoba semicostata
	O. acicula
	Rissoa parva
	Rissoidae, sp. no. 1
Polyplacophora	*Lepidochitona cinerea*
Bivalvia	*Lasaea rubra*
Crustacea	*Carcinus maenas* > 15 mm
	C. maenas < 15 mm
	Cancer pagurus
	Unidentified crab
	Eliminius modestus
	Semibalanus balanoides
	Hyale stebbingi
	Hyalidae, sp. no. 2
	Chaetogammarus marinus
	Jaera forsmanii
	Melita palmata
	Idotea pelagica
	Caprellidae
	Tanais dulongi
Insecta	*Anurida maritima*
	Clunio marinus
	Unidentified dipteran larva
	Isotomidae
	Tipulidae
Arachnida	Ameronothidae
	Bdellidae
Anthozoa	*Actinia equina*
Polychaeta	*Lepidonotus squamatus*
	Syllis gracilis
	Ophelia bicornis
	Perinereis cultrifera
	Pygospio elegans
	Capitellidae
	Cirratulidae
	Glyceridae, sp. no. 1
	Glyceridae, sp. no. 2
	Juvenile nereidae
	Unidentified polychaete no. 1
	Unidentified polychaete no. 2
Oligochaeta	Oligochaeta
Phylum Nemertea	*Lineus* sp.
	Palaeonemertea
	Unidentified nemertean no. 1
	Unidentified nemertean no. 2
Phylum Nematoda	Nematoda

Table 2. Regressions analyses testing relationships (a) between SFG, habitat variables (sediment characteristics, mussels, fractal dimension) and diversity and (b) between SFG and aspects of mussel population structure. See text for details of independent variables

(a)	Dependent variable							
	Species richness				Shannon diversity			
Independent variable	Mean square	F	P	r^2	MS	F	P	r^2
SFG	134·6	8·6	0·04	68·0	1·91	9·2	0·04	69·7
Dry mass of sediment < 63 μm	8·07	0·17	0·7	4·1	0·16	0·25	0·65	5·8
Dry mass of sediment 63 μm–1 mm	0·12	0	0·96	0·1	0·001	0·00	0·97	0
Dry mass of sediment 1–6·7 mm	66·9	2·05	0·23	33·9	0·89	1·92	0·24	32·4
Dry mass of sediment > 6·7 mm	64·85	1·96	0·23	32·8	0·84	1·76	0·26	30·5
Total dry mass of sediment	40·74	1·04	0·37	20·6	0·46	0·81	0·42	16·9
% organic content of dry mass of sediment	27·15	0·64	0·47	13·7	0·22	0·34	0·59	7·9
No. of mussels	11·3	0·24	0·65	5·7	0·11	0·15	0·71	3·7
Biomass of mussels	10·13	0·22	0·67	5·1	0·18	0·27	0·63	6·4
Fractal dimension	46·3	1·22	0·33	23·4	0·44	0·77	0·43	16·2

(b)	Independent variable			
	SFG			
Dependent variable	MS	F	P	r^2
No. of mussels	34191	1·08	0·36	21·2
Biomass of mussels	2026	0·34	0·59	7·9
No. < 5 mm	11354	0·86	0·40	17·7
No. 10–20 mm	3463	0·87	0·40	17·9
No. 20–40 mm	1·2	0·01	0·93	0·2
No. > 40 mm	68·35	0·86	0·31	0·4
Proportion > 40 mm	0·0006	0·09	0·77	2·3
Proportion < 10 mm	0·03	0·76	0·43	16

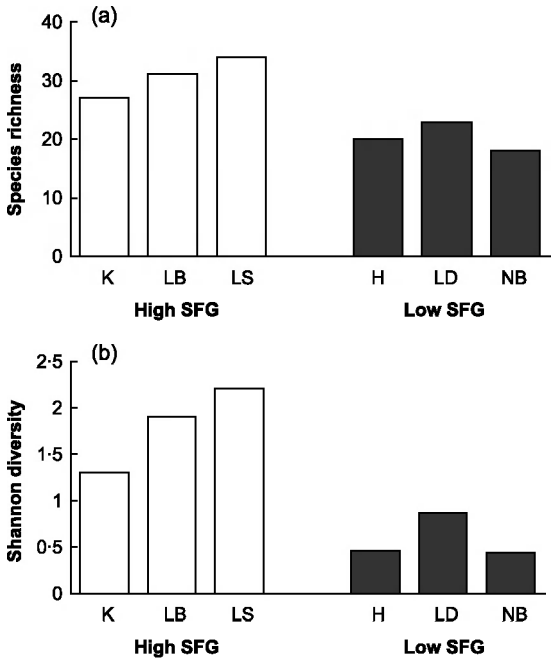

Fig. 2. Variation in relation to SFG of (a) species richness; (b) the Shannon–Wiener index. Values are based on pooled data from all four samples at each site. Sites with high SFG (> 15 J g^{-1} h^{-1}; Table 5) were Kirkholm (K), Llanbedrog (LB) and Loch Striven (LS); sites with low SFG (< 10 J g^{-1} h^{-1}; Table 5) were Heysham (H), Llandudno (LD) and New Brighton (NB).

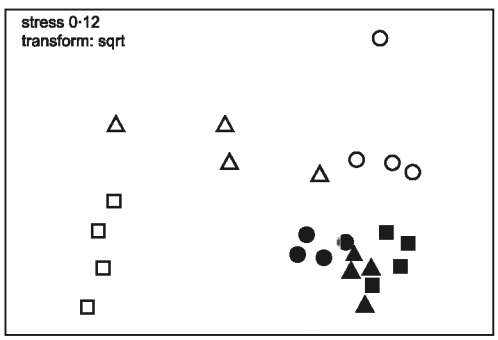

Fig. 3. nMDS representation of ranked dissimilarities between assemblages from plots at each location (Clarke 1993). The three locations with low SFG each have different shaped filled symbols; the three locations with high SFG each have different shaped open symbols; $n = 4$ plots. See Fig. 1 to relate shapes of symbols to specific sites.

Table 3. NP-MANOVA analysis of differences in community structure in samples from the different sites. Data were square root transformed

Source of variation	d.f.	MS	F	P
SFG	1	11556·93	3·14	0·0034
Site (SFG)	4	3685·99	5·03	0·0002
Residual	18	732·77		

Table 4. SIMPER analysis of taxa contributing to dissimilarity between sites with low and high SFG. The taxa listed accounted for 80% of the dissimilarity between the two groups of sites. Based on square root transformed data. Diss/SD = measure of the variation in contribution to dissimilarity (Clarke 1993); % contribution = percentage contributed to the overall Bray Curtis dissimilarity between communities at sites from the two groups (64·3%)

Taxon	Average abundance High SFG	Average abundance Low SFG	Average dissimilarity contributed by each taxon	Diss/SD	% contribution
Elminius modestus	72·2	676·8	19·6	2·9	30·4
Semibalanus balanoides	73·0	18·2	5·1	1·3	7·9
Cirratulids	33·9	1·6	3·6	0·8	5·6
Carcinus maenas < 15 mm	5·7	23·2	2·9	1·7	4·5
Oligochaeta	10·8	14·1	2·9	1·5	4·4
Littorina saxatilis < 5 mm	22·8	3·3	2·4	1·1	3·7
Pygospio elegans	7·5	4·4	2·2	1·0	3·5
Jaera forsmanii	7·3	1·9	1·8	1·2	2·8
Nematoda	4·3	3·8	1·5	0·9	2·4
Chaetogammarus marinus	4·8	0·3	1·4	0·8	2·2
Littorina littorea > 5 mm	1·9	0·7	1·4	1·2	2·2
L. littorea < 5 mm	4·5	0·3	1·4	0·9	2·1
Clunio marinus	3·8	2·7	1·4	1·3	2·1
Lineus sp.	3·5	2·1	1·2	1·3	1·9
Cingula trifasciata	8·6	0	1·1	0·5	1·6
Actinia equina	2·2	0·2	1·0	1·1	1·6

SMALL-SCALE RELATIONSHIPS BETWEEN COMMUNITY STRUCTURE AND HABITAT CHARACTERISTICS

BIOENV showed that the best relationships between habitat variables and biota on a plot-by-plot basis occurred when the matrix of habitat variables contained only (i) fractal dimension and mass of 1–6·7 mm sediment ($\rho_w = 0{\cdot}64$) or (ii) mass of 1–6·7 mm sediment and total biomass of mussels ($\rho_w = 0{\cdot}61$). No other combinations of habitat variables yielded correlations of $\rho_w > 0{\cdot}6$. It should be stressed that fractal dimension was correlated with chain and adjusted area indices of step lengths 4 and 8, and that mass of 1–6·7 mm sediment was correlated with total mass of sediment (see the Methods).

RELATIONSHIP BETWEEN SFG AND CONTAMINATION WITH UCM

The relationship between SFG and UCM was generally good. Sites with relatively high SFG all had negligible concentrations of UCM (Table 5). Of the sites with low SFG, two had high concentrations of UCM. The third, Llandudno, did not (Table 5). This site had relatively high levels of sewage input, which may explain its low SFG (Widdows *et al.* 2002).

Table 5. Mean concentrations of aliphatic and aromatic hydrocarbon UCM found in mussel tissues at each of the locations sampled. SFG data are also included (from Widdows *et al.* 2002). Values are means (range), $n = 2$. ND, the UCM were below the detection limit of the technique

Site	SFG (J h^{-1} g^{-1})	Aliphatic UCM (μg g^{-1})	Aromatic UCM (μg g^{-1})
Kirkholm	18·3	ND	ND
Loch Striven	15·9	ND	ND
Llanbedrog	18·9	ND	ND
Llandudno	8·2	26·5 (53)	ND
Heysham	5·5	533·5 (177)	163·5 (13)
New Brighton	8·7	736 (56)	250 (30)

Discussion

The value of SFG as a rapid sensitive integrated biological measure of environmental quality has often been postulated and supported with ecotoxicological studies and field-based sampling of contaminants (Anderlini 1992; Widdows & Page 1993; Widdows *et al.* 1995; but see Buhringer & Danischewski 2001). A field-based relationship between SFG and biodiversity or community structure had not been demonstrated prior to the current study. In this study, differences in SFG and concentrations of UCM were clearly reflected in differences in diversity and structure of communities of organisms living among intertidal mussels. Only six sites were sampled, however, and these were carefully selected to represent a particular habitat type: moderately sheltered, fully marine rocky shores. Further sampling is needed to test the relationships at a larger number of sites of this type and for a wider range of habitats and communities (e.g. fully sheltered shores, meiofaunal communities, communities associated with algae). If the relationship is general, SFG would be a valuable indicator of biotic impacts at a range of levels of organization. It may be of particular value in the implementation of the European Union (EU) Water Framework Directive (European Union 2000), which requires cost-effective biotically based measures of ecological quality.

The study documented a reduction in diversity through loss of rarer species at polluted sites. While such patterns have long been recognized in other systems (Gray 1989), comprehensive multivariate studies on rocky shore communities are rare (Crowe *et al.* 2000). A common pattern in other systems is an increase in abundance of opportunistic species at polluted sites (Gray 1989; Smith & Simpson 1998). In the current study, polluted sites were characterized by high abundance of the opportunistic and invasive barnacle *Elminius modestus* (Harms 1999) and by relatively large numbers of oligochaetes, often opportunistic tubificids (B. Healy, personal communication). Isopods and amphipods are particularly sensitive to oil pollution (Bonsdorff & Nelson 1981; Suchanek 1993) and were reduced in abundance at polluted sites. Similarly, several species of gastropods present at sites with high SFG were rare or absent at sites with low SFG. Nagelkerken & Debrot (1995) also documented reductions in density and diversity of molluscs at shores chronically affected by oil pollution (Smith & Simpson 1998). Changes to gastropod grazer communities often affect growth and community structure of algae (Hawkins & Hartnoll 1983; Underwood, Denley & Moran 1983; McQuaid 1996), with potential impacts on ecosystem functioning (Crowe, in press).

The rocky shore communities studied here included a number of organisms that would normally occur in soft sediment. They were present because of the sediment trapped among the mussel shells. The distribution of such organisms is affected by characteristics of the sediment (Gray 1981; Little 2000). Evidence from regression, however, showed that variation in sediment structure and abundance did not contribute significantly to patterns of variation among sites and so did not confound the large-scale relationship between SFG and diversity.

In our analyses, there was no evidence that pollution influenced mussel population structure. This is perhaps surprising, given evidence of severe effects of some pollutants on mussel larvae (Hoare, Beaumont & Davenport 1995; Hoare, Davenport & Beaumont 1995). Nevertheless, the current evidence suggests that the relationship between SFG and community structure is probably driven by direct effects of pollution on the organisms associated with the mussels rather than by indirect effects mediated by changes in the habitat provided by the mussels.

At smaller spatial scales, PRIMER's BIOENV routine (Clarke & Ainsworth 1993) showed that community structure was related to the fractal dimension of the surface of the beds, the biomass of mussels and the sediment trapped between them (either the total mass of sediment or the 1–6·7-mm fraction). Community structure has been linked to complexity of mussel beds (Seed 1996), but complexity has rarely been measured precisely in isolation from potentially confounding variables. Fractal dimensions have been used before to quantify the topographic complexity attributable to mussel beds (Commito & Rusignuolo 2000; Kostylev & Erlandsson 2001), but no published studies have assessed effects on associated communities. In other assemblages, diversity and community structure have been linked with topographic complexity, as indicated by fractal dimension (Beck 1998, 2000; Davenport, Butler & Cheshire 1999; Finlay & Fenchel 2001), even at larger spatial scales (Olff & Ritchie 2002; Whitehouse *et al.* 2002). One would expect fractal dimension of the surface of a mussel bed to relate to population structure of the underlying mussels. Although overall mussel biomass was an influential variable, mussel population structure was unrelated to the structure of associated communities. There is, however, some evidence of links between age structure of mussels and community structure (Seed 1996; T. Crowe, unpublished data). Potential direct effects of contaminants on mussel population structure and indirect effects on structure of associated communities therefore merit further investigation.

The apparent influence of 1–6·7-mm sediment (which was correlated to total mass of sediment) on community structure is not surprising, given the well-known relationship between the distribution of soft-sediment biota and sediment character (Gray 1981; Little 2000). The fact that no single descriptor of habitat characteristics provided a good relationship with community structure probably reflects the diversity of organisms present and their wide range of responses to different habitat characteristics.

SFG is affected by a range of contaminants (Widdows 1985) and relating community-level effects to specific contaminants is difficult. Declines in SFG have been linked to inhibition of clearance rate caused by high levels of toxic hydrocarbons in the North Sea (Widdows *et al.* 1995) and Irish Sea (Widdows *et al.* 2002). Although work to date suggests that aliphatic UCM exert only minor toxicity, laboratory and field studies suggest that monoaromatic and oxidized aliphatic UCM components are toxic to *Mytilus edulis* (Thomas, Donkin & Rowland 1995; Rowland *et al.* 2001; Donkin, Smith & Rowland 2003). Direct links between reduced diversity at polluted sites and concentrations of some UCM hydrocarbons are therefore possible. Our findings do not rule out this possibility: elevated levels of UCM at two of the locations (Heysham and New Brighton) corresponded to reductions in SFG and diversity relative to the three control locations. No previous field-based data have related UCM contamination to community-level effects. The average concentrations of aromatic UCM found at Heysham (164 $\mu g\ g^{-1}$) and New Brighton (250 $\mu g\ g^{-1}$) were similar to those found by Rowland *et al.* (2001) at contaminated sites at Cleethorpes and Teesmouth (102 and 136 $\mu g\ g^{-1}$, 83 and 94 $\mu g\ g^{-1}$, respectively) and somewhat less than at Whitby ($410 \pm 61\ \mu g\ g^{-1}$; $n = 4$; years 1990, 1995).

Evidence from the present study and from Widdows *et al.* (2002) indicates that SFG can be suppressed by different contaminants at different sites. For example, at Llandudno SFG was low but UCM concentrations were not high. Widdows *et al.* (2002) suggested that

sewage inputs may account for some of the unexplained portion of SFG suppression at sites including Llandudno. Indeed Widdows *et al.* (2002) considered a range of contaminants, including metals, organometals and organic pollutants. However, metals and organometals played a relatively minor role so that a significant proportion of reduced SFG at many sites was attributable to what they termed '2 and 3-ringed aromatic hydrocarbons' measured by HPLC with ultraviolet (UV) detection. Rowland *et al.* (2001) showed that such HPLC fractions were in fact dominated by 'monoaromatic' UCM compounds when examined by GC-MS (Murray, Gibbs & Kavanagh 1983). Donkin, Smith & Rowland (2003) showed that of all the hydrocarbon components detectable in polluted mussel extracts by HPLC-UV the most toxic fractions were those comprising 'monoaromatic' hydrocarbons. Recently, using two-dimensional GC-time of flight-MS, hundreds of alkyldimethylbenzenes were detected in these toxic fractions (P.A. Sutton, E.L. Smith, A.M. Booth, A.M. Lewis, A.C. Lewis, & S.J. Rowland, unpublished data) but it is not known if these structures were responsible for the toxicity. Concentrations of UCM at polluted sites are often many times greater than those of the polycyclic aromatic hydrocarbons (PAH) peaks resolved by GC, but their potential effect is rarely considered.

Overall, our findings are a significant step towards establishing field-based relationships between contaminants, biotic indices of stress (SFG) and community level effects. Such ground-truthing is essential in the development of practical indicators of environmental impact that are integrated across a range of levels of biological organization.

Acknowledgements

This work was undertaken as part of a NERC Postodoctoral Fellowship (GT59807MS) held by T. P. Crowe in Steve Hawkins' research group at the University of Southampton. Analysis of hydrocarbon contamination was funded by British Ecological Society Small Ecological Project Grant no. 1729 and DETR grant CDEP 84/5/267. We would like to thank John Widdows for providing SFG data before it was published (and for valuable comments on an earlier draft), Paul Somerfield for advice on sample processing and analysis, Simon Bray, Alison Arbuthnott and Jane Stout for help with sample sorting, and Martin Sheader, Alex Rogers, Louise Allcock and Brenda Healy for identifying specimens. Steve Ormerod and two anonymous referees made helpful comments on an earlier draft. This paper is dedicated to the memory of Peter Donkin.

References

Anderlini, V.C. (1992) The effect of sewage on trace metal concentrations and scope for growth in *Mytilus edulis aoteanus* and *Perna canaliculus* from Wellington Harbour, New Zealand. *Science of the Total Environment*, **125**, 263–288.

Anderson, M.J. (2001) A new method for non-parametric multivariate analysis of variance in ecology. *Austral Ecology*, **26**, 32–46.

Bayne, B.L. (1985) Ecological consequences of stress. *The Effects of Stress and Pollution on Marine Animals* (eds B.L. Bayne, D.A. Brown, K. Burns, D.R. Dixon, A. Ivanovici, D.R. Livingstone, D.M. Lowe, M.N.S.A. R.D. Moore & J. Widdows), pp. 141–157. Praeger, New York, NY.

Beck, M.W. (1998) Comparison of the measurement and effects of habitat structure on gastropods in rocky intertidal and mangrove habitats. *Marine Ecology Progress Series*, **169**, 165–178.

Beck, M.W. (2000) Separating the elements of habitat structure: independent effects of habitat complexity and structural components on rocky intertidal gastropods. *Journal of Experimental Marine Biology and Ecology*, **249**, 29–49.

Bonsdorff, E. & Nelson, W.G. (1981) Fate and effects of Ekofisk crude oil in the littoral of a Norwegian fjord. *Sarsia*, **66**, 231–240.

Buhringer, H. & Danischewski, D. (2001) Laboratory studies on the scope for growth in blue mussels, *Mytilus edulis* L. *Archive of Fishery and Marine Research*, **49**, 61–68.

Caley, M.J. & Schluter, D. (1997) The relationship between local and regional diversity. *Ecology*, **78**, 70–80.

Clarke, K.R. (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, **18**, 117–143.

Clarke, K.R. & Ainsworth, M. (1993) A method of linking multivariate community structure to environmental variables. *Marine Ecology Progress Series*, **92**, 205–219.

Clarke, K.R. & Gorley, R.N. (2001) *PRIMER v5*. PRIMER-E Ltd, Plymouth, UK. www.primer-e.co.uk.

Commito, J.A. & Rusignuolo, B.R. (2000) Structural complexity in mussel beds: the fractal geometry of surface topography. *Journal of Experimental Marine Biology and Ecology*, **255**, 133–152.

Crowe, T.P. (in press) What do species do in intertidal ecosystems? *The Intertidal System. Proceedings of a Royal Irish Academy Symposium* (ed. J.G. Wilson). Royal Irish Academy, Dublin, Ireland.

Crowe, T.P., Thompson, R.C., Bray, S. & Hawkins, S.J. (2000) Impacts of anthropogenic stress on rocky intertidal communities. *Journal of Aquatic Ecosystem Stress and Recovery*, **7**, 273–297.

Davenport, J., Butler, A. & Cheshire, A. (1999) Epifaunal composition and fractal dimensions of marine plants in relation to emersion. *Journal of the Marine Biological Association of the United Kingdom*, **79**, 351–355.

Donkin, P., Smith, E.L. & Rowland, S.J. (2003) Toxic effects of unresolved complex mixtures of aromatic hydrocarbons accumulated by mussels, *Mytilus edulis*, from contaminated sites. *Environmental Science and Technology*, **37**, 4825–4830.

European Union (2000) *Directive 2000/60/EC of the European Parliament and of the Council. Establishing a Framework for Community Action in the Field of Water Policy*. European Union, Brussels, Belgium. www.europa.eu.int/eur-lex.

Finlay, B.J. & Fenchel, T. (2001) Protozoan community structure in a fractal soil environment. *Protist*, **152**, 203–218.

Goldberg, E.D. (1975) The mussel watch: a first step in global marine monitoring. *Marine Pollution Bulletin*, **6**, 111.

Gray, J.S. (1981) *The Ecology of Marine Sediments*. Cambridge University Press, Cambridge, UK.

Gray, J.S. (1989) Effects of environmental stress on species rich assemblages. *Biological Journal of the Linnean Society*, **37**, 19–32.

Harms, J. (1999) The neozoan *Elminius modestus* Darwin (Crustacea, cirripedia): possible explanations of its successful invasion in European waters. *Helgolander Meeresuntersuchungen*, **52**, 337–345.

Hawkins, S.J. & Hartnoll, R.G. (1983) Grazing of intertidal algae by marine invertebrates. *Oceanography and Marine Biology Annual Review*, **21**, 195–282.

Hoare, K., Beaumont, A.R. & Davenport, J. (1995) Variation among populations in the resistance of *Mytilus edulis* embryos to copper: adaptation to pollution? *Marine Ecology Progress Series*, **120**, 155–161.

Hoare, K., Davenport, J. & Beaumont, A.R. (1995) Effects of exposure and previous exposure to copper on growth of veliger larvae and survivorship of *Mytilus edulis* juveniles. *Marine Ecology Progress Series*, **120**, 163–168.

Kostylev, V. & Erlandsson, J. (2001) A fractal approach for detecting spatial hierarchy and structure on mussel beds. *Marine Biology*, **139**, 497–506.

Little, C. (2000) *The Biology of Soft Shores and Estuaries*. Oxford University Press, Oxford, UK.

McQuaid, C.D. (1996) Biology of the gastropod family Littorinidae. II. Role in the ecology of intertidal and shallow marine ecosystems. *Oceanography and Marine Biology Annual Review*, **34**, 263–302.

Murray, A.P., Gibbs, C.F. & Kavanagh, P.E. (1983) Estimation of total aromatic hydrocarbons in environmental samples by high pressure liquid chromatography. *International Journal of Environmental Analytical Chromatography*, **16**, 167–195.

Nagelkerken, I.A. & Debrot, A.O. (1995) Mollusc communities of tropical rubble shores of Curaçao: long-term (7+ years) impacts of oil pollution. *Marine Pollution Bulletin*, **30**, 592–598.

O'Connor, T.P. (2002) National distribution of chemical concentrations in mussels and oysters in the USA. *Marine Environmental Research*, **53**, 117–143.

Olff, H. & Ritchie, M.E. (2002) Fragmented nature: consequences for biodiversity. *Landscape and Urban Planning*, **58**, 83–92.

Paine, M.D., Chapman, P.M., Allard, P.J., Murdoch, M.H. & Minifie, D. (1996) Limited bioavailability of sediment PAH near an aluminium smelter: contamination does not equal effects. *Environmental Toxicology and Chemistry*, **15**, 2003–2018.

Radziejewska, T. (1986) On the role of *Mytilus edulis* aggregations in enhancing meiofauna communities off the southern Baltic coast. *Opehelia*, **4** (Supplement), 211–218.

Rowland, S., Donkin, P., Smith, E. & Wraige, E. (2001) Aromatic hydrocarbon 'humps' in the marine environment: unrecognised toxins? *Environmental Science and Technology*, **35**, 2640–2644.

Seed, R. (1996) Patterns of biodiversity in the macro-invertebrate fauna associated with mussel patches on rocky shores. *Journal of the Marine Biological Association of the United Kingdom*, **76**, 203–210.

Smith, S.D.A. & Simpson, R.D. (1998) Recovery of benthic communities at Macquarie Island (sub-Antarctic) following a small oil spill. *Marine Biology*, **131**, 567–581.

Suchanek, T.H. (1993) Oil impacts on marine invertebrate populations and communities. *American Zoologist*, **33**, 510–523.

Thomas, K.V., Donkin, P. & Rowland, S.J. (1995) Toxicity enhancement of an aliphatic petrogenic unresolved complex mixture (UCM) by chemical oxidation. *Water Research*, **29**, 379–382.

Underwood, A.J., Denley, E.J. & Moran, M.J. (1983) Experimental analyses of the structure and dynamics of mid-shore rocky intertidal communities in New South Wales. *Oecologia*, **56**, 207–219.

Whitehouse, M.E.A., Shochat, E., Shachak, M. & Lubin, Y. (2002) The influence of scale and patchiness on spider diversity in a semi-arid environment. *Ecography*, **25**, 395–404.

Widdows, J. (1985) Physiological measurements. *The Effects of Stress and Pollution on Marine Animals* (eds B.L. Bayne, D.A. Brown, K. Burns, D.R. Dixon, A. Ivanovici, D.R. Livingstone, D.M. Lowe, M.N.S.A.R.D. Moore & J. Widdows), pp. 3–45. Praeger, New York, NY.

Widdows, J. & Donkin, P. (1992) Mussels and environmental contaminants: bioaccumulation and physiological aspects. *The Mussel Mytilus: Ecology, Physiology, Genetics and Culture* (ed. E. Gosling), pp. 383–424. Elsevier, Amsterdam, the Netherlands.

Widdows, J. & Page, D.S. (1993) Effects of Tributyltin and Dibutyltin on the physiological energetics of the mussel *Mytilus edulis*. *Marine Environmental Research*, **35**, 233–249.

Widdows, J., Donkin, P., Brinsley, M.D., Evans, S.V., Franklin, A., Law, A.J. & Waldock, M.J. (1995) Scope for growth and contaminant levels in North Sea mussels, *Mytilus edulis*. *Marine Ecology Progress Series*, **127**, 131–148.

Widdows, J., Donkin, P., Staff, F.J., Matthiessen, P., Law, R.J., Allen, Y.T., Thain, J.E., Allchin, C.R. & Jones, B.R. (2002) Measurement of stress effects (scope for growth) and contaminant levels in mussels (*Mytilus edulis*) collected from the Irish Sea. *Marine Environmental Research*, **53**, 327–356.

Widdows, J., Nasci, C. & Fossato, V.U. (1997) Effects of pollution on the scope for growth of mussels (*Mytilus galloprovincialis*) from the Venice Lagoon, Italy. *Marine Environmental Research*, **43**, 69–79.

Received 26 August 2003: final copy received 13 November 2003