Helgol Mar Res (1999) 53:195–208

ORIGINAL ARTICLE

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The use of fish metabolic, pathological and parasitological indices in pollution monitoring

II. The Red Sea and Mediterranean

Received: 25 February 1999 / Received in revised form: 15 May 1999 / Accepted: 20 May 1999

Abstract The complex interactions between parasites, hosts and the environment are influenced by the stability of the ecosystem. Heteroxenous parasites, with complex, multiple-host life cycles, can persist only in habitats where the full range of their required hosts are present. Conversely, in impoverished environments such as those impacted by environmental stress, monoxenous species that have simple, single-host life cycles are likely to predominate. In the present study, we analyzed the ratio between heteroxenous and monoxenous (H/M) parasites as well as parasite species richness (S_H/S_M) and species diversity in rabbitfish (*Siganus rivulatus*) collected from several sites in the Red Sea. The rabbitfish is a Suez Canal immigrant, well established in the eastern Mediterranean, and fish were also collected from a site on the Mediterranean coast of Israel. Separate treatment of the micro- and macroparasite components of the rabbitfish parasite communities in the Red Sea suggested that macroparasites only – monogenea and gut parasites – were better indicators than the parasite community as a whole. Quantification of macroparasites is accurate, saves time and effort, produces more accurate data and better differentiates between sites. Higher H/M ratios and S_H/S_M ratios were found in the rabbitfish collected at the ecologically stable habitat of the coral reef compared to rabbitfish from sandy habitat or mariculture-impacted sandy habitat. The results of the study emphasized the negative impacts of cage mariculture on the environment. The rabbitfish collected near the mariculture farms supported the poorest and least diverse parasite communities of all sampled sites, with virtual depletion of heteroxenous species, and even reduction of gill monogenean infections on the hosts. When results from the Mediterranean sites were compared with those of the Red Sea, the data showed full representation of monoxenous parasites (all but one of Red Sea origin), while heteroxenous species were completely absent. We may therefore regard the Mediterranean as a simulation model for a severely environmentally deteriorated, impoverished habitat, in which all or part of the intermediate host species have been depleted, enabling survival of the monoxenous parasite species only. Parasitological investigations were supplemented by testing the activity of cytochrome P 450-dependent mono-oxygenase EROD as a measure of exposure, and lysosomal stability as a measure of toxic effect in the liver of rabbitfish. The results underline the parasitological findings, showing that fish caught at the impacted sandy beach location in the Red Sea have significantly higher EROD activity and a decreased membrane stability compared with animals from the coral reef. In comparison, EROD activity values in rabbitfish from the Mediterranean Sea were double, while lysosomal membrane stability was half that measured at the most impacted Red Sea location.

Key words Pollution monitoring · Red Sea · Mediterranean · Heteroxenous and monoxenous parasites · Rabbitfish (*Siganus rivulatus*) · EROD · Lysosome stability

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Introduction

The assessment of ecosystem health in terms of applied ecological criteria is becoming increasingly important in the attempt to define the human impact beyond the simple measurement of anthropogenic pollutants in the envi-

ronment. The health of an ecological system can be measured by the health of its individual components expressed as biochemical parameters, or by its resilience to disturbance and its level of biodiversity, employing various ecological parameters. Venturing from chemical to biological monitoring towards the application of biochemical parameters in biological effects monitoring has marked a milestone in the approach to environmental quality assessment. In addition, the consequent application of a still more holistic approach will inevitably lead from the use of established biochemical and biological parameters (such as EROD activity) to a better understanding of human impact effects through the elucidation of ecological processes.

The impact of human activities should be visible at all ecological levels and concomitantly with the conservative biomarkers such as used in chemical and biological monitoring, and even in biological effects monitoring at the subcellular level, effects should be detectable on higher organizational levels to the extent that whole ecosystems, or changes therein, may be used as a biomarker for anthropogenic impact. In the present study, host-specific parasite communities are considered as the ecosystem under question and their reactions are compared with conservative and histochemical biomarkers.

Parasites, due to the great diversity of their life history strategies, appear to be extremely sensitive bio-indicators in the ecosystem (Overstreet 1997; Paperna 1997). Heteroxenous parasites, with complex, multiple-host life cycles, can apparently persist only in habitats where the full range of required hosts persist – the implication of this assumption is that transmission can be constrained in impoverished environments with low diversity (Bartoli and Boudouresque 1997). In such environments, monoxenous species that have simple developmental strategies restricted to a single host will predominate (D'Amelio and Gerasi 1997). The basic hypothesis in this study is that the ratio between the heteroxenous and monoxenous parasite community indices – or H/M (numerically or as population parameters) – is indicative of the biotic stability of the ecosystem. In the present study, this evaluation was employed parallel to an analysis of the diversity parameters of the parasite community as a whole.

In an impacted habitat, the analysis of parasitic infections in fishes is dependent on our ability to detect natural variations in the infection patterns, such as those caused by, say, seasonal changes. Factors that can complicate results from fish parasite studies were noted by Overstreet (1997) who pointed out that normal seasonal fluctuations of a parasite or atypical extreme environmental conditions such as extreme tides, gales, floods, storms or long periods of draught may complicate interpretation of data. The interaction between parasite, host and the environment is complex and dynamic. Parasite levels of infection are subject to naturally occurring, nonrandom spatial and temporal variations, as recently discussed in detail by Kennedy (1997) and McVicar (1997), who listed additional critical areas of variation on top of natural variability: biases arising from variability in sampling, detection, identification, data recording and analysis.

To test whether the results obtained from the parasitological data were due to the influence of environmental contamination, two biological effect monitoring parameters at the molecular and subcellular level of biological organization were measured in the same rabbitfish of the Mediterranean Sea and the Red Sea. The activity of the CYP1A-dependent mono-oxygenase EROD in fish liver was used as a biomarker of exposure of specific lipophilic, planar compounds like polyaromatic hydrocarbons (PAHs) and polychlorinated bi-phenyls (PCBs), while the stability of hepatocyte lysosomal membranes was tested as a biomarker for nonspecific toxic response. Both parameters are recommended by the ICES Advisory Committee on the Marine Environment (ACME) for application in biological effect monitoring and have been tested extensively in the North Sea for their utility (Anonymous 1996).

Materials and methods

The model fish for our study was the rabbitfish (*Siganus rivulatus*). It is abundant in the Gulf of Eilat, the Red Sea, and has also migrated via the Suez Canal into the east Mediterranean Sea (Ben Tuvia 1953). Rabbitfish occupy a variety of coastal habitats and their associated parasite community components are relatively well known (Diamant 1985, 1989a,b, Diamant and Paperna 1986). In the northern Gulf of Eilat, samples were regularly obtained during 1995–1998 from five stations representing a diversity of habitats (Fig. 1): site 1, a coastal coral reef at the Underwater Observatory and Inter-University Institute (OBS-IUI); site 2, a sandy beach (north beach, NB); site 3, a mariculture site on the sandy northern beach (AR); site 4, Eilat harbor (EH), site 5, a coral reef site at the oil terminal (DK). The latter two sites provided relatively small numbers of fish, and were considered in the analyses only in some cases as additional groups for comparison, see map (Fig. 2) in the introductory paper to the MARS 1 project (this volume). In the Mediterranean most fish were obtained from Ashdod harbor, near a mariculture cage fish farm, and a few additional fish were obtained from two other sites, at Atlit and in Haifa Bay (Kishon Harbor).

For the application of the biochemical and histochemical tests, parallel to parasitology on the same individual, fish were caught in November 1995 in the Mediterranean Sea (Ashdod) and in the Red Sea (NB). The Red Sea location NB was also sampled in April and October 1997 to test whether seasonal differences occur in a temperate sea. The Mediterranean Sea location Ashdod was sampled in April 1996 again for the same reason. Site-specific differences in the Red Sea were tested in April 1997, when fish were caught at three Red Sea locations: sandy beach (NB), coral reef (OBS-IUI) and Eilat harbour (EH).

Parasitological data processing

Parasitological counts included both microparasites, i.e. protozoa and Myxosporea, which are not readily countable, and macroparasites, i.e. monogenea and gut helminths, which can be accurately enumerated. Parasite identification was carried out based on past taxonomic analysis (Diamant 1985). The captured fish were transferred live to the respective laboratories and held in aerated flow-through seawater tanks until examination (always within 72 h) or placed on ice and brought to the laboratory and processed immediately in a similar manner. The fish were then killed and examined for presence of both external and internal parasites. Freshly pre-

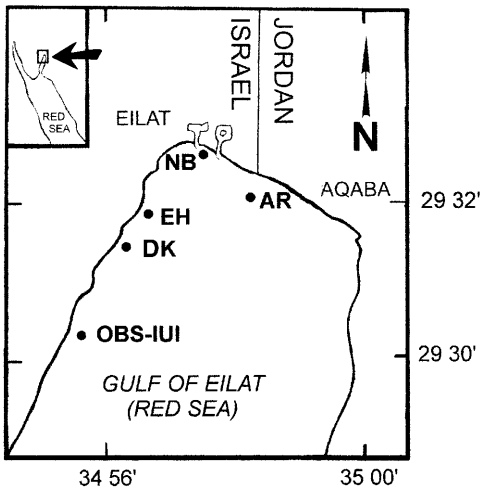


Fig 1 Sampling sites at the Red Sea location. *OBS-IUI* A coastal reef site; *DK* a coral reef site at oil terminal; *EH* Eilat Harbor; *NB* a north beach; *AR* a mariculture site

pared skin, gill, intestinal mucous, urinary and gall bladder smears were studied under microscope and, where relevant, air dried, fixed and stained. Adult helminths were usually identified unfixed under a cover slip. Representative specimens were fixed and preserved by pressing between glass slides in ethyl alcohol 70%. Where required, specimens were stained with hematoxylin-eosin or basic fuchsin.

In the Mediterranean, the fish were placed on ice and brought to the laboratory (Faculty of Agriculture, Rehovot, Israel) where they were processed immediately in a similar manner. Sample size for the parasite diversity analyses needed to be sufficiently large to ensure detection of less abundant parasites (minimum prevalence level of 5%, at a confidence level of 95%). We initially estimated this to be in the range of 30–50 individuals per homogeneous fish sample (per site, season, host size, etc.), and this was tested by analyzing the relationship between host sample size and parasite species richness per site. Taking into consideration dynamic qualitative changes occurring in the parasite assemblages with fish growth, fish were divided into three size groups according to their fork length (size group 1, under 150 mm; size group 2, 150–220 mm; size group 3, over 220 mm) and the relationship between fish size and parasitological parameters was tested.

We attempted to overcome seasonal bias by combining year-round and year-by-year data, in order to even out seasonal variation. The evaluation of parasite distribution patterns was performed by analyzing the population parameters of prevalence, abundance and importance (=relative abundance) (Janion 1968; Pence and Eason 1980) and the ecological indices of richness, diversity and equitability (Shannon and Weaver 1949; Pielou 1978) for the entire parasite assemblage as well as separately for the monoxenous and heteroxenous species. The analyses were carried out after ensuring that there were no year-to-year variations in parasite prevalence.

The ratio between heteroxenous and monoxenous species focused on the following parameters:

1. Species richness (S), the number of parasite species present in a given number fish sampled from a population.
2. Prevalence of infected fish (Pi), the relative number of fish infected by individual parasites belonging to species “i” in the fish population.
3. H/M ratio, the ratio between the sums of heteroxenous and monoxenous parasite individuals per host fish.
4. S_H/S_M ratio, the ratio between heteroxenous and monoxenous parasite species richness found on the host fish.

When plotting the number of host individuals examined vs. the number of parasite species recovered, an exponential function emerges in which the asymptote represents that level at which further additions of hosts to the sample will not yield any new information (parasite species), and thus depicts the “true” species richness (Walther et al. 1995). A basic assumption of the model is that it portrays the species richness of a given host or habitat, which is dependent on the sample size. D’Amelio and Gerasi (1997) employed this model to determine the host sample size required to reliably characterize a population – the number of fish corresponding to the point where the curve approaches the plateau level. In the proposed model, parasite species richness is described by an asymptotic curve of the type: $S(x)=a(1-e^{-bx})/b$, where coefficient a represents the increase in parasite species at the beginning of the sampling process and b determines the asymptote. Coefficient a is strongly influenced by the parasite species richness and prevalence of infection with these parasites. The “true” species richness (R) equals the proportion of these two coefficients: R=a/b. The host sample size required to determine the “true” species richness (C) is readily determined from the asymptote. In the present study, exponential species accumulation curves were fitted separately to the heteroxenous and monoxenous parasite species, considering species with a prevalence of >3% at a 90% confidence limit.

Biochemical and histochemical tests

Rabbitfish were killed by a blow to the head and the liver was removed immediately, without damaging the gall bladder, and weighed. A central part of the liver (about 1 cm^3) was snap frozen in liquid nitrogen and stored in a freezer (at –80°C) until further processing for the histochemical test of lysosomal membrane stability. The remainder of the liver was similarly frozen and maintained for the biochemical investigation of EROD activity.

Lysosomal stability

Lysosomal stability was measured as described elsewhere in this volume (Broeg et al; this Volume), according to the methods of Köhler (1991). Because the activity of the lysosomal enzyme n-acetylhexosamidase, measured by Köhler in North Sea flounder (a carnivorous species), was very low in the herbivorous rabbitfish, we had to modify the procedure. Instead of this enzyme we used acid phosphatase for the determination of destabilization time in *Siganus rivulatus* hepatocyte lysosomes. The point of destabilization was regarded as the moment of membrane breakdown following acid labilization. This point in time is demonstrated by the most intensive density of the formazan product of an azo-coupled enzyme/substrate complex and determined by computer-assisted image analysis according to Chieco et al. (1994).

EROD activity

EROD activity was measured according to Krüner et al. (1996), as described elsewhere in this volume (Broeg et al., this Volume).

Statistics

Data of parasitic infections in the sampled fish were recorded by number of specimens of each parasite species per individual fish and later compiled and sorted according to size class group, date and location using Microsoft Excel. Statistical testing was carried out using Jump Statistical Package. Analyses of the biochemical and histochemical tests were performed with Microsoft Statistica. Site-specific and seasonal differences for more than two locations were tested with the nonparametric Kruskal–Wallis test, followed by the Nemenji post hoc test. For the differences between two sampling locations and seasonal differences at the Mediterranean Sea location Ashdod (two seasons were compared), the Mann–Whitney *U*-test was applied. Differences were considered statistically significant at $P<0.05$.

Table 1 The parasites found on the rabbitfish *Siganus rivulatus* in the Red Sea and Mediterranean Sea

Taxonomic group	Parasite species	Development[a]	Target organ/tissue	Red Sea[b] prevalence (%)	Mediterranean[c] prevalence (%)
Microspora[d]	*Nosema ceratomyxae*	M	Gall bladder	38.8	9.2
Myxosporea	*Ceratomyxa* sp.	H[e]	Gall bladder	92.9	21.5
	Zschokkella icterica	H[e]	Gall bladder/liver	3.4	–
	Ortholinea sp.	H[e]	Urinary bladder	10.2	Rare (<0.1%)
Diplomonadinae	*Octomitus* sp.	M	Hindgut	96.6	43.8
Amoebida	*Entamoeba* sp.	M	Hindgut	74.3	29.6
Ciliophora	*Balantidium sigani*	M	Hindgut	35.9	19.7
	Trichodina sp.	M	Anus	26.4	–
	Cryptocaryon irritans	M	Gills and Skin	–	8.6
Monogenea	*Pseudohaliotrematoides polymorphus eilaticus*	M	Gills	0.9	–
	Pseudohaliotrematoides polymorphus suezicus	M	Gills	41.7	–
	Pseudohaliotrematoides polymorphus indicus	M	Gills	Rare (<0.1%)	–
	Pseudohaliotrematoides polymorphus "nagatyi"	M	Gills	30.3	4.0
	Pseudohaliotrema plectocirra	M	Gills	33.1	–
	Polylabris sigani	M	Gills	20.1	–
Digenea	*Hexangium sigani*	H	Gut	34.6	–
	Gyliauchen volubilis	H	Hindgut	9.3	–
	Opisthogonoporoides hanumanthai ("sp.1")	H	Gut	29.9	–
	Opisthogonoporoides ("sp. 2")	H	Gut	0.8	–
	Prosorchis sp.	H	Oesophagus	0.1	–
	Metacercaria	H	Muscle	2.8	–
Nematoda	*Cucullanus sigani*	H	Hindgut	16.9	–
	Procamallanus elatensis	H	Anterior gut	12.4	–
Acanthocephala	*Sclerocolum rubrimaris*	H	Anterior gut	39.2	–

[a] H Heteroxenous; M monoxenous
[b] *n*=563 host fish
[c] *n*=152 host fish
[d] Hyperparasite of the myxosporean *Ceratomyxa* sp.
[e] Status of this Myxosporean as a heteroxenous parasite species is uncertain

Table 2 Details of Red Sea rabbitfish samples (>150 mm fork length) per season of capture and per sampling site; *numbers in brackets* are total samples, including fish under 150 mm fork length. *AR* Mariculture cage site; *NB* north beach site; *EH* Eilat Harbor; *DK* oil terminal site; *OBS-IUI* Underwater Observatory and Inter-University Institute

Sampling site	Spring	Summer	Autumn	Winter	Total
AR	29 (29)	4 (14)	32 (46)	37 (41)	102 (130)
NB	46 (51)	50 (58)	28 (72)	25 (55)	149 (236)
EH	32 (39)	8 (8)	0 (0)	0 (0)	40 (47)
DK	3 (3)	2 (2)	0 (0)	12 (12)	17 (17)
OBS-IUI	48 (48)	34 (34)	28 (28)	23 (23)	133 (133)
Total	158 (170)	98 (116)	88 (146)	97 (131)	441 (563)

Results

Parasitology

In the Red Sea, 563 rabbitfish (*Siganus rivulatus*) were collected, yielding a total of 22 parasite species. In the Mediterranean samples, 152 rabbitfish yielded a total of 9 parasite species. Details on the parasites and their prevalences are given in Table 1. In the Mediterranean, a total of 152 rabbitfish (*S. rivulatus*) were examined, of which 146 were from Ashdod, 2 from Atlit and 4 from Haifa Bay. In the Red Sea details of the rabbitfish samples per site and season are presented in Table 2.

The distribution of host size in the samples was heterogeneous: different sites supported host populations with differing combinations of size groups. The fish at OBS-IUI were all larger than 150 mm (i.e; of length groups 2 and 3). Conversely, approximately 35% of the fish sampled at NB were <150 mm and thus belonged to size group 1.

Parasite accumulation is known to increase with the time of host exposure to infection, and therefore the age (length) of the fish is an important factor when parasite assemblages of fish individuals are compared. The parasite species richness in the combined Red Sea rabbitfish samples examined is shown in Fig. 2. The data demonstrate an increase in value with fish length.

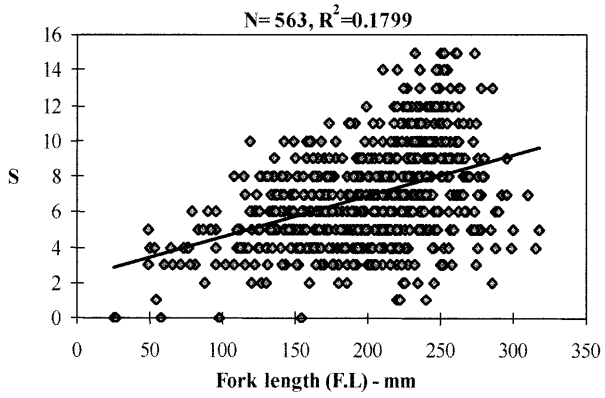


Fig. 2 Relationship between number of parasite species recovered (*S*), or species richness, and rabbitfish *Siganus rivulatus* size (*FL*) in Red Sea samples. R^2 value is significant for this large sample size (*z* distribution)

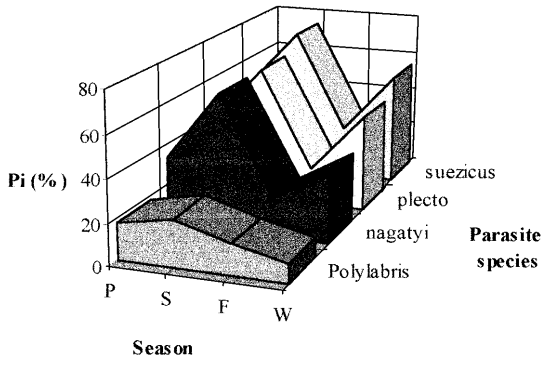


Fig. 4 Gill monogenean (all monoxenous species) prevalence (*Pr*) in the Red Sea *S. rivulatus* samples per season. *P* Spring; *S* summer; *F* fall; *W* winter; *Polylabris Polylabris sigani*; *nagatyi Pseudohaliotrematoides polymorphus "nagatyi"*; *plecto Pseudohaliotrema plectocirra*; *suezicus Pseudohaliotrematoides polymprhus suesicus*

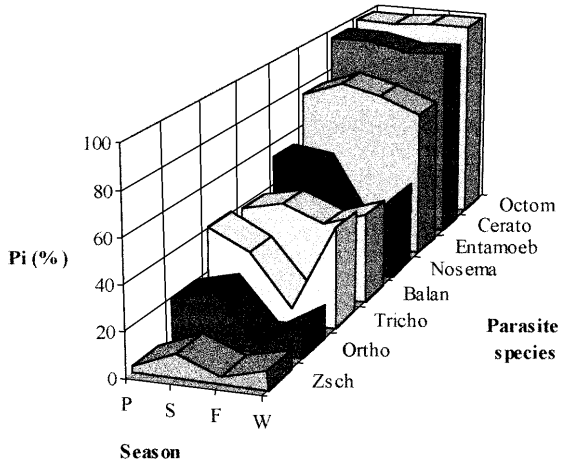


Fig. 3 Microparasite prevalence (*Pr*) in the Red Sea *S. rivulatus* samples per season. *P* Spring; *S* summer; *F* fall; *W* winter; *Zsch Zchokkella icterica*; *Ortho Ortholinea*; *Trich Trichodina* sp.; *Balan Balantidium sigani*; *Nosema Nosema ceratomyxae*; *Entamoeb Entamoeba*; *Cerato Ceratomyxa*; *Octom Octomitus*

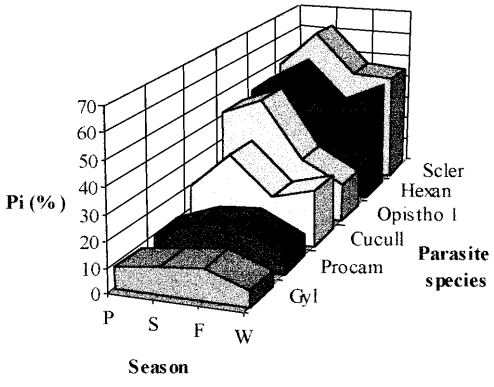
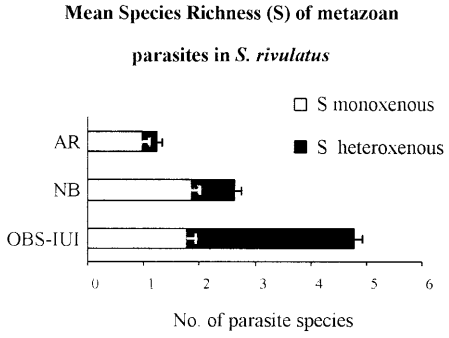


Fig. 5 Gut helminth (all heteroxenous species) prevalence (*Pr*) in Red Sea *S. rivulatus* samples per season. *P* Spring; *S* summer; *F* fall; *W* winter; *Gyl Gyliauchen volubilis*; *Procam Procamallanus elatensis*; *Cucull Cucullanus sigani*; *Opistho 1 Opisthogonoporoides hanumanthai* "sp. 1"; *Hexan Hexangium sigani*; *Scler Sclerocollum rubrimaris*

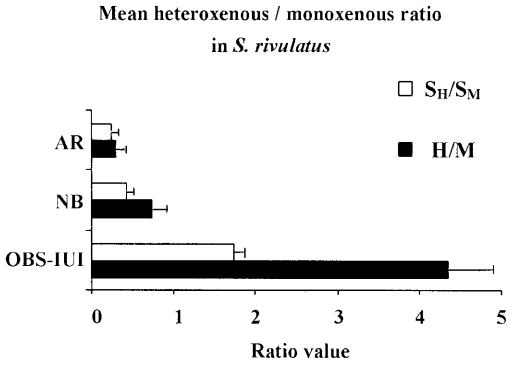
The quantitative attributes of the microparasite, monogenean and gut helminth populations infecting rabbitfish per season of capture are given in Figs. 3, 4 and 5. Microparasite infections varied considerably between species and between seasons. Some species (*Zschokkella icterica* and *Ortholinea* sp.) displayed low prevalence values (up to approximately 20%). *Ceratomyxa* sp., *Octomitus* sp. and *Entamoeba* sp. had a relatively uniform, high prevalence of 65–100% year-round, while others (*Ortholinea* sp., *Trichodina* sp., *Balantidium sigani* and *Nosema ceratomyxae*) had fluctuating prevalence levels, most of them with a similar seasonal pattern: increase in the spring/summer and decrease in the autumn (Fig. 3). As a general trend, monogenean prevalence was highest during summer months (Fig. 4). An increase in the infection intensities during the winter months was noted in the dactylogyrid monogeneans (*Pseudohaliotrematoides* spp.), but not with regard to the microcotylid *Polylabris sigani*. Gut helminths similarly displayed a high summer prevalence of infection (Fig. 5). We found higher gut helminth prevalence at the OBS-IUI coral reef site when compared with all other sites. In some extreme cases, virtually no gut worms (e.g. *Gyliauchen volubilis*, *Procamallanus elatensis* and *Cucullanus sigani*) were found in the host fish at the sandy sites. The representation of all heteroxenous parasites in the parasite community was far greater at the reef site.

Parasite species richness in each of the three main sampling sites is shown in Fig. 6, clearly showing that the overall value was highest at the reef site. Gut helminth species richness clearly distinguished the sandy sites from the reef, but monogenean species richness at the mariculture (AR) site was lowest, while the sandy north beach (NB) and OBS-IUI reef site were not significantly different. When the overall, monoxenous and heteroxenous assemblages were considered, NB displayed a significantly higher species richness than the AR site. The respective H/M ratios (both quantitative and species richness ratio) are shown in Fig. 7. In both cases, the



	ANOVA	t-test
S	$p < 0.0001$	OBS-IUI > NB > AR
S monoxenous	$p < 0.0001$	NB > AR
S heteroxenous	$p < 0.0001$	OBS-IUI > NB > AR

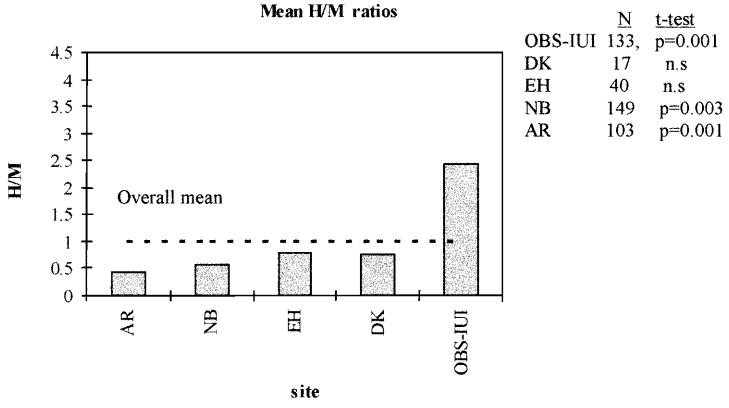
Fig. 6 Mean parasite species richness (*S*) in each of the Red Sea sampling sites. "Monoxenous" includes monogenea only; "Heteroxenous" includes gut helminths only. *AR* mariculture site; *NB* north beach site; *OBS-IUI* Underwater Observatory and Inter-University Institute



	ANOVA	t-test
S_H/S_M	$p < 0.0001$	OBS-IUI > NB, AR
H/M	$p < 0.0001$	OBS-IUI > NB, AR

Fig. 7 Heteroxenous/monoxenous mean parasite species richness ratio (S_H/S_M) and quantitative ratio (H/M) at the different Red Sea sampling sites. *AR* Mariculture site; *NB* north beach site; *OBS-IUI* Underwater Observatory and Inter-University Institute

Fig. 8 Mean H/M ratios of the Red Sea rabbitfish host samples during the study. *Broken line* indicates calculated overall mean ratio (H/M) of 0.989



OBS-IUI reef site displayed values that were highly significant: the coral reef rabbitfish samples supported parasite assemblages that both were richer in species and had heavier infection loads than the mariculture and north beach sites. The H/M ratios of the various sampling sites compared with the overall mean are shown in Fig. 8. The analyses produced statistically significant differences between the reef and sandy sites. However, when microparasites alone were considered, no significant differences between the sites were detected.

The separate analyses of microparasites and macroparasites show that microparasites failed to provide any comparative basis, and in fact masked the differences reflected by the macroparasites (Figs. 9 and 10). On the other hand, the macroparasite analyses clearly demonstrated that the H/M ratio at the coral reef was significantly higher than the mean H/M for all sites, while the values for all other sites were below the mean, with statistically significant lower values for both the mariculture and sandy sites.

Heteroxenous species accumulation curves for the Red Sea samples (including rare species with a prevalence of <3%) clearly differentiated between the coral reef and sandy sites, with the OBS-IUI site having a steeper initial slope of the curve (parasite) and a significantly higher asymptote value (species richness). The differences between the sandy and mariculture sites, however, revealed no statistically significant differences (Fig. 11).

When comparing the Red Sea and Mediterranean rabbitfish parasite assemblages, analysis of the Mediterranean data yielded a very low species richness value, due to the virtual absence of heteroxenous species from the Mediterranean fish (Fig. 12). The corresponding H/M value calculated for the Mediterranean rabbitfish was 0.286, which was lower than any of the Red Sea sites (compare with Fig. 8) . Therefore, the Mediterranean data were plotted for comparison with the monoxenous parasite species only at each of the Red Sea sites (Fig. 13). The curves were similar, with the main differences

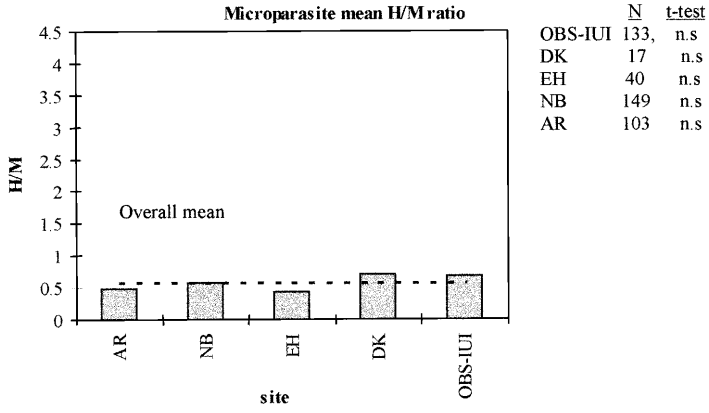


Fig. 9 Mean H/M ratio for Red Sea rabbitfish microparasites only. *Broken line* indicates calculated overall mean ratio for microparasites of 0.573

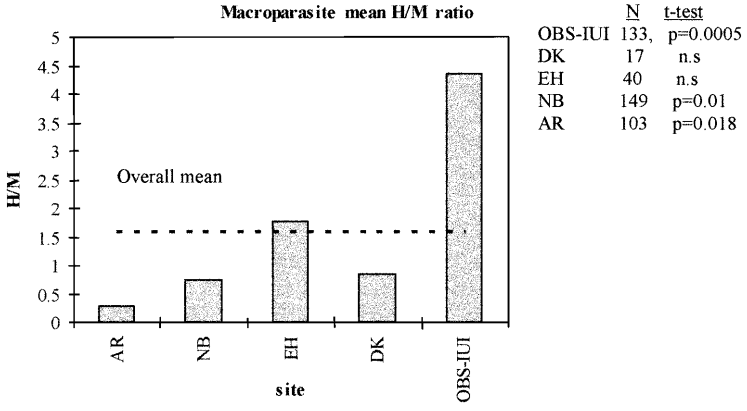


Fig. 10 Mean H/M ratio for Red Sea *S. rivulatus* macroparasites only. *Broken line* indicates the calculated overall mean ratio for macroparasites of 1.59

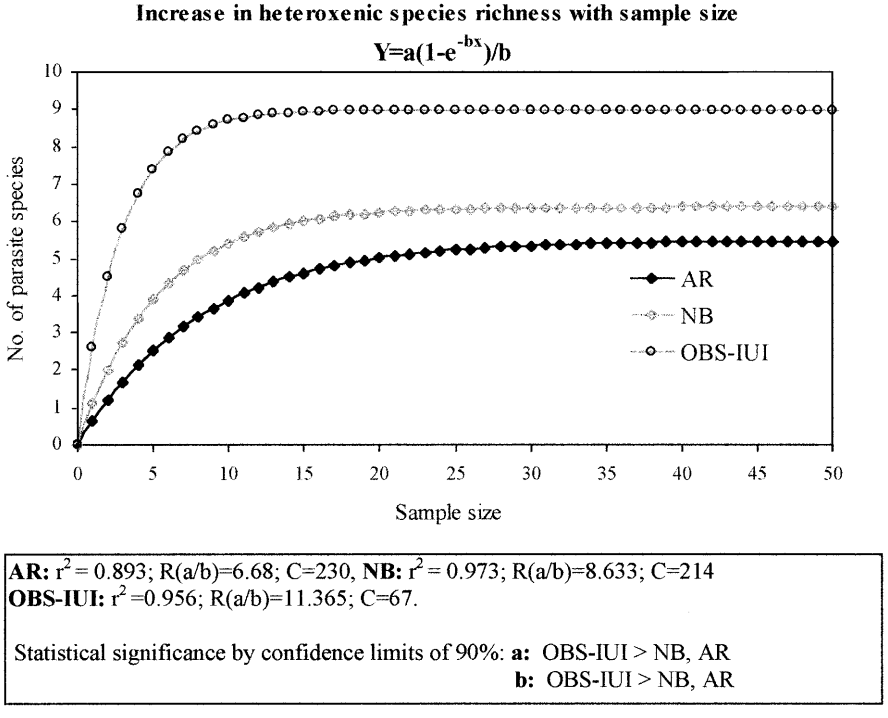


Fig. 11 Heteroxenous parasite species richness in Red Sea rabbitfish as a function of the number of hosts examined. Data plotted according to the exponential species accumulation model proposed by Walther et al. (1995). *AR* Mariculture cage site; *NB* North Beach; *OBS-IUI* Underwater Observatory and Inter-University Institute. r^2 Regression coefficient; *R*(*a*/*b*) calculated "true" species richness; *C* capacity or number of hosts needed to reach "true" species richness

being a considerably lower abundance of the monoxenous parasites in the Mediterranean, reflected by a moderate slope (parasite incidence) and a considerably higher required host sample size of C=68 for determining the "true" species richness.

EROD activity and lysosomal membrane stability

For EROD activity, a direct dependence on the length of *Siganus rivulatus* was observed in fish over 21 cm in length. Therefore larger animals were not taken into con-

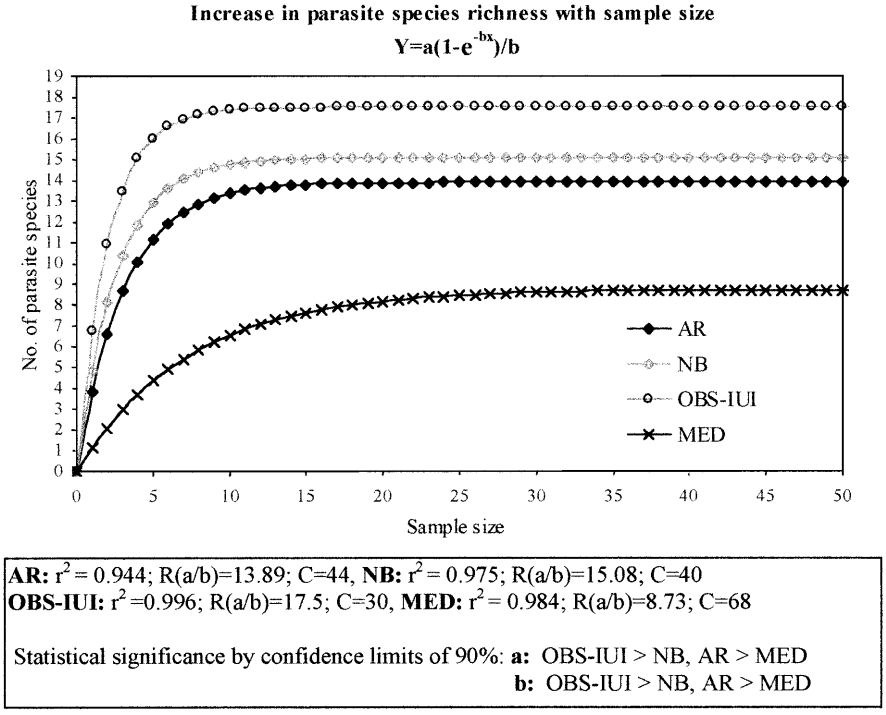


Fig. 12 Entire parasite assemblage for each site as a function of number of hosts examined. *AR* Mariculture cage site; *NB* North Beach site; *OBS-IUI* Underwater Observatory and Inter-University Institute; *MED* Mediterranean Sea (see Fig. 11 for other explanations)

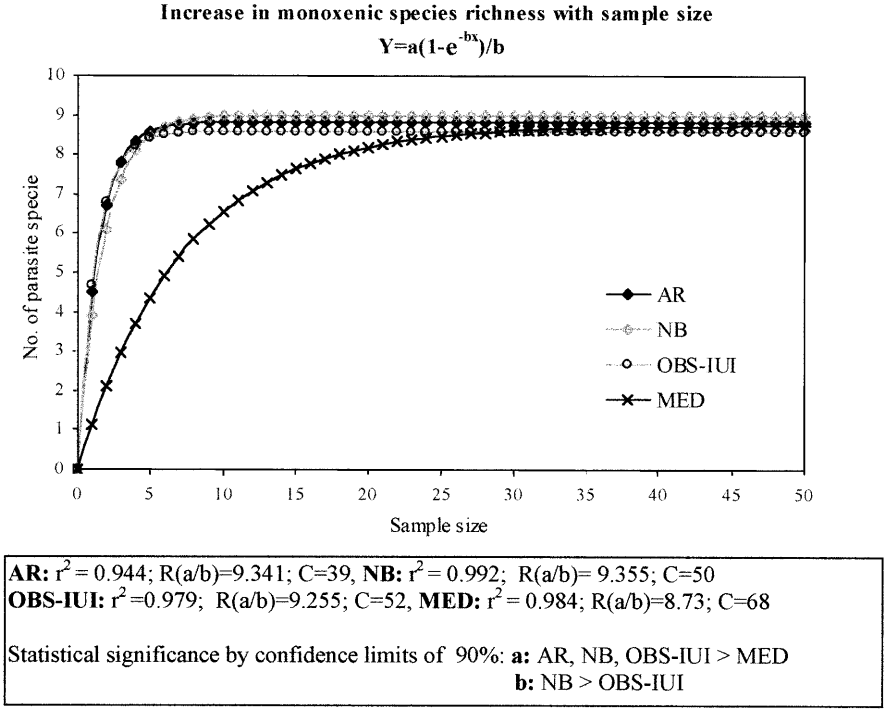


Fig. 13 Monoxenous parasite assemblage for each site as a function of number of hosts examined. *AR* Mariculture cage site; *NB* North beach site; *OBS-IUI* Underwater Observatory and Inter-University Institute; *MED* Mediterranean Sea (see Fig. 11 for explanations)

sideration for the statistical evaluation. Lysosomal stability was found to be independent of fish size.

Site-specific differences in the Red Sea (Fig. 14)

The comparison between three locations in the Gulf of Aqaba showed significant site-specific differences in EROD activity. Fish caught at the tourist area at North Beach (NB) exhibited significantly higher EROD activity than fish caught at the coral reef (OBS-IUI) and fish caught at Eilat harbor (Kruskal–Wallis ANOVA, $P<0.001$). Lysosomal stability was higher in animals from the coral reef and Eilat harbour when compared with fish from North Beach. However, the site differences of lysosomal stability were not statistically significant.

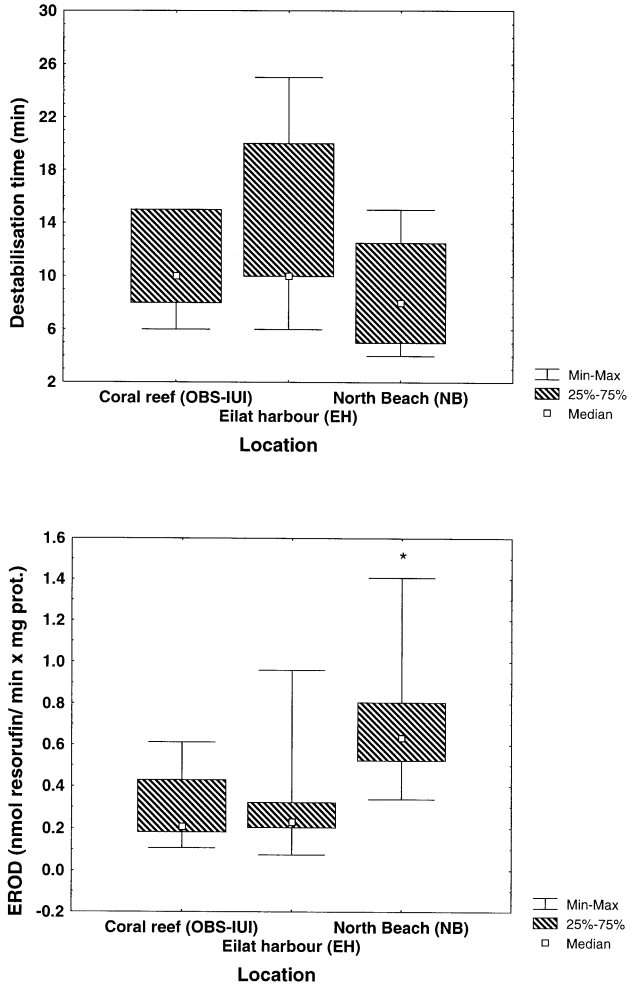


Fig. 14 Site differences of lysosomal stability and EROD activity in Red Sea *Siganus rivulatus* (* statistically significant differences, Kruskal–Wallis ANOVA, $P<0.001$)

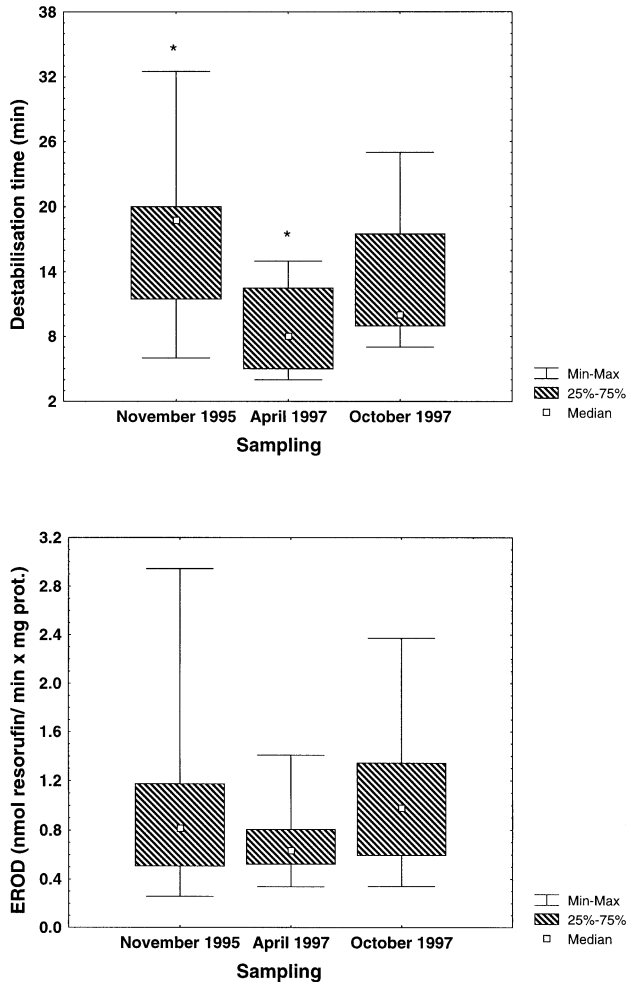


Fig. 15 Seasonal and annual differences of lysosomal stability and EROD activity at the Red Sea location North Beach (* statistically significant differences, Kruskal–Wallis ANOVA, $P<0.05$)

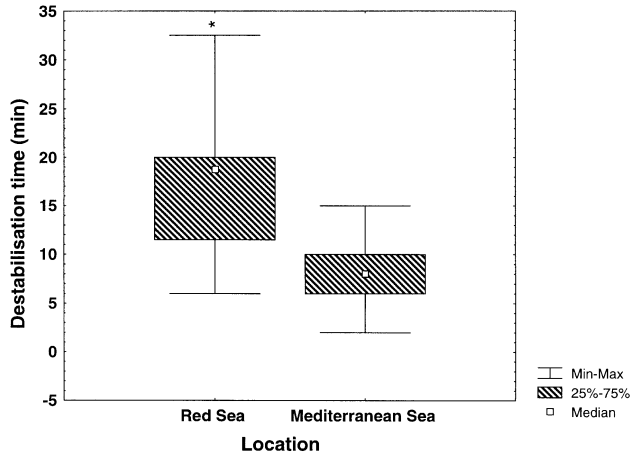
Seasonal and annual differences in the Red Sea (Fig. 15)

Although EROD activity showed no statistically significant differences between spring and autumn samplings, activity was slightly lower in spring. Lysosomal stability was significantly lower in spring 1997 than in autumn 1995 (Kruskal–Wallis ANOVA, $P<0.05$). Comparing the data of membrane stability in spring 1997 with the data obtained in October 1997, the seasonal differences were not significant. A comparison between 1995 and 1997 autumn samplings showed no significant differences. However, it is noteworthy that mean EROD values were higher and mean membrane stability values lower in 1997, suggesting an increase of anthropogenic impact from 1995 to 1997 at the North Beach sampling site.

Differences between the Red Sea and Mediterranean Sea (Fig. 16)

Comparing EROD activity in fish from the Mediterranean harbour Ashdod with fish from the tourist region at North Beach in Eilat, the Red Sea, mean values are almost double in Mediterranean rabbitfish. The observed differences are highly significant (Mann–Whitney *U*-test, $P<0.0001$). Even more discernible are the lysosomal stability values between Red Sea and Mediterranean Sea rabbitfish. The mean destabilization time in animals from Ashdod was 8 min, whereas the membrane breakdown of lysosomes from Red Sea fish occurred after 18 min of acid labilization.

The results clearly indicate that *Siganus rivulatus* in the Mediterranean harbour of Ashdod exhibit liver cell damage. In five animals, destabilization time went below 6 min, a value that is thought to represent irreversible changes in liver cell structure in North Sea flounder (Köhler and Pluta 1995). This may include functional disorder of biotransformation in the cell and the loss of EROD activity. In these five rabbitfish, EROD activity was also extremely low (Fig. 17) as compared to fish that exhibit average or "normal" membrane stability (>6 min).



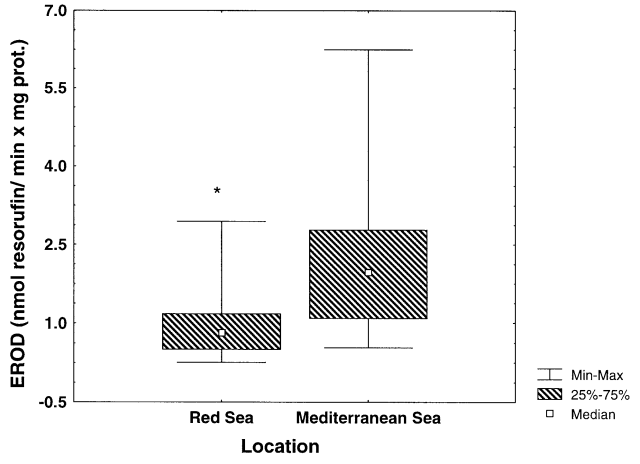


Fig. 16 Lysosomal stability and EROD activity in *Siganus rivulatus* from the Red Sea (North Beach) and Mediterranean (Ashdod Harbor) (* statistically significant differences, Mann–Whitney *U*-test, $P<0.0001$)

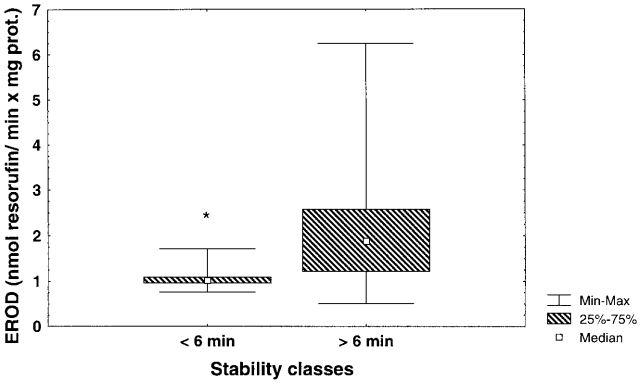
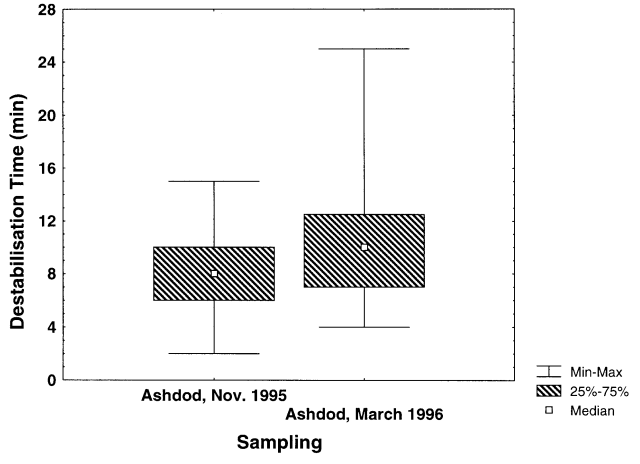


Fig. 17 EROD activity in two classes of lysosomal stability (destabilization time <6 min, >6 min) in *Siganus rivulatus* from Ashdod Harbor (* statistically significant differences, Mann–Whitney *U*-test, $P<0.001$)



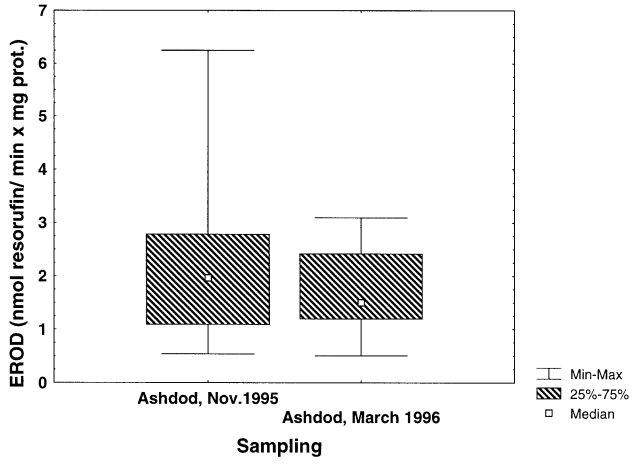


Fig. 18 Seasonal differences of lysosomal stability and EROD activity in *Siganus rivulatus* from Ashdod Harb

Seasonal differences in the Mediterranean Sea (Fig. 18)

The Mediterranean harbour of Ashdod was sampled twice, in November 1995 and April 1996, to obtain information on potential seasonal differences of biomarker response. For both parameters, EROD activity and lysosomal stability, no significant differences were detected between autumn and spring samples, although mean lysosomal stability was higher and EROD activity was lower in April when compared to the November sampling.

Discussion

Parasitological parameter evaluation

In the present study we employed the same approach taken by Gelnar et al. (1997) and D'Amelio and Gerasi (1997), using an asymptotic curve to estimate species richness of a given population. Our results were backed by data analyses employing species diversity and rich-

ness indices as proposed by Soberon and Llorente (1993) and Walther et al. (1995) specifically for parasitological models. The results verified our presented working hypothesis: we could demonstrate distinct values of heteroxenous (H) vs. monoxenous (M) parasites between the study areas. The higher ratio of heteroxenous over monoxenous parasites was evident in the data obtained from the more ecologically stable habitats (coral reef) as against the sandy beach location, and even more so, the mariculture site located on a sandy habitat. Our working hypothesis was further validated when data from the Mediterranean (Red Sea immigrant) parasite community of *S. rivulatus* was compared with those of the Red Sea (autochthonous) sites. The Mediterranean population of this fish serves as an excellent conceptual model for a situation when one fragment at a particular trophic level is excluded from the habitat, as is expected to occur under pollution stress. Of the nine parasite species found in Mediterranean rabbitfish, all but one were Red Sea immigrants that accompanied the rabbitfish host into the Mediterranean. The ninth species, *Cryptocaryon irritans*, an ectoparasitic ciliate, is a ubiquitous species which occurs in both seas and has been associated with sea bream mortalities in Mediterranean mariculture facilities (Diamant et al. 1991). Our parasitological data showed a complete absence of heteroxenous species in the Mediterranean rabbitfish.

The "interference" of background factors, such as season of host capture and host age, is an unavoidable problem in field studies such as the present investigation. It would seem that as far as short-term effects of environmental stress are concerned, these would probably be difficult or even impossible to detect when data are summarized as year-round compilations. Therefore, comparison of parasite communities would best be carried out during a given season, since periodic deviations from normal patterns (e.g., summer peaks or winter low abundance of monogenea) would be easier to recognize.

A close correlation between host size and parasite richness has been previously demonstrated in rabbitfish (e.g., Grutter 1994; Martens and Moens 1995). This relationship is particularly relevant as far as the heteroxenous species are concerned (Digeneans, Acanthocephala and many species of nematodes) since they accumulate but cannot proliferate on the host. Unfortunately, little is known on the biology of these parasites, the longevity in the fish host is not known, and the life cycles have yet to be elucidated. Nevertheless, considering taxonomically related species, a multiple-host life cycle may be assumed in all of them (Pearson 1972: Lotz and Corkum 1984). On the other hand, ectoparasites (monoxenous species) have been shown to be generally stable and unchanged by such factors (Poulin 1995). Therefore, community indices such as species richness and species diversity of parasites with heteroxenous and monoxenous developmental cycles are useful for parasite assemblage analyses to assess changes due to habitat destruction. This is so not only because of the soundness of the underlying concept, but also because these indices have been widely used and are today generally well understood (Washington 1984).

When microparasites and macroparasites were analyzed separately, the results indicated that only the latter were particularly subject to seasonal fluctuation. The microparasite populations, on the other hand, that represented a relatively large group of species displayed little if any seasonal changes which may be related to these parasites' (consisting mostly of monoxenous species) life histories. The sheer size of most microparasite populations which largely outnumber individual metazoans could seriously bias the calculations of parasite diversity, although species richness remains unaffected (D'Amelio and Gerasi 1997). Overall, the separate H/M analyses clearly indicated that exclusion of the microparasites, namely protozoa and microscopic parasites such as myxosporeans, significantly improved expression of differences between sites. Since microparasites are both difficult to quantify and (almost certainly) possess a considerably shorter lifespan than macroparasites, we concluded that macroparasite species alone (eight gut helminths and six monogeneans) were more suitable to represent the rabbitfish parasite assemblages.

In the Red Sea, when heteroxenous and monoxenous species were analyzed separately, the abundance of heteroxenous species displayed a peak during spring months, corresponding to the pre-spawning intensive feeding period characterized by heavy grazing. It is during this season that the fish have a greater probability of exposure (by ingestion) to infective stages of gut parasites (Diamant 1989a). The peak abundance of the monoxenous species (predominantly monogenea), during the summer months, may have been due to increased proliferation on the host at high ambient summer temperatures as well as host post-spawning stress, which could have reduced host immunity and enhanced infections (see Yeomans et al. 1997). The lowest prevalence of gut parasites occurred during autumn months, which corresponds with decreased feeding rates, possibly coupled with decreased abundance of infective stages. This pattern has been proposed to explain the dynamics of acanthocephalan *S. rubrimaris* gut infections in rabbitfish (Diamant 1989a), and similar patterns also reported in eels and other fish hosts (Kennedy 1997).

A noteworthy outcome of the parasitological analyses in the Red Sea sites is the highlighting of the ecologically adverse impact of cage mariculture. Its evident impacts, namely eutrophication, low redox potential and promotion of spread of pathogens, are a growing concern in the Eilat region (Popper 1995). The sandy sea bed below the cages, littered with anaerobic mats of decaying organic material and normally with increased concentrations of dissolved organic matter in the water results in a corresponding decline of benthic faunal diversity, to the point of species disappearance and vanishing of seagrass meadows (Popper 1995; Porter et al. 1996). The rabbitfish near the mariculture farm supported the poorest and least diverse heteroxenous parasite communities of all sampled sites. Curtis (1988) showed that an affinity ex-

ists between fish parasite community changes and effects of mariculture and described it as a long-term process, while Karasev et al. (1997) showed that fish that fed on commercial feed displayed a dramatic change in their parasite fauna. At the Ardag mariculture site, the rabbitfish fed copiously on the artificial pellets that dropped through the cage nets and accumulated on the bottom. This feeding behavior could explain the low heteroxenous gut parasite abundance in the rabbitfish at this site, in comparison with hosts from other sites, that presumably fed on their natural diet, which included intermediate hosts.

Interestingly, the detrimental effect of the mariculture by-products was not restricted to the interference in transmission of heteroxenous parasites, but appeared to suppress also the reproduction of monogenean species. Indeed, the lowest values of species richness of monogenea in this study were found at the mariculture site. This may perhaps be partly explained by the eutrophication at the site, which may have directly impacted transmission of ectoparasites. Monogenean eggs that settle on the organically enriched, largely anaerobic sediment below the farm are probably lost. Even if the oncomiracidium develops and hatches, the viability of the free-swimming-stage and the chances of finding a host can be expected to be very low in that environment. Also, activity of "cleaner fish" that aggregate around the cages and remove ectoparasites – such as pomacentrids and wrasses – may further reduce monogenean abundance at this site (see review in Rohde 1993). Remarkably, in contrast to the results of the present work, parasitological studies carried out at mariculture facilities in Norway and Japan indicated that monogenea proliferated in both farmed and wild fish (Egidius et al. 1991; Ogawa 1996).

Biochemical and histochemical parameters

EROD activity represents a sensitive indicator of early cell response following exposure to specific lipophilic compounds, such as polyaromatic hydrocarbons, coplanar PCB congeners and dioxins (Goksoyr and Förlin 1992; Boer et al. 1993; Sleiderink et al. 1995). Thus, it can serve as a suitable biomarker for a general characterization of marine environments (i.e. polluted–unpolluted). Experimental and field studies investigating the applicability of EROD activity in bio-effect monitoring have hitherto been focused on flatfish species, such as dab (*Limanda limanda*) and flounder (*Platichthys flesus*) from northern seas. Relatively scant information is available for fish species from temperate and tropical seas, where the need to conduct biological-effect monitoring programmes is often not yet evident due to the relatively low degree of coastal industrialization and urbanization. Nevertheless, even relatively clean and undisturbed areas like the Red Sea may provide important information on the onset of changes in biomarker response due to the influence of anthropogenic impact.

In the present study, the rabbitfish *Siganus rivulatus* proved to be a suitable species for the application of biochemical and histochemical biomarker tests. A close correlation existed between fish size and EROD activity in larger animals displaying a lowered induction of the cytochrome P 450-dependent monooxygenase (see also Addison 1984); therefore these were excluded from the analytic evaluations. Although the reason for this has not been proven, physiological and metabolic processes, as well as cell turnover, change with age, and it is therefore not surprising that biotransformation, a process at the molecular level of biological organization, would develop continuously during the lifetime of the individual animal. Lysosomal stability, in contrast, was found to be independent of fish length.

In agreement with the parasitological community indices, there were significant site-specific differences in EROD activity between the Red Sea locations at North Beach and the coral reef site. Differences in lysosomal stability existed as well but were not significant. The fact that a high EROD activity corresponds with a low lysosomal stability at the sandy beach location NB suggests an influence of xenobiotics. EROD is a biomarker of exposure and is induced by certain substances before a toxic effect at the subcellular level has been manifested. Lysosomal stability is relatively high in comparison with the Mediterranean Sea values measured in the same fish species (Fig. 15). Thus, the only clear site-specific difference was observed at the lowest level of biological organization, the molecular level. According to the hierarchical and concentric organization of biological systems (Wolfe 1992) and compared with the findings of Köhler and Pluta (1995) in livers of relatively healthy flounder from the North Sea, the disturbance of the biological system in the Red Sea was at a very early stage. The comparison between Mediterranean Sea and Red Sea *Siganus* demonstrated a highly significant difference in both tested biomarkers. In the Mediterranean Sea, lysosomal stability values were below 6 min, which in flounder liver was found to represent a high degree of liver cell damage (Köhler and Pluta 1995). Also in this study, EROD activity, which was found to be absent from healthy livers, increased considerably with the onset of liver changes and dropped again with degenerative, preneoplastic and neoplastic lesions. The findings of low EROD activity in *Siganus* from the Mediterranean Sea and parallel extremely decreased lysosomal stability in the same individual may therefore be caused by a progression of liver lesions, a high degree of liver damage, caused by the influence of anthropogenic pollution.

Conclusion

In conclusion, the evaluation of the H/M analyses of the rabbitfish parasite communities in this study was useful when employed to compare two distinctly different habitats (e.g; coral reef vs. sand bottom). Species richness, although being a qualitative index, produced results that

were consistent with the quantitative H/M ratio and in fact helped unmask certain differences between sites, such as winter differences between the sand bottom and mariculture sites. Both quantitative H/M ratios and S_H/S_M species richness ratios clearly demonstrated high values in an ecologically stable habitat, and low values at anthropogenically impacted environments. These findings were validated by the biochemical and histochemical biomarkers, EROD activity and lysosomal stability, leading to the conclusion that changes in parasitological parameters are sensible tools for the reflection of environmental changes at the onset of disturbance due to the influence of anthropogenic pollution.

Acknowledgements This study was supported by the German–Israel BMBF MARS 1/2 project, contract no. 03F0159B, coordinated by GKSS Geesthacht and the Ministry of Sciences and Arts, Israel.

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Communicated by H. v. Westernhagen