ORIGINAL ARTICLE

Eran Gefen · Lewi Stone · Michael Rosenfeld ·
Hein von Westernhagen

# Biological indicators in marine and coastal waters: a statistical and modelling analysis of the MARS campaign

**Abstract** We perform a statistical analysis of ecological data collected in the MARS project from marine environments of the Red and Mediterranean Seas in Israel, and the North Sea in Germany. In the study of fish parasite data, we examine the basic premise that the complex host lifecycles of heteroxenous ($h$) parasites make them more sensitive to pollution than monoxenous ($m$) parasites which have only one host. Unlike results from analyses of earlier survey data, we find that the pooled $h/m$ index for a community is not a clear indicator of environmental stress as originally hypothesized. This is due to the relatively large seasonal variability at the sampling sites over the course of the 3-year survey. Other possible indicators for water pollution gradients are checked, including species richness, diversity and prevalence of parasite populations. A theoretical model of host-parasite population dynamics that makes the presence of ecological thresholds transparent is offered to help explain the abrupt transitions prominent in the distributions of parasites at different sites under different pollution loadings. Heavy metal and chlorinated hydrocarbon data collected from fish tissue samples and molluscs of the three seas are assessed as bioindicators for pollution monitoring. The xenobiotic loads and indices of defence system activity and tissue damage are shown to have great potential as monitoring bioindicators.

**Keywords** Pollution · Parasites · Fish · Molluscs · Bioindicators

E. Gefen (✉) · L. Stone · M. Rosenfeld
Department of Zoology,
Tel Aviv University,
69978 Ramat Aviv , Israel
e-mail: erangef@post.tau.ac.il

H. von Westernhagen
Alfred-Wegener-Institut für Polar- und Meeresforschung,
Biologische Anstalt Helgoland,
Columbusstraße, 27568 Bremerhaven, Germany

## Introduction

The two-phase MARS 1 (1995–1998) and MARS 2 (1999–2000) projects are part of a long-term German-Israeli collaboration in studying and developing techniques to monitor the ecology of the marine environment in three seas: the Red Sea and Mediterranean Sea in Israel, and the North Sea in Germany. Our main aim was to assess a set of quantitative tools designed by the different MARS groups for the detection of environmental stress in these seas. This was to be accomplished by integrating data from several sub-projects: (1) the use of fish parasitological indices in pollution monitoring; (2) fish tissue residues analysis; and (3) the use of marine molluscs for biomarker-based pollution monitoring. Together, these three sub-projects test for possible influences of pollution on population and community structure, as well as the effect of exposure to varying pollution intensities at the organismic and cellular levels.

The three subprojects have, in total, generated a large amount of field data that has proved to be interesting in its own right in the context of each specific project. In this paper, we report a summary overview of a more detailed statistical analysis comparing trends between the three different seas (Stone et al. 2002), while at the same time tying this together with established results presented both in this volume and earlier studies from the MARS 1 project. Our goal is thus to report the main trends seen in the enormous amount of data collected. We are inspired by the earlier challenge of Diamant and von Westernhagen (1999), who wrote: "Clearly the usefulness of any contribution, including that of the present [MARS] project, is measured by our capacity to comprehend, integrate and generalize details, with the objective of developing means for predicting consistent and valid endpoints. Thus, the accumulation of a large body of information, such as generated here, is the prerequisite for an in depth comprehension of the phenomena under study." This motivates our attempt to explore and synthesize results from the different projects and place them together under the umbrella of a well-defined

multivariate statistical analysis. Since almost all of the different MARS projects sampled the same sites, conducting the comparative analysis both within and across the three different regions is easier. Furthermore, each region has its own associated 'reference' site that was determined to be relatively clean or unpolluted compared to all other sites in the region. This made it possible to test the performance of bioindicators in sites with different kinds and varying levels of pollution against that found in the cleaner reference sites.

The parasitological data was studied through the application of several standard ecological indices taking advantage of conventional [see Diamant et al. 1999; Dzikowski et al. 2003 (this issue)] and multivariate analyses (Stone et al. 2002). A basic premise behind the analysis is that heteroxenous parasites have complex multiple-host lifecycles and, as a result, are expected to be more sensitive to pollution than monoxenous parasites, which have only one host (Diamant et al. 1999). Heteroxenous parasites can only survive when the full range of conditions required by all their hosts persists, a criterion which could be much more demanding than that for monoxenous parasites, whose existence depends on a single host species. Indeed, the earlier MARS 1 data shows that the number of heteroxenous parasites ($h$) decreases with pollution levels while the number of monoxenous parasites ($m$) remains relatively unchanged (Diamant et al. 1999). Note, though, that this is not a clear-cut rule. For example, pollution can sometimes increase the abundance of intermediate hosts or render the main host more susceptible to parasitism, either of which could act to increase $h$ (Lafferty and Kuris 1999). Also, monoxenous species may be affected by, for example, direct impact of pollutants on infective stages.

As emphasized in MARS 1, the $h/m$ ratio provides a quantitative evaluation of environmental stress in the MARS systems, with 'clean' or 'stable' sites characterized by higher $h/m$ values than 'polluted' or 'perturbed' sites. The MARS 2 project continues to examine this hypothesis, and tests other possible indicators of water pollution gradients, including species richness, diversity and prevalence of parasite populations. These indices are, in any event, standard in the parasitological literature and are routinely applied. It is well known that parasite communities are likely to be species poor in the period immediately following disturbance events, and changes in pollution should influence these basic indices (Poulin 1997).

The population dynamics of host-parasite relationships are explored from a modelling perspective. We review some of the theoretical literature (Anderson and May 1978; Dobson 1989) and also analyse a simple model that makes transparent ecological threshold mechanisms intrinsic to host-parasite communities. This could have important implications for the study of bioindicators. In the presence of such ecological thresholds, it is unlikely that bioindicators will respond in a linear manner to pollution levels. Instead, abrupt transitions may well be observed as soon as the effects of pollution reduce a parameter to a sub-threshold level. A perturbation that even slightly reduces a parasite's transmission rate to sub-threshold could have drastic effects.

Marine organisms, including fish and molluscs, accumulate pollutants such as heavy metals and chlorinated hydrocarbons from the environment, and have therefore been used as biomonitors for pollution (Bresler et al. 1999). Exposure of marine organisms to anthropogenic xenobiotics, such as organochlorides, was found in MARS 1 to be correlated with changing parasite community structures, along with effects at the cellular and sub-cellular levels (Broeg et al. 1999). These included increased EROD activity, and decreased lysosomal membrane stability. However, levels of heavy metal in fish tissues have not always been in accordance with environmental gradients determined for the various sampling sites (Kress et al. 1999), sometimes making it difficult to detect pollution gradients. We test whether residue levels of various pollutants in fish tissues are a true reflection of environmental pollution gradients. These organic and inorganic compounds, generally termed xenobiotics, stress ecosystems and communities by interacting with the organism's physiology. The most marked impact of these pollutants usually occurs in the shallow waters of marine habitats. Thus marine organisms (especially molluscs living in intertidal sandy and rocky habitats) have the potential to be good biomarkers and can provide early warning in a habitat under xenobiotic stress.

## Methods

### Sampling sites, selection of reference sites, and environmental gradients

The comparative analysis comprises study sites from three different marine systems located in: (1) the Red Sea, Israel; (2) the Mediterranean Sea, Israel; and (3) the North Sea, Germany. The environmental status of each site was initially determined on the basis of the earlier MARS 1 Environmental Analysis (MEA), being collectively based on information in Stone et al. (1998) and Herut et al. (1999), consultations (Herut and Kress, personal communications) and discussions with MARS participants. For each sea it was possible to identify a relatively clean 'reference' site in which the effects of pollution were mild in comparison to the other, 'polluted' study sites in the region. Evaluations and comparisons in sensitivity and performance of the various bioindicators are considered with respect to these 'reference' sites and the MEA's hypothesized environmental gradients in each region. This also provides a means of reconfirming the validity of the hypothesized MEA's environmental gradients, thereby providing scope for ensuring a good degree of self-consistency.

### Red Sea

The Israeli Red Sea sites are located at the most northern extension of the Gulf of Aqaba (Eilat). The southwestern shore of the Gulf mainly consists of beach-rock formations, paralleled offshore by a fringing coral reef. Located on this shore is Israel's Inter University Institute for Marine Sciences (IUI), a site known to be relatively clean in terms of water quality (MEA). The IUI was therefore taken as the Red Sea's reference site. The two other Red Sea sampling sites are located in the northern part of the gulf and are mainly sandy habitats. In the offshore northern part is a mariculture site

**Table 1** Summary of the organisms sampled

| Sea | Fish | Molluscs |
| --- | --- | --- |
| Red Sea | *Siganus rivulatus* | *Cellana rotha* |
| Mediterranean Sea | *Liza ramada*; *L. aurata* | *Donax trunculus* |
| North Sea | *Platichthys flesus* | *Mytilus edulis* |

referred to as the 'Fish Farm' (FF), which is considered to be relatively polluted (MEA). Furthermore, it is the focus of an ongoing environmental controversy in which the fish cages at the site are believed to be a potential pollution hazard. A heavy organic pollution load at the site has the potential to cause damage to Eilat's coral reefs, which depend on a clear, transparent and oligotrophic water column. The third Red Sea site is located to the northeast of the fish farms and is referred to as the North Beach (NB) site. The hypothesised environmental gradient of the Red Sea sites, according to the MEA, is thus:

$$IUI < NB \leqslant FF$$

That is, the pollution levels are expected to increase when passing from IUI to NB and FF.

### The Mediterranean Sea

Mediterranean organisms were sampled from sites situated within Haifa Bay (HB) namely Shemen beach and Frutarom, that demonstrate by far the heaviest levels of organic and inorganic pollution of all sites in both the Red Sea and the Mediterranean (MEA). Sites situated outside of HB, namely Caesarea (Ca) and Ma'agan Michael (MM), were relatively clean and therefore taken as reference sites in the Mediterranean. The hypothesised environmental gradient of the Mediterranean Sea sites according to the MEA is thus:

$$MM = CA < HB$$

### The North Sea

All North Sea samplings of fish and molluscs were carried out in the following four sites, several of which are known to be polluted by heavy metals, chlorinated hydrocarbon and nutrients (Büther 1990; Herut et al. 1999): Elbe (polluted); Eider (moderately polluted); and both Helgoland and Spiekeroog as reference sites. The environmental gradients were taken from Schmolke et al. (1999) and von Westernhagen (personal communication). The hypothesised environmental gradient of the North Sea sites, according to the MEA is thus:

$$Helgoland = Spiekeroog < Eider < Elbe$$

Table 1 summarizes the organisms sampled in the different seas in the MARS projects that were examined in this statistical study.

## Ecological indices and statistical methodology

### Parasitology

Formally, we use the following notation:

$h$ = no. of heteroxenous individuals
$m$ = no. of monoxenous individuals
$S_h$ = no. of heteroxenous species

referring to the number of counts from all sampled fish. Individual counts were available for macroparasites only, as microparasites were quantified by their levels of infection. Parasitological values were corrected for individual fish size, in order to account for host size effect on the parasite load (Diamant et al. 1999).

Although we have examined some ten different indices, we restrict our analysis to the three most successful, which we define as:

1. The ratio of heteroxenous to monoxenous individuals = $h/m$
2. Heteroxenous species richness = $S_h$
3. Shannon-Wiener Diversity Index for heteroxenous:

$$H'_h = \sum_1^n p_i \ln p_i$$

### Fish tissue residues

Multivariate PCA analysis was used to analyse residues comprising heavy metals and organochlorines that had accumulated in the muscle tissue of the fish sampled in each of the three seas. PCA is a method of extracting new principal components by rotation of the original variable space (here different residue levels) to a point which maximizes the variance of the newly formed components (Legendre and Legendre 1998). This in turn greatly enhances the ability to discriminate between specific groupings of the variables (sites, seasons). Component loadings of the original variables (residues) on the new principal components reflect the correlation coefficients between them. Each particular principal component is to be associated with its own particular subset of residues. The goal of the PCA analysis was to determine whether the reference site could be differentiated from the other sites in multidimensional space in terms of the different residues.

### Molluscs

The molluscs sampled at the Israeli sites were analyzed by specifically designed biochemical tests aimed at checking the amount of tissue damage and defence system activity of these organisms. The tests were based on methods described in Bresler et al. (1999) and used in vivo and in vitro fluorescent measurement techniques. The first type of test assessed the ability of organisms to deal with xenobiotic loads as reflected in the activity of two active transport systems: MXRtr (multi xenobiotic resistance transporter) and SATOA (system of active transport of organic anions). A positive correlation is expected between each of these two variables and environmental pollution. The second type of test reflected the extent of damage caused to the organism as a result of its inability to handle the high pollutants levels, over and above the defence systems already mentioned. These included the MNT test, an estimate of DNA nucleation, and damage caused to the epithelial layer estimated by measuring its permeability using a fluorescent anion marker (Bresler et al. 1999). MNT and epithelial permeability are expected to be positively correlated with pollution levels.

Statistical analyses of the above parameters were conducted via one-way analysis of variance (ANOVA) after first checking basic assumptions required for carrying out ANOVA (normality of data and homogeneity of variances). All statistical analyses, throughout this study, were carried out using STATISTICA for WINDOWS, version 5.0.

## Model

A mathematical model was designed to compare the stability of heteroxenous versus monoxenous host-parasite systems. The model was based on a differential equation formulation and integrated using Matlab version 5.3. The model was tested for ecological thresholds, and for biological and environmental scenarios that give exact theoretical conditions that allow parasites to invade their host populations.

## Results

Parasitology

All parasitological data were derived from fish samples collected over four campaigns in the three regions, as summarized in Table 2. We begin by providing a rapid summary of the performance of the parasitological indices. The overview is given in Fig. 1a–c where the indices $h/m$, $S_h$ and $H'_h$ are plotted for each campaign/region of the North Sea. In all figures, the cleaner reference sites are indicated by the filled marker symbols. Figure 1b, c show without ambiguity that the heteroxenous species richness ($S_h$) and community diversity indices ($H'_h$) take their highest values at the cleaner reference sites (filled symbols). This is easily visualized because the filled symbols sit at the highest points of the graph for all sampling dates forming a 'ceiling' over the graphs. Hence Fig. 1b, c readily attests to the usefulness of these parasitological indices in distinguishing the cleaner reference sites in the North Sea. In contrast, Fig. 1a shows that the $h/m$ index fails to distinguish reference from polluted sites.

A more detailed analysis of the parasitological indices is provided in Table 3 which uses the Kruskal-Wallis test and a nonparametric multiple comparison test to check difference in means between sites. Separation of the North Sea sites by the $h/m$ index was not possible during the MARS 2 project. While all four sites did not differ significantly in autumn 2000, the $h/m$ index for the Outer Eider was highest in autumn 1999 and one of the lowest in the following campaign, spring 2000.

Furthermore, $h/m$ failed to separate the heavily polluted Elbe from the reference site of Helgoland in all of the four sampling campaigns (Fig. 1a). Both $S_h$ and $H'_h$ yield a significant difference between Helgoland and Elbe in all of the four MARS 2 sampling campaigns (Table 3; Fig. 1b–c).
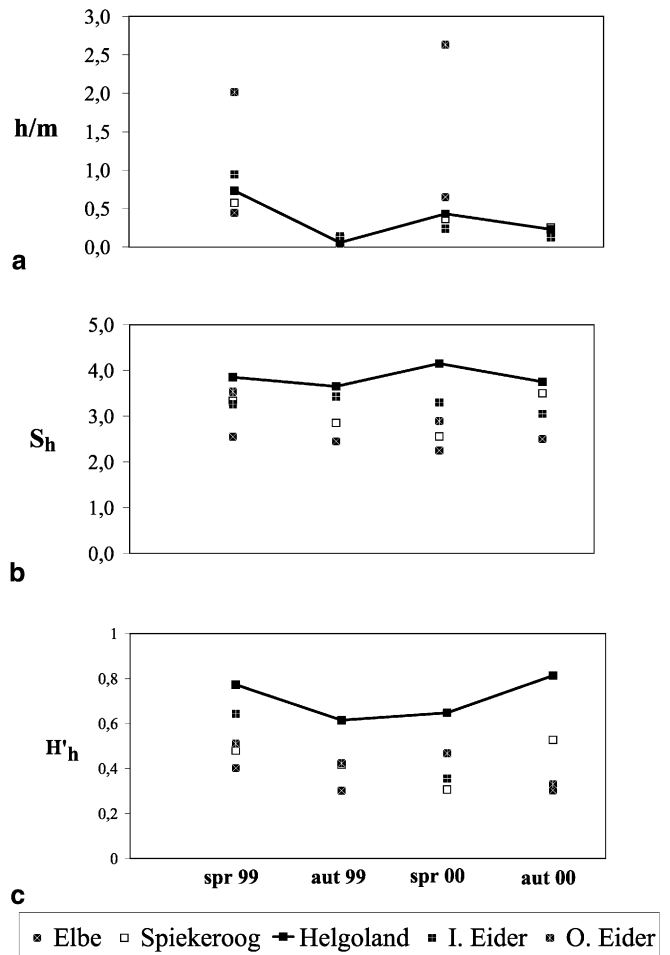


**Fig. 1** Mean $h/m$ (**a**), $S_h$ (**b**) and $H'_h$ (**c**) values for sampling sites of the North Sea. The four columns represent the four MARS 2 sampling campaigns (left to right: spring 1999, autumn 1999, spring 2000, autumn 2000). The clean reference site, Helgoland, is represented by the *filled symbols*

**Table 2** The number of fish sampled and analyzed in each MARS 2 sampling campaign in the three seas. Numbers in parenthesis represent numbers of fish from each sample analyzed for muscle tissue residue levels

| Sea | Site | Spring 1999 | Autumn 1999 | Spring 2000 | Autumn 2000 |
|---|---|---|---|---|---|
| Red | Inter Uni. Inst. (IUI) | 13(7) | 28(9) | 20(10) | 20(10) |
| | North Beach (NB) | 16(13) | 20(8) | 20(10) | 18(10) |
| | Fish Farms (FF) | 5(5) | 28(20) | 20(10) | 18(10) |
| Med. | Ma'agan Michael (MM) | 13(10) | 16(10) | 20(10) | 20(10) |
| | Haifa | 8(8) | 19(16) | 19(10) | 11(8) |
| North | Elbe | 20(17) | 20(17) | 20(10) | 20(14) |
| | Helgoland | 20(10) | 20(10) | 20(10) | 20(10) |
| | Inner Eider | 15(10) | 0 | 18(10) | 0 |
| | Outer Eider | 19(10) | 21(10) | 20(10) | 20(10) |
| | Spiekeroog | 9(9) | 20(9) | 9(9) | 20(10) |

**Table 3** Comparison of parasitological indices between sampling sites within the North Sea using Kruskal-Wallis and nonparametric multiple comparisons. Abbreviations for North Sea sites: *He* Helgoland; *IE* Inner Eider; *S* Spiekeroog; *OE* Outer Eider. Sites within brackets are not statistically different from each other (NS). Significant differences are represented by a > sign with He>Elbe implying that the index associated with the He site is greater than that of the Elbe with statistical significance

| | Spring 1999 | Autumn 1999 | Spring 2000 | Autumn 2000 |
|---|---|---|---|---|
| $h/m$ | NS | NS | NS | NS |
| $S_h$ | He>Elbe | He>Elbe | He>(IE=S=Elbe) | He>Elbe |
| $H'_h$ | He>(Elbe=OE) | He>rest | He>(Elbe=IE) | He>rest |
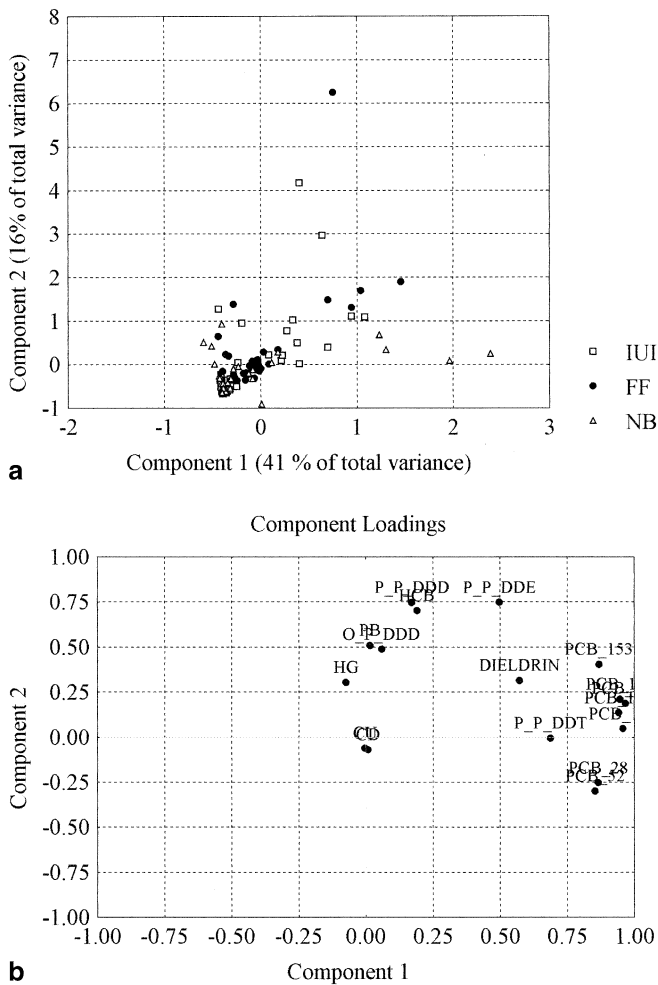
**Fig. 2 a** Muscle tissue residues as analyzed from Red Sea fish. Each point represents an individual fish. **b** Component loadings can be interpreted as correlation coefficients between the new principal components and the analyzed residues (i.e. the original variables). *IUI* Inter University Institute; *FF* Fish Farm; *NB* North Beach

## Red and Mediterranean Seas

Due to constraints regarding data accessibility, we are unable to present formal results of parasite analyses made at the Israeli sites. Fortunately, since the main trends are already known [see Stone et al. 2002; Dzikowski et al. 2003 (this issue)], we are still able to make the comparison without loss of generality (see Discussion).
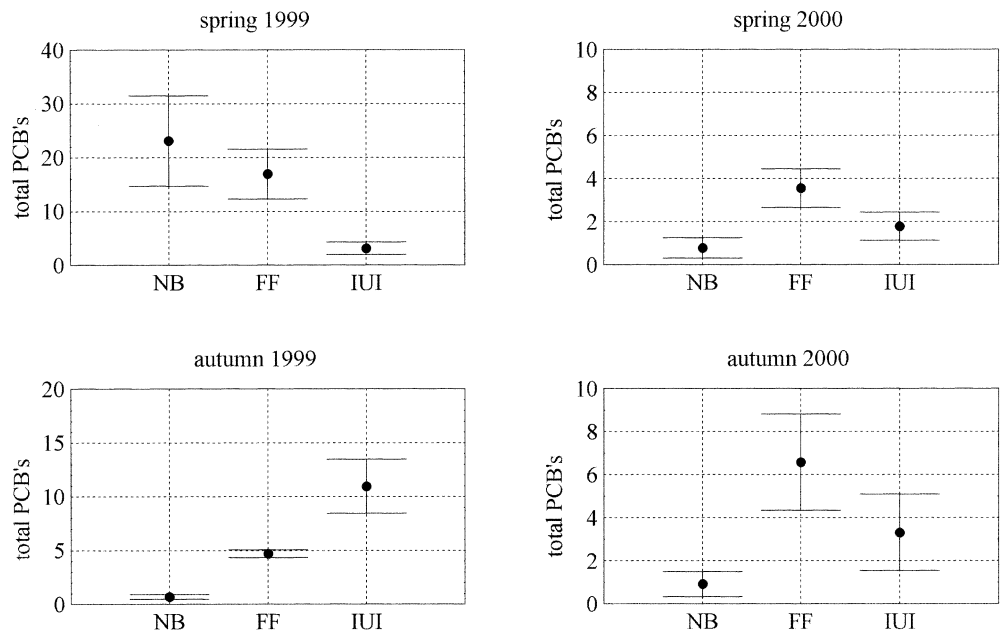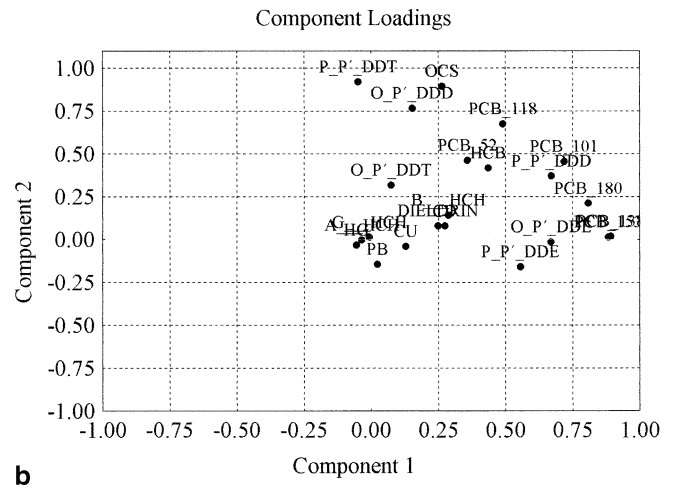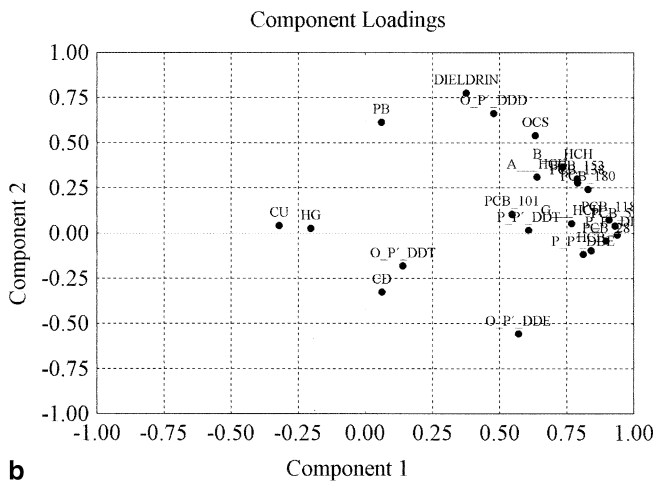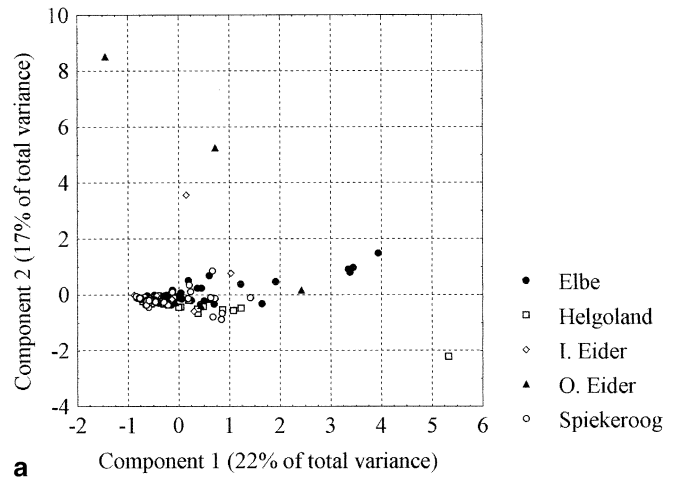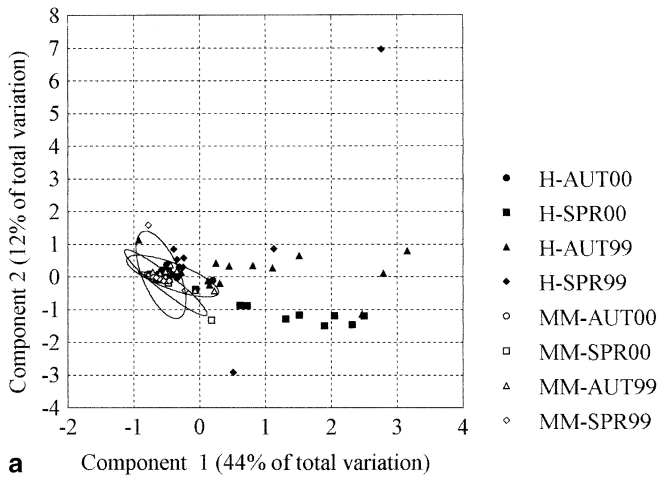
### Fish tissue residues

All fish tissue residue data were derived from fish samples collected over four campaigns in the three regions, as summarized in Table 1. PCA analysis was used to examine the residues levels in fish muscle tissue (Figs. 2, 3, 5). Each point in a PCA plot represents an individual fish located at its appropriate position in multivariate space.

### Red Sea

The PCA analysis of tissue residues from fish sampled in the Red Sea is presented in Fig. 2. In this analysis, the data were pooled over all seasons (i.e. spring 1999 and 2000; autumn 1999 and 2000). Evidently, the two main principal components, 1 and 2, are not useful for separating the three Red Sea sites in multidimensional space. We examined the data at a finer temporal (seasonal) resolution to seek reasons why this separation was difficult to obtain. First, note that in Fig. 2, principal component 1 explains 41% of the total variation, and is highly correlated with levels of polychlorinated biphe-niles (PCB) in muscle tissue (Fig. 2b). This is evident because all PCBs are characterized by high component loadings (>0.8) and are thus highly correlated with



**Fig. 3** Mean values (±1SE) of calculated total PCB as µg/kg wet weight in fish sampled from the three Red Sea sampling sites in each of the four MARS 2 sampling campaigns

Fig. 4 a Muscle tissue residues as analyzed from Mediterranean fish. Each point represents an individual fish. Each sampling campaign is represented by a different symbol. Ellipses represent 95% confidence limits. *H* Haifa Bay; *MM* Ma'agan Michael; *AUT* autumn; *SPR* spring. **b** Component loadings can be interpreted as correlation coefficients between the new principal components and the analyzed residues (i.e. the original variables)

Fig. 5 a Muscle tissue residues as analyzed from North Sea fish. Each point represents an individual fish. Each site is represented by a different symbol. **b** Component loadings can be interpreted as correlation coefficients between the new principal components and the analyzed residues (i.e. the original variables)

principal component 1. The total PCBs were calculated as the sum of PCBs 28, 52, 101, 138, 153 and 180, and are presented in Fig. 3. Kruskal-Wallis tests, followed by nonparametric multiple comparisons, show significant differences in the ordering of site residue levels over the four sampling campaigns of MARS 2. While total PCB values at NB are higher than those at the control site (IUI) in spring 1999, NB values were significantly lower than those at FF and IUI in the following sampling campaign, autumn 1999. In both 2000 campaigns NB values were lower than those of fish from FF. The shifting residue levels found in fish tissues from different sites and sampling seasons could result from either fluctuating pollution levels at the three sites, or fish migration between sites. In this respect, it should be noted that all three Red Sea sites are within a few miles of each other.

## Mediterranean Sea

Figure 4 summarizes the Mediterranean analysis (ellipses represent 95% confidence areas for MM samples). The first principal component, which accounts for 44% of the total variation, is highly correlated with levels of PCBs and other organic pollutants, such as DDD, DDE and HCH (Fig. 4b). The two sites are clearly divided on the first principal component, as the different pollution levels in the water of the Haifa Bay area and the control site, MM, are reflected in residue levels in the muscle tissue. The high water pollution levels in the Haifa Bay area resulted in consistently elevated component scores for most fish sampled in the area compared to those of MM. The highest residue levels were recorded in autumn 1999 and spring 2000 (Fig. 4).

**Table 4** Number of molluscs collected from different sampling sites during this study. For site acronyms see text

| Organism | Sea | Site | 1999 | | 2000 | |
|---|---|---|---|---|---|---|
| | | | Spring | Autumn[a] | Spring | Autumn[b] |
| *C. rotha* | Red Sea | FF | 10 | 15 | 19 | 23 |
| | | IUI | 10 | 19 | 14 | 21 |
| *D. trunculus* | Mediterranean Sea | HB | 40 | 40 | 25 | 15(20) |
| | | MM | 20 | 20(20) | 20 | 6(12) |
| *M. edulis* | North Sea | Eider | 9 | 10 | 10 | 10 |
| | | Elbe | 10 | 10 | 10 | 10 |
| | | Helgoland | 10 | 10 | 10 | 10 |

[a] Additional sampling in June at one site (in parentheses)
[b] Additional sampling in December at two sites (in parentheses)

## *North Sea*

The PCA results for the North Sea residue data (Fig. 5) are similar to those of the Red Sea; there is only a weak separation of the sampling sites on either of the two main principal components. Principal component 1 is highly correlated with several PCBs, but both components fail to explain a substantial proportion of the total variance.

## Molluscs

The data in each region (Table 4) were initially screened by a preliminary statistical analysis. Using analysis of variance followed by a posterior test (least significant difference test), the only significant difference was found between sites. No significant differences were found between sampling campaigns within a certain site. Therefore, in what follows we confine ourselves to demonstrating the differences between sites by pooling the values for all seasons within a site.

## *Red Sea*

The values of all measured parameters (MXRtr, MNT, SATOA and epithelium permeability) in *Cellana rotha* were significantly higher at the FF site ($P<0.05$), than at the IUI reference site (Fig. 6a, d, g, j). Similar differences were obtained from the bivalve *Pteria aegyptia*, and are reported elsewhere in this volume. When these data are compared over the entire MARS 1 and MARS 2 campaigns from November 1995 to December 2000, the examined markers clearly separate the reference site from polluted sites (Fig. 7a, b).

## *Mediterranean Sea*

*Donax trunculus* sampled in FRU (Frutarom), situated inside the polluted Haifa Bay, demonstrated significantly higher values ($P<0.05$) in all the measured parameters (i.e. MXRtr, MNT, SATOA and epithelium permeability) than organisms sampled from MM, the reference site (Fig. 6b, e, h, k). Similar differences were observed in

other species of molluscs (*Patella caerulea* and *Brachidontes pharaonis*) sampled from the polluted SH site in the Haifa Bay, and the reference site, CA, and are reported elsewhere in this volume. The significant differences between the polluted and reference sites were consistent throughout the entire MARS project, from November 1995 to December 2000 (Fig. 7c, d).
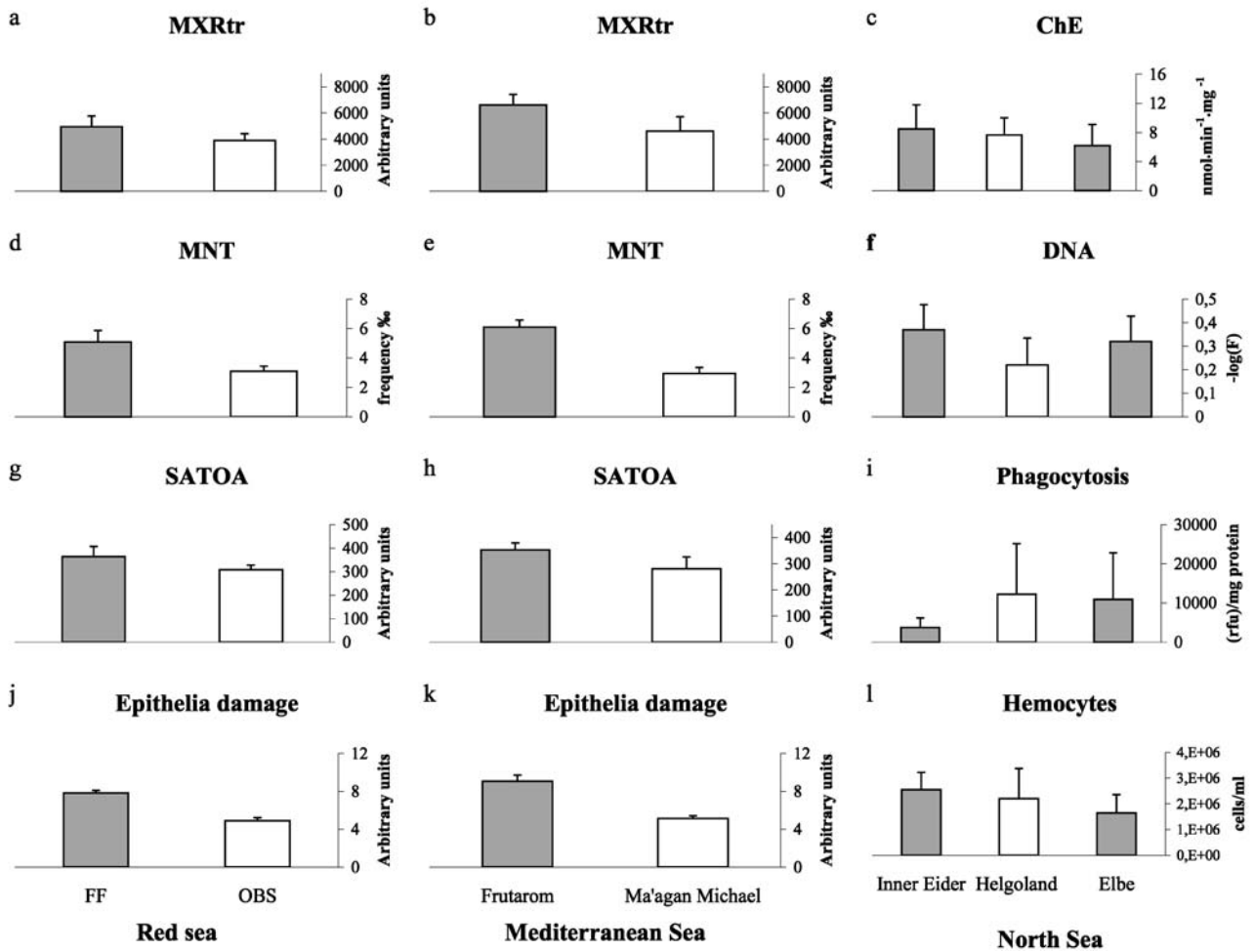
## *North Sea*

The parameters measured from samples of *Mytilus edulis* in the North Sea were unable to significantly differentiate between sites (Fig. 7e, f). In some sampling campaigns significant differences were observed but, in the larger picture, no site was consistently different as was found (e.g. for the reference sites) in the Red and Mediterranean Seas (Fig. 6i, l, 7e, f).

# Host-parasite model: bioindicator response given ecological threshold effects

Theoretical considerations help explain some of the results observed in this wide-ranging study of bioindicators that test pollution levels in marine and coastal waters. We initially focus our argument on host-parasite systems, reviewing and expanding several key concepts introduced in the literature, and discussing their implications in the context of applying bioindicators for environmental assessment. We attempt to consider the implications of ecological threshold effects in regard to assessing the effectiveness of bioindicators. Consider a simplified model describing the dynamics of the direct lifecycle of monoxenous macroparasites (May and Anderson 1978). At time $t$, let $H_t$ represent the numbers of the fish-host population, $P_t$ the numbers of the adult parasites in the host, and $W_t$ the numbers of parasites in their free-living stage:

$$dH/dt = (a - b)H - \alpha P dP/dt$$
$$= \beta WH - (\mu + \alpha + \beta)P - \alpha(k + 1)P^2 \cdot$$
$$\cdot (kH)dW/dt = \lambda P - \gamma W - \beta WH. \qquad (1)$$

**Fig. 6a–l** Data collected during the MARS 2 project in 1999 and 2000 from *Cellana rotha* sampled in the Red Sea (**a**, **d**, **g**, **j**), *Donax trunculus* sampled in the Mediterranean Sea (**b**, **e**, **h**, **k**) and *Mytilus edulis* sampled in the North Sea (**c**, **f**, **i**, **l**). The variables presented are: MXRtr (**a**, **b**); cholinesterase activity in the organism's gill (**c**); MNT (**d**, **e**); DNA unwinding in the organism's gill (**f**); SATOA (**g**, **h**); phagocytosis activity in the organism's haemolymph (**i**); epithelia filtering qualities for anionic marker (**j**, **k**); and number of haemocytes in the organism's haemolymph (**l**)

Here $b$, $\mu$ and $\gamma$ are the natural mortality rates of hosts, free-living parasites and adult parasites respectively, $a$ is the birth rate of the host (fish) population and $\lambda$ is the fecundity of the parasite population. The rate at which free-living parasites locate or come into contact with their hosts is given by $\beta$. The parasite causes mortality to the host at rate $\alpha$ to the host which should be small for the benign parasites in the Israeli sites. It is also assumed that parasites are aggregated in their hosts according to a negative binomial distribution where $k$ is the 'clumpiness' of this aggregation, and $P/H$ is the mean. There is little understanding as to why parasites are generally distributed according to the negative binomial distribution, nevertheless it is a common empirical observation (Anderson and May 1978). The model's nonlinearity makes it difficult to analyse in its present form. Instead, it is convenient and not unrealistic to make the assumption that the timescale of transmission from the parasite's free-living stage to the host is shorter than any other timescale of the model. We can thus set $dW/dt=0$ and assume that $W$

sits close to the equilibrium $W^*=\lambda P/(\gamma+\beta H)$. The three equations thus collapse to:

$$dH/dt = (a-b)H - \alpha P \qquad (2a)$$

$$dP/dt = \lambda\beta PH/(\beta H + \gamma)$$
$$-(\mu+\alpha+\beta)P - \alpha(k+1)P^2/(kH) \qquad (2b)$$

At equilibrium, all rate equations are zero ($dH/dt=dP/dt=0$). The equilibria are thus:

$$H* = \gamma d/[\beta(\lambda-d)], \quad P* = (a-b)/\alpha H* \qquad (3)$$

where $d=\mu+\alpha+b+(a-b)(k+1)/k$. Of course, this equilibrium can only make sense if $H^*$ and $P^*$ are positive, i.e. if $\lambda-d>0$ or, in full:

$$\lambda > \mu + \alpha + \beta + (a-b)(k+1)/k \qquad (4)$$

A linear stability analysis about the equilibrium shows that if both $H^*>0$ and $P^*>0$, the equilibrium is necessarily stable (see Anderson and May 1978). Thus, criterion 4

**Fig. 7a–f** Time series of data collected during the entire MARS project from 1995 to 2000. Presented here are MXRtr and MNT values of *C. rotha* sampled in the Red Sea (**a**, **b**), MXRtr and MNT values of *D. trunculus* sampled from the Mediterranean Sea (**c**, **d**), Cholinesterase Activity in gill and DNA Unwinding in gill of *M. edulis* sampled from the North Sea (**e**, **f**)
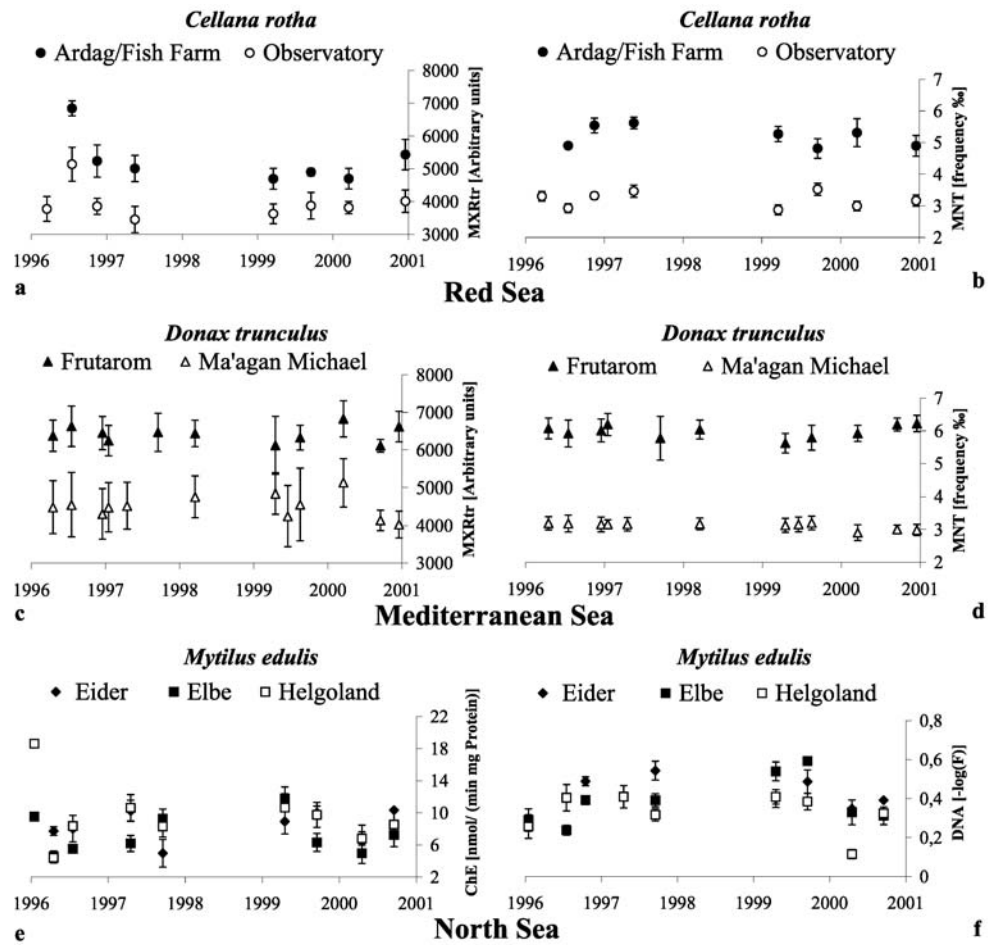


above is the main constraint on whether there is a feasible stable equilibrium for Eqs. 2a, 2b. Figure 8a plots the model host-parasite system (Eq. 1) and shows that a stable equilibrium is reached when condition 4 holds. Figure 8b, c gives examples of the trajectory when condition 4 is not satisfied; neither the parasite nor host reaches a positive equilibrium level and either goes extinct or grows exponentially. Note that although condition 4 was derived from the simplified model 2a, 2b, it makes accurate predictions concerning the simulations performed on the original model 1.

We now investigate the important condition for a parasite to invade a virgin host population. A necessary and sufficient condition is that the parasite's growth rate $(1/P \, dP/dt)$ is positive when the parasite itself is very low in numbers. From Eq. 2b, the condition is thus:

$$\lambda\beta H/(\beta H + \gamma) - \mu + \alpha + \beta > 0 \tag{5}$$

where the term $P/H$ in Eq. 2b has been neglected since it is assumed that there are only a small number of monoxenous parasites attempting to invade. This motivates defining

$$R_0(\text{Monoxenous}) = \lambda\beta H/[(\beta H + \gamma)(\mu + \alpha + \beta)] \tag{6}$$

Obviously then, from Eq. 5, the parasite can only invade a population if

$$R_0(\text{Monoxenous}) > 1. \tag{7}$$

In more general notation, following Dobson (1989), we can write

$$R_0(\text{Monoxenous}) = T_1/(M_1 M_2) \tag{8}$$

where $T_1 = \lambda\beta H$ is the total parasite transmission rate; $M_1 = (\mu + \alpha + b)$ is the host's parasite's mortality rate; $1/M_1$ is the life expectancy of the host's parasite. $M_2 = (\beta H + \gamma)$ is the per capita mortality rate of the free-living parasites; $1/M_2$ is the life expectancy of the free-living parasite.

That is, $R_0$ can be considered as the ratio of the parasite's transmission rate to its effective mortality rate. If the ratio is larger than unity, transmission dominates and the parasite can invade.

$R_0$ is a crucial parameter in host-parasite systems. It can also be interpreted as the parasite's reproductive rate and is equivalent to the average number of offspring per parasite that have the potential to infect a host. In practice, a parasite cannot invade a population unless, on average, it can reproduce itself at least once and live to infect at least one host (Anderson and May 1978). This translates to the condition $R_0(\text{Monoxenous}) > 1$.

As May and Anderson (1978) noted, when $H$ itself is very large, $\lambda\beta H/(\beta H + \gamma) \sim \lambda$, and the expression for $R_0$ (Eq. 6) simplifies to:
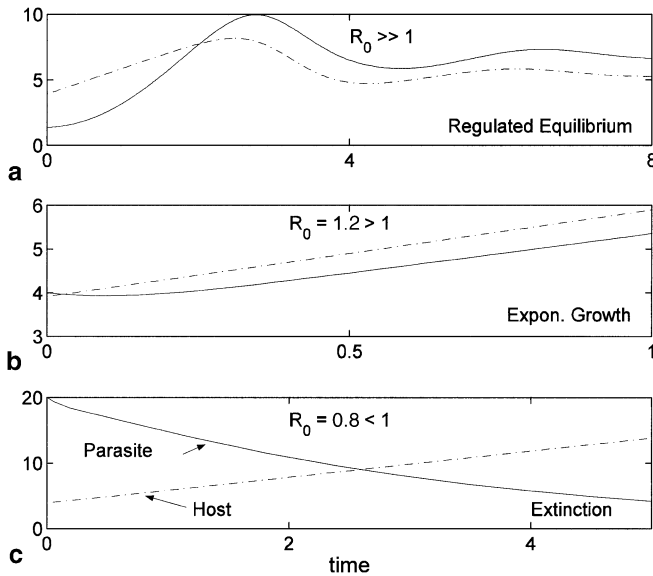
**Fig. 8a–c** Simulation of host-parasite model Eq. 1. **a** A stable equilibrium is reached when $\lambda=5.35$ is large enough such that condition 4 holds. Parameters: $\mu=0.1$; $a=3$; $b=1$; $k=2$, $\alpha=0.5$; $\beta=0.1$; $\gamma=15$ so that $R_0=7.6$. **b** When $\lambda=1.92$ (so that condition 4 does not hold) then $R_0=1.2>1$. The parasite invades but the host population grows and remains unregulated. **c** When $\lambda=1.28$ (so that condition 4 does not hold) then $R_0=0.8<1$. The parasite fails to invade at all. Parameters: $\mu=0.1$; $a=3$; $b=1$; $k=2$, $\alpha=0.5$; $\beta=0.1$; $\gamma=1$

$$R_o(\text{Monoxenous}) = \lambda/[\mu + \alpha + \beta] \qquad (9)$$

Figure 8b provides a simulation from model 1 where $R_0$(Monoxenous)=1.2 (using Eq. 9). Since $R_0>1$, the parasite invades. Fig. 8c displays the model populations when $R_0$(Monoxenous)=0.8. Since $R_0<1$, the introduced parasite fails to invade the host population. Again, note that although condition 9 was derived from the simplified models 2a, 2b, it makes accurate predictions concerning the original model 1.

In summary, two threshold conditions may be identified:

1. Criterion 4 states that the condition for the host parasite system to be maintained at a stable equilibrium is $\lambda>\mu+\alpha+b+(a-b)(k+1)/k$.
2. Criterion 9 states that the condition for the parasite to invade a host population is that the reproductive rate $R_0>1$ (or, equivalently, $\lambda>\mu+\alpha+b$).

Note that, in the range $\mu+\alpha+b<\lambda<\mu+\alpha+b+(a-b)(k+1)/k$, the host-parasite system is unstable but the parasite is nevertheless able to invade. In such a situation, the parasite is unable to regulate the host population, but its transmission characteristics are nevertheless high. This might correspond to the parasites in the Red, Mediterranean and North Seas which are reasonably benign (i.e. small $\alpha$) with limited capacity to cause damage to their host population.

The above analysis summarizes the case of monoxenous parasites and is presented to give basic insights for

this simpler case. What is of more importance for us here is that the same exercise can be repeated for a model that describes heteroxenous parasites that utilize one or more intermediate hosts. It can be shown (e.g. Dobson 1989) that, for parasites with multiple life stages, in analogy to Eq. 8, the arguments extend naturally so that:

$$R_o(\text{Heteroxenous}) = T_1T_2/(M_1M_2M_3M_4) \qquad (10)$$

Here $T_1$, $T_2$ and $M_1$, $M_2$, $M_3$, $M_4$ represent the transmission rates and mortality rates of the parasite in each stage of its lifecycle and the notation extends that used for the monoxenous case above. Heteroxenous parasites are able to invade a virgin host population if:

$$R_o(\text{Hetereoxenous}) > 1 \qquad (11)$$

These theoretical considerations lead us to make two important observations. Firstly, we see that heteroxenous parasites are likely to be more fragile than monoxenous parasites. This is because, all things being relatively equal, the conditions 10, 11 required to maintain $R_0$(Heteroxenous)>1 are far more stringent and demanding than the conditions 7, 8 to maintain $R_0$(Monoxenous)>1. Furthermore, with the introduction of any form of variability, be it environmental or biological, one might expect that there is far more scope for pushing $R_0$(Heteroxenous) below unity than $R_0$(Monoxenous). In this respect, the model indicates the obvious: that the more complex lifecycle of heteroxenous parasites makes them more fragile than the more 'robust' monoxenous parasites. This, in fact, is the basic assumption behind the $h/m$ index advocated in Diamant et al. (1999).

Secondly, the model points out the threshold effect in which neither heteroxenous nor monoxenous parasites can establish themselves unless their basic reproductive rates $R_0$ exceed unity. This threshold condition has the important implication that, should the effects of pollution or environmental stress be such as to modify transmission rates (see e.g. Lafferty and Kuris 1999) to a point that $R_0$ is reduced slightly below unity, the entire parasite population will disappear (Fig. 8c). Indeed, in the datasets analysed in the Mediterranean, heteroxenous parasites were almost absent. This might explain why the $h/m$ index is successful at indicating extreme levels of pollution, but is less sensitive otherwise.

## Discussion

We begin by discussing the performance of the parasitological indices over all three seas. Unfortunately, the Israeli data have not been made available for publication here and we are only able to report qualitative trends (see Stone et al. 2002 for full analysis). Figure 9a–c plots the indices $h/m$, $S_h$ and $H_h'$ for each campaign/region, and sketches how we see the 'big picture' based on our present conceptualization. Nevertheless, they mirror the main features and provide useful comparative insights. Dzikowski et al. (2003, this issue) and Stone et al. (2002)
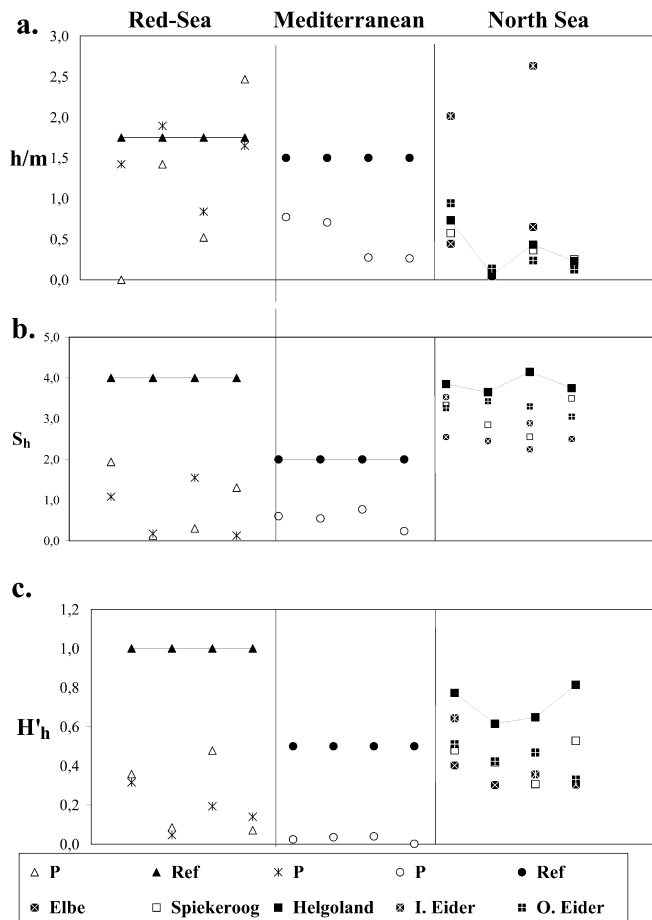
**Fig. 9** A comparison that characterizes our current conceptualization of **a** mean $h/m$, **b** $S_h$ and **c** $H'$ values for sampling sites of all three seas for polluted (P) versus reference (R) sites. Unlike the North Sea (authentic data), the graphs of Israeli sites are qualitative trends (see text), as reported in Stone et al. 2002. Each figure is separated into three sections representing (from left to right) the Red Sea, the Mediterranean and the North Sea. Within each section there are four columns, which stand for the four sampling campaigns (left to right: spring 1999, autumn 1999, spring 2000, autumn 2000; cf. Fig. 1)

should be consulted for full details of the Israeli sites. The graphs representing the North Sea trends, however, are generated from the authentic data. In all parts, the cleaner reference sites are indicated by the filled marker symbols. Figure 9b, c shows that the species richness ($S_h$) and community diversity indices ($H'_h$) have their highest values at the cleaner reference sites (filled symbols). This is easily visualized because the filled symbols sit at the highest points of the graph for all seas and sampling dates, and the reference sites appear as filled symbols that form a 'ceiling' over the graphs. Hence, Fig. 9b, c suggests that these parasitological indices are useful in distinguishing the cleaner reference sites. In contrast, Fig. 9a shows that the $h/m$ index is unsuccessful in the North Sea and probably in the Red Sea.

The $h/m$ index does not appear to be a sensitive early warning indicator for habitat degradation as previous

MARS 1 results suggested for both the North Sea (as reported here) and the Red Sea [Dzikowski et al. 2003 (this issue), see also Stone et al. (2002)]. In the case of the Mediterranean, it is likely that $h/m$ is a good indicator of high pollution gradients [Stone et al. 2002; Dzikowski et al. 2003 (this issue)]. Pollution gradients between Mediterranean sites are higher than those in the Red Sea, where the polluted sites are considered to be only 'slightly contaminated' (Herut et al. 1999). It is possible that, for detection of low pollution gradients, a more sensitive indicator is needed. It may be that the $h/m$ index is best capable of detecting coarse trends that arise from, say, ecological thresholds intrinsic to host-parasite systems, but is less able to accurately pick out smooth monotonic trends.

In contrast, both $S_h$ and $H'_h$ proved to be more sensitive than $h/m$ at following pollution gradients in the North Sea, and also in the Israeli sites [Stone et al. 2002; Dzikowski et al. 2003 (this issue)]. Reference sites showed consistently elevated values of both parameters in comparison to 'polluted' sites. The apparent advantage of using $H'_h$ may stem from the fact that its calculation is based on macroparasite species only. In contrast, the index $S_h$ is simply the total number of heteroxenous species, both macroparasites and microparasites. It has been shown before that microparasites are of limited comparative value, and could even mask differences reflected by macroparasites (Diamant et al. 1999). However, the advantage of using $H'_h$ may also be because it takes into account both species richness and the distribution of individuals among species. That is, it is often a better guide of community structure.

The modelling analysis revealed another aspect that needs consideration when using indices of community structure for environmental assessment. The dynamics of host-parasite communities (and many other ecological systems) are governed by delicate nonlinear ecological thresholds. One important threshold parameter is the reproductive growth rate, $R_0$ (Eqs. 8, 10), a parameter which effectively swallows all of the main factors that lead to the transmission of the parasite into the host community. If levels of pollution are able to push $R_0$ so that it is sub-threshold ($R_0 < 1$), only to a very minor degree, the parasite species will nevertheless rapidly disappear. Since each species is characterized by its own particular value of $R_0$, as pollution loads increase parasite populations could disappear one by one in a staggered manner governed by the individual species particular threshold levels. Or if the parasites have similar $R_0$s, the species could all become extinct almost simultaneously. Either way, under such conditions community indices cannot be expected to behave in a linear fashion in the presence of ecological thresholds. This helps explain some of the discrepancies seen in the MARS parasite analyses. In particular, the population and community indices seemed to be most sensitive when pollution gradients were relatively high (e.g. in the Mediterranean) where threshold effects are more likely to kick in. When the pollution gradient between two sites was intermediate,

the indices were often unable to differentiate the sites from one another (e.g. FF and NB in the Red Sea; sites in the North Sea). Again, this could be expected if the pollution indices were less responsive to intermediate pollution levels which are unable to trigger threshold effects.

We now discuss bioindicators that are not specifically based at the population or community level. Moving, then, to the results concerning fish tissue residues, we first note that the analysis failed to yield a separation of sites as clear cut as the parasitological results just discussed. The ambiguous interpretation of the PCA plot of residue levels in the Red Sea is a result of the seasonal changes (Fig. 3) in levels of the main pollutants (see Fig. 2b). Seasonal changes in fish tissue residue levels may have their origins in either changing pollution levels, or seasonal migration between Red Sea sampling sites (which are only a few miles away from each other). However, these seasonal changes were not reflected in the other parasitological analyses.

Mediterranean sites were clearly divided by the PCA plot (Fig. 4a), where fish from the Haifa Bay area showed elevated levels of muscle tissue residues. On the other hand, the North Sea results plotted in Fig. 5 are far less conclusive, and fail to show separation of the sampling sites. In conclusion, while muscle tissue residue data are useful only when pollution gradients are rather high (as in the case of Mediterranean sites), indices of changing parasite community structure (e.g. $S_h$ and $H_h'$) appear to be more sensitive indicators for pollution levels of the marine environment. The markers used in studying the mollusc data show two different patterns. In the Red and Mediterranean Seas a clear and regular separation is observed between the polluted and reference sites whereas, in the North Sea, the polluted and reference sites could not be distinguished by the measured parameters (Fig. 7). Two explanations may settle this difference in patterns observed. First, it is possible that the nature of the pollution combined with the special oceanographic settings governing the Elbe estuary system, which are different to those in the Red and Mediterranean Seas, contribute significantly to the different patterns discovered in this study. Second, the variables measured in the North Sea were different to those measured in the Red and Mediterranean Seas. It is therefore possible that, since the variables measured in the Red and Mediterranean Seas are specifically designed to assess both the activity and integrity of the first lines of defence available to these organisms, they would be able to detect even the smallest difference in pollution loads as manifested in the organism's physiology, enabling us to detect cryptic early responses and pre-pathological alterations before they can be noticed by other means.

The findings of this comparative study make clear that the MARS bioindicators were to a large extent successful. Although simplistic, and possibly even crude, some of the standard ecological indices proved very effective in testing hypotheses regarding changes in community structure. Their effectiveness was especially enhanced through the use of multivariate statistical tools such as Principal Component Analysis. The other bioindicators, such as those based on biochemical tests designed to assess an organism's defence mechanisms and measure damage to the organisms pathology, were also found to be sensitive indicators of pollution.

Finally, we mention that the analysis of the MARS 2 bioindicators reconfirmed the initial environmental gradients obtained from the earlier MARS 1 analyses (Stone et al. 1998) and gives further support to the hypothesis that the reference sites chosen were all indeed the cleaner of the regional sites. Assignment of reference sites was a controversial issue that was queried many times throughout the MARS project. Our results do not contradict the initial assignment, although it was difficult to discriminate an environmental gradient in the North Sea.

## References

Anderson RM, May RM (1978) Regulation and stability of host-parasite population interactions: I: Regulatory processes. J Anim Ecol 47:219–247

Bresler V, Bissinger V, Abelson A, Dizer H, Sturm A, Kratke R, Fishelson L, Hansen PD (1999) Marine molluscs and fish as biomarkers of pollution stress in littoral regions of the Red Sea, Mediterranean Sea and North Sea. Helgol Mar Res 53:219–243

Broeg K, Zander S, Diamant A, Körting W, Krüner G, Paperna I, von Westernhagen H (1999) The use of fish metabolic, pathological and parasitological indices in pollution monitoring. I. North Sea. Helgol Mar Res 53:171–194

Büther H (1990) Spatial and temporal trends in organochlorine contamination of dab (*Limanda limanda*) and flounder (*Platichthys flesus*) in the North Sea. Arch Fisch Wiss 40:133–152

Diamant A, von Westernhagen H (1999) Biological indicators for the detection of natural and man-made changes in coastal waters—an introduction to the MARS project. Helgol Mar Res 53:148–153

Diamant A, Banet A, Paperna I, von Westernhagen H, Broeg K, Krüner G, Koerting W, Zander S (1999) The use of fish metabolic, pathological and parasitological indices in pollution monitoring. II. The Red Sea and Mediterranean. Helgol Mar Res 53:195–208

Dobson AP (1989) The population biology of parasitic helminths in animal populations. In: Levin SA, Hallam TG, Gross LJ (eds) Applied mathematical ecology. Springer, Berlin Heidelberg New York, pp 145–175

Dzikowski R, Paperna I, Diamant A (2003) Use of fish parasitological species richness indices in analyzing anthropogenically impacted coastal marine systems. Helgol Mar Res (in press)

Herut B, Kress N, Shefer E, Hornung H (1999) Trace element levels in molluscs from clean and polluted coastal marine sites in the Mediterranean, Red and North Seas. Helgol Mar Res 53:154–162

Kress N, Herut B, Shefer E, Hornung H (1999) Trace element levels in fish from clean and polluted coastal marine sites in the

284

Mediterranean Sea, Red Sea and North Sea. Helgol Mar Res 53:163–170

Lafferty KD, Kuris AM (1999) How environmental stress affects the impacts of parasites. Limnol Oceanogr 44:925–931

Legendre P, Legendre L (1998) Numerical ecology. Developments in environmental modelling 20. Elsevier, Amsterdam

May RM, Anderson RM (1978) Regulation and stability of host-parasite population interactions: II Destabilizing processes. J Anim Ecol 47:249–267

Poulin R (1997) Species richness of parasite assemblages: evolution and patterns. Annu Rev Ecol Syst 28:341–58

Schmolke SR, Broeg K, Zander S, Bissinger V, Handen PD, Kress N, Herut B, Jantzen E, Krüner G, Sturm A, Körting W, von Westernhagen H (1999) Multivariate statistical approach to the temporal and spatial patterns of selected bioindicators observed in the North Sea during the years 1995–1997. Helgol Mar Res 53:257–266

Stone L, Bhasin H, Gefen E (1998) Biological indicators of natural and man-made changes in marine and coastal waters. MARS I statistical analysis: Israeli report

Stone L, Gefen E, Rosenfeld M (2002) Biological indicators of natural and man-made changes in marine and coastal waters. MARS II statistical analysis: Israeli report