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ORIGINAL ARTICLE

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Use of fish parasite species richness indices in analyzing anthropogenically impacted coastal marine ecosystems

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Abstract The diversity of fish parasite life history strategies makes these species sensitive bioindicators of aquatic ecosystem health. While monoxenous (single-host) species may persist in highly perturbed, extreme environments, this is not necessarily true for heteroxenous (multiple-host) species. As many parasites possess complex life cycles and are transmitted through a chain of host species, their dependency on the latter to complete their life cycles renders them sensitive to perturbed environments. In the present study, parasite communities of grey mullet *Liza aurata* and *Liza ramada* (Mugilidae) were investigated at two Mediterranean coastal sites in northern Israel: the highly polluted Kishon Harbor (KH) and the relatively unspoiled reference site, Ma'agan Michael (MM). Both are estuarine sites in which grey mullet are one of the most common fish species. The results indicate that fish at the polluted site had significantly less trematode metacercariae than fish at the reference site. Heteroxenous gut helminths were completely absent at the polluted sampling site. Consequently, KH fish displayed lower mean parasite species richness. At the same time, KH fish mean monoxenous parasite richness was higher, although the prevalence of different monoxenous taxa was variable. Copepods had an increased prevalence while monogenean prevalence was significantly reduced at the polluted site. This variability may be attributed to the differential susceptibility of the parasites to the toxicity of different pollutants, their concentration, the exposure time and possible synergistic effects. In this study, we used the cumulative species curve model that extrapolates "true" species richness of a given habitat as a function of increasing sample size. We considered the heteroxenous and monoxenous species separately for each site, and comparison of curves yielded significant results. It is proposed to employ this approach, originally developed for estimating the "true" parasite species richness for a given habitat, in the characterization of communities of differentially impacted coastal marine ecosystems.

Keywords Diversity indices · Species richness · Mediterranean · Heteroxenous-monoxenous parasite ratio · Grey mullet

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Introduction

The inherent complexity and diversity of parasite life history strategies implies that their communities may integrate adverse effects and stresses that influence other components of the ecosystem. Parasite communities can thus be regarded as comprehensive bioindicators of ecosystem health and environmental stability (Paperna 1997; Overstreet 1997). As heteroxenous parasites with complex, multiple host life cycles are expected to persist only in stable habitats in which both the free-living stages and intermediate hosts are capable of survival, extreme conditions may be predicted to allow the persistence of mainly monoxenous parasite species with simple, single-host life cycles. This is the conceptual background for the use of parasites as ecological bioindicators as researchers become increasingly aware of their potential as sensitive probes in environmental impact studies (Paperna 1997). Consequently, the monitoring of anthropogenic impacts by using fish parasite ecological indices has been used in a variety of habitats: oceans, seas, lakes and rivers. Studies on this subject were communicated in a symposium carried out within the framework of the VII

European Multicolloquium of Parasitology in Parma, Italy, 1996 (Paperna 1997).

Both hosts and parasites may be affected by pollution in a variety of ways. While in some cases the negative effect of the parasitic infection may be aggravated, in others the stressor may work to ameliorate the effects of the parasites (Lafferty and Kuris 1999). Pollution impact may be estimated in a variety of ways, for example by following the modulation of the host immune response (Hoole 1997), or by examining the lethal effect of waterborne pollutants on parasites within the fish (Riggs et al. 1987). There are many case reports on the negative effects of pollution on parasite communities, which are often reflected by a decline in species richness and diversity (Khan and Thulin 1991; Poulin 1992; MacKenzie et al. 1995; Lafferty 1997; MacKenzie 1999). However, pollutants may not necessarily influence fish parasites directly, but may act in an indirect way, such as by affecting free-living parasite stages (swimming larva) or stages developing in invertebrate intermediate hosts, such as molluscs, copepods and others (Evans 1982; Munkittrick and Dixon 1988; Siddall et al., 1993). Whatever the nature of the effect, quantitative measurement of pollution impact may be implemented by following the size fluctuation of specific fish parasite populations. This has been done with trichodinids, trypanosomes, monogeneans and others (Paperna and Overstreet 1981; Khan 1987, 1990; Khan and Kiceniuk 1988; Hoole 1997; Khan and Payne 1997; Overstreet 1997; Yeomans et al. 1997). Alternatively, this can also be done by analyzing the structure of whole parasite communities (D'Amelio and Gerasi 1997; Landsberg et al. 1998; Broeg et al. 1999; Diamant et al. 1999).

In this paper, we present results of the German-Israeli cooperation in marine science (MARS) program initiated in 1995. The study was undertaken to test the described approach as a practical tool for distinguishing between habitats subjected to differing degrees of adverse anthropogenic impact on the Mediterranean and Red Sea coasts of Israel. The results of the first phase, dealing with the parasite communities of rabbitfish *Siganus rivulatus* (Siganidae) in the Gulf of Aqaba, Red Sea, have been published elsewhere (Diamant et al. 1999). For the studies in the Mediterranean, grey mullet (*Liza ramada* and *Liza aurata*, Mugilidae) were chosen as model fish. These two common species are widely distributed along the Israeli Mediterranean coast; their parasite fauna has been previously studied by Paperna (1975) and Paperna and Overstreet (1981) and is consequently well known. The presented data were collected from grey mullet exceeding 150 mm in total length (TL) from two marine habitats subject either to low and or to high anthropogenic impacts. The data were also compared with those available from an earlier study carried out during 1993–1995, on parasites of juvenile grey mullet (30–150 mm TL) captured from the same habitats (see Gerasi 1996).

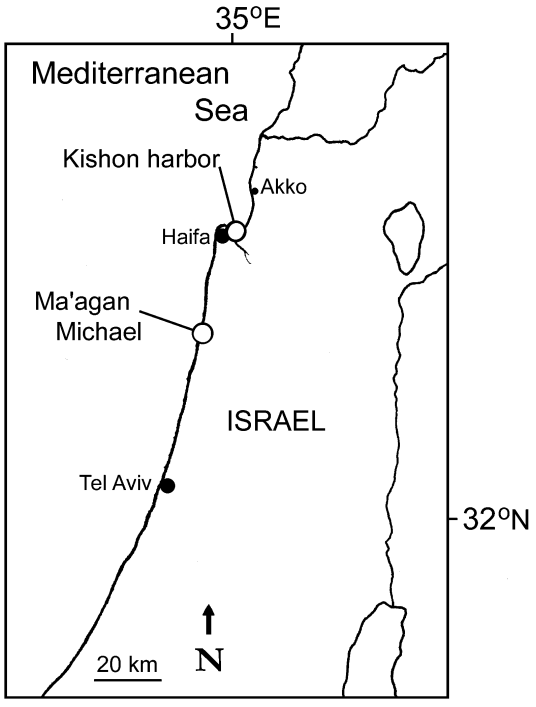


Fig. 1 Map of northern Israel showing Kishon Harbor (*KH*) and Ma'agan Michael (*MM*) grey mullet sampling sites

Methods

Study sites and sampling design

The work was carried out on the eastern Mediterranean coast of northern Israel (Fig. 1): at Kishon Harbor (KH), a polluted estuarine site, and at Ma'agan Michael (at Jisr el Zarka) (MM), a reference estuarine site. Fish were caught by gill nets over four seasonal samplings in spring and fall of 1999 and 2000.

Parasite data processing

The collected fish were brought to the laboratory and kept alive in seawater tanks until they were dissected (within 48 h of capture) for parasitological examination. The skin, gills, viscera and internal organs of each fish were carefully examined for the presence of parasites. Freshly prepared skin, gill and intestinal mucosa scrapings, wet impressions of kidney, heart, liver, muscle and gall bladder wall and lumen contents were examined using light microscopy (LM). Suspected infected smears were air-dried, fixed in absolute methanol and stained with Giemsa. Blood films were stained by the same procedure. Smears containing myxosporeans were fixed in Bouin's solution and after rinsing in 70% alcohol were stained with Giemsa. Following the preliminary identification of adult digeneans and metacercariae by LM, selected specimens were fixed in 70% alcohol under a cover slip and stained with hematoxylin or acetocarmine.

The following ecological indices were utilized in this study:

1. Species richness (S)
2. Margalef's index of diversity (D, Margalef 1958)
3. Shannon Weiner index of diversity (H′)
4. Index of evenness (J)
5. Heteroxenous\monoxenous ratio (Sh/Sm)
6. "True" species richness extrapolated by $Y=a(1-e^{-bx})/b$ (Walter et al 1995)

Table 1 Parasite species recovered from grey mullet (*Liza ramada, Liza aurata*) collected from the Mediterranean coast of Israel during 1999–2000

Taxon	Species name	Ectoparasite	Endoparasite	Heteroxenous	Monoxenous
Bacteria	Epitheliocystis		+		+
Algae	*Amyloodinium ocellatum* (Brown,1934)	+			+
Protozoa					
Ciliophora	*Trichodina puytoraci* Lom, 1962	+			+
	Tripartiella sp.	+			+
Mastigophora	*Hexamita* sp.		+		+
Myxosporea	*Myxobolus* sp.1		+	+	
	Myxobolus sp.2		+	+	
	Myxobolus sp.3		+	+	
	Sphaerospora sp.		+	+	
	Zschokkella mugilis Sitja–Bobadilla and Alvarez–Pellitero, 1993		+	+	
	Ceratomyxa sp.		+	+	
	Ortholinea sp.		+	+	
Metazoa					
Monogenea	*Ligophorus vanebendenii* (Parona and Perugia, 1890)	+			+
	Ligophorus szidati (Euzet and Suriano, 1977)	+			+
	Metamicrocotyle sp.	+			+
Digenea	*Haploporus benedenii* (Stossich, 1987) Looss, 1902		+	+	
	Haploporus lateralis Looss, 1902		+	+	
	Haplosplanchnus pachysomus (Eysenhardt, 1829) Looss, 1902		+	+	
	Schikobalotrema sp.		+	+	
	Dicrogaster contructus Looss, 1902.		+	+	
	Hemiurus appendiculatus (Rudolphi, 1802) Looss, 1902		+	+	
	Lecithaster confusus Odhner, 1905		+	+	
Digenea metacercariae	*Heterophyes heterophyes* (Siebold, 1853) Stile and Hassall, 1900		+	+	
	Parascocotyle sp.		+	+	
	Haplorchis sp.		+	+	
	Stictodora sawakiensis Looss, 1899		+	+	
	Bucephalid sp.		+	+	
	Cryptocotyle sp.		+	+	
	Metacercariae 1 (Liver)		+	+	
	Metacercariae 2 (Kidney)		+	+	
	Metacercariae 3 (Strigeidae)		+	+	
	Metacercaria 4 (Gall bladder)		+	+	
	Mesocercariae (intestine)		+	+	
Cestoda	*Scolex polymorphus* (Mueler, 1788)		+	+	
Nematoda	*Contracaecum* sp.		+	+	
	Cucullanus sp.		+	+	
Acanthocephla	*Neoechinorhynchus* sp.		+	+	
Crustacea	*Colobomatus mugilis* Raibaut, Caillet and Ben Hassine, 1978	+			+
	Gnathia sp.	+			+
	Caligus apodus (Brian, 1924)	+			+
	Caligus pageti Russel, 1925.	+			+
	Eubrachiella mugilis Kabata, Raibaut and Ben Hassine, 1971	+			+

Long-term changes were investigated by comparing the grey mullet parasite data with similar records collected at the same sampling sites during the years 1993–1995. The processing and analyses were carried out with similar procedures to those previously outlined in Diamant et al. (1999).

Statistical analysis

A database was established and all parasite data, per fish/per sampling site/per season were entered. Previous work has demonstrated that the parasite community indices of *Liza ramada* and *L. aurata* are not significantly different (see Gerasi 1996). Analysis of our data (not shown) confirmed that no significant differences are found between these host species. Thus, we pooled all data

Table 2 Parasitological parameters for grey mullet (*Liza ramada, Liza aurata*) sampled from the Israeli Mediterranean coast in 1999–2000. Results are presented±SE. Sh/Sm index was calculated after 1+log(x+1) transformation. *S*, Species richness; *D*, species richness after Margalef (1951); *S* (heteroxenous), heteroxenous parasite species richness; *S* (monoxenous), monoxenous parasite species richness; *Sh/Sm*, heteroxenous/monoxenous ratio; *H′*, Shannon Weiner index of diversity; *J*, index of evenness. *H′* was calculated only for metazoa

	Kishon Harbor	Ma'agan Michael	*P* value
Total no. of fish	121	124	
Total no. of parasites	19 (9)	34 (27)	
Mean fish weight (g)	182.5±8.5	196.8±8.1	n.s.
Mean fish length (cm)	23.2±0.3	23.5±0.3	n.s.
Condition factor (K=weight/length^3*100)	1.4±0.02	1.5±0.02	$P<0.05$
Diversity indices			
S	1.8±0.2	3.1±0.2	$P<<0.001$
D (Margalef)	0.2±0.03	0.5±0.03	$P<<0.001$
S (heteroxenous)	0.7±0.12	2.3±0.11	$P<<0.001$
S (monoxenous)	1.1±0.1	0.8±0.1	$P<0.05$
Sh/Sm	0.96±0.02	1.2±0.02	$P<<0.001$
H′ (Shannon Wiener)	0.2±0.04	0.4±0.04	$P<0.01$
J (evenness)	0.26±0.04	0.43±0.04	$P<0.05$

collected from these two host species at the KH site. In order to minimize bias, monogenean species of the genus *Ligophorus*, which showed a high degree of host specificity (different species infect *Liza ramada* and *L. aurata*) (Euzet and Suriano 1977), were pooled to the genus level as *Ligophorus* sp. Host condition factor, K=100*weight/length^3 was calculated according to Bolger and Connolly (1989).

All indices were calculated and sorted using Microsoft Excel. "True" species richness and confidence limits were calculated using the SlideWrite Plus software package. Differences between sampling sites were tested using two-way ANOVA (location×season). Differences in prevalence and relative representation of the main taxa were tested using a χ^2 test. All statistics were carried out with the JMP statistical software package with significance levels set at $P<0.05$.

Results

A total of 121 fish (67 *Liza aurata* and 54 *L. ramada*) were caught at the polluted site KH, and 124 (all *L. aurata*) at the reference site MM. Nineteen species of parasites were recovered from fish sampled at KH compared to 34 at MM (Tables 1, 2). Although no significant differences were found between the mean fish weight and length at both sites, condition factor K was significantly higher at MM (Table 2).

The overall prevalence of heteroxenous parasites was significantly higher at MM (88.4%) than at KH (46.9%). Conversely, the prevalence of monoxenous parasites was significantly higher at KH (70.4%) than at MM (47%) (Fig. 2a). The prevalence values for Digenea (both adult and metacercariae), Myxosporea and Monogenea were also significantly higher at MM. The prevalence of the monoxenous parasitic copepods *Colobomatus mugilis* that invade the inner surface of the host operculum and chalimi of *Caligus apodus* that attach to the host gills were 65.4% at KH and significantly higher than the 27.9% found at MM (Fig. 2b).

When parasite taxa were considered, the relative representation of parasitic copepods, in relation to the entire parasite community, was 57% at KH, whereas that of Myxosporea, digenean metacercariae and adult helminths (trematodes, nematodes and acanthocephalans) was as low as 20.4%, 9.7% and 0%, respectively. At MM,

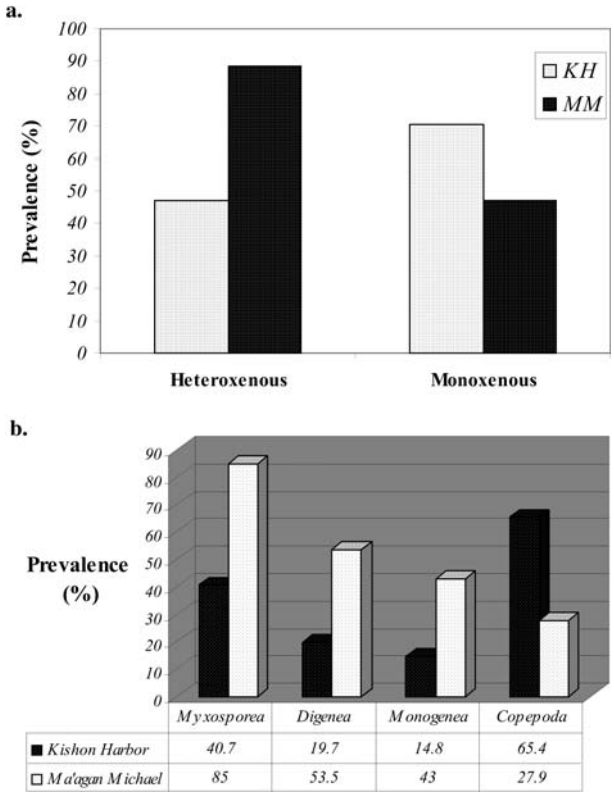


Fig. 2 **a** Prevalence of infection of heteroxenous and monoxenous parasites and **b** of the main parasite taxa from the Israeli Mediterranean coast during 1999–2000 (*KH* Kishon Harbor, *MM* Ma'agan Michael)

the parasite community composition was different. The relative representation of Myxosporea, metacercariae and gut helminths was 42.4%, 20.7% and 10.7%, respectively. Only 9.1% of the hosts were infected with parasitic copepods (Table 3).

Mean parasite species richness and diversity indices (S, D, H′ and J), had significantly higher scores at MM than KH. Sh and the Sh/Sm ratio displayed the same trend in which values at MM were significant higher than at

Table 3 Relative representation (%) of the main parasite taxa of the entire parasite community infecting grey mullet sampled from the Israeli Mediterranean coast during 1999–2000

Parasite taxa	Kishon Harbor	Ma'agan Michael
Myxosporea	20.4	42.4
Copepoda	57	9.1
Monogenea	6.5	14.5
Protozoa	2.7	0.4
Algea	3.8	2.2
Bacteria	0	0.7
Nematoda	0	1.4
Acanthochephala	0	0.7
Cestoda	0	0.4
Trematoda (adult)	0	7.6
Trematoda (metacerceriae)	9.7	20.7

KH. On the other hand, Sm values at KH were significantly higher than those at MM (Table 2).

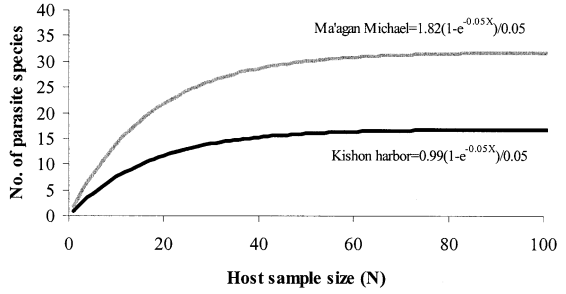
The "true" species richness of the parasite communities in the grey mullet sampled at the sampling sites, as extrapolated from the cumulative model $Y=a(1–e^{–bx})/b$, generated two curves with a significantly ($P<0.05$) higher value for the MM communities when compared with those of KH (Fig. 3a). Where calculated for heteroxenous parasites, only the differences between "true" species richness values were also significant (Fig. 3b).

When calculated separately for monoxenous parasites, grey mullet sampled at KH had a significantly higher species richness value than those from MM ($P<0.05$) (Fig. 3c). Based on the model calculation of the ratio between "true" species richness values of heteroxenous vs. monoxenous, a value of 4.29 at MM was significantly greater than 0.8 at KH.

Ecological indices of the KH samplings demonstrated seasonal variation. Mean Sm, Sh/Sm and J values at KH varied significantly between spring and fall, while such variations were insignificant at MM (Fig. 4). During fall, the mean Sm value (±SE) at KH was 1.38±0.16, while during spring it was only 0.91±0.14. As a result, the Sh/Sm index decreased significantly during fall (0.88±0.04) as compared with spring (1.01±0.04). Moreover, evenness (J) was 0.41±0.07 during fall and only 0.14±0.06 during spring.

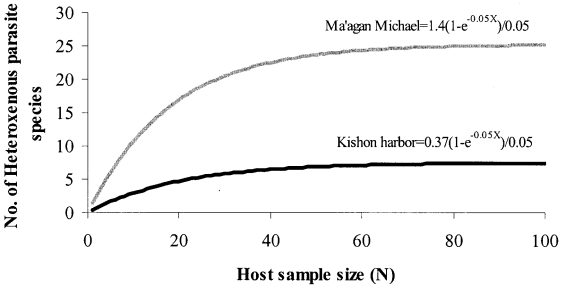
Comparison of these data with those of grey mullet parasite communities sampled at the same two sites in the mid 1990s (1993–1995) revealed that gut helminths found in fish (*Haploporus benedenii* and *Saccocoelium tensum*) at KH in the past (Gerasi 1996) were absent in the fish collected in 1999–2000. At the same time, the prevalence of gut helminths in grey mullet caught at MM increased significantly, from 12.7% to 25.9%. However, the increased prevalence was accompanied by a significant decline in the mean intensity of infection, from 11 worms per fish to 6.5 worms at the end of the decade (Table 4). A significant decrease in the "true" species richness value over the same time period was found at KH, while no notable changes were noted at MM (Fig. 5).

a



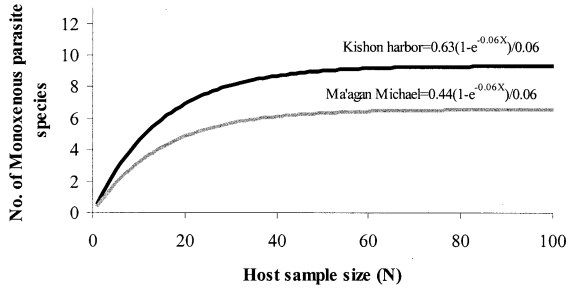
	r^2	R (a/b)	a	b	c
Kishon harbor	0.95	16.87	0.99[b]	0.05	51
Ma'agan Michael	0.97	31.86	1.82[a]	0.05	63

b



	r^2	R (a/b)	a	b	c
Kishon harbor	0.93	7.52	0.37[b]	0.05	55
Ma'agan michael	0.98	25.23	1.4[a]	0.05	85

c



	r^2	R (a/b)	a	b	c
Kishon harbor	0.94	9.34	0.63[a]	0.06	49
Ma'agan michael	0.95	6.57	0.44[b]	0.06	37

Fig. 3 Grey mullet (*Liza ramada*, *Liza aurata*) parasites species richness by sample size $Y=a(1–e^{–bx})/b$ from the Israeli Mediterranean coast during 1999–2000

Table 4 Comparison of the prevalence and intensity of gut helminths of grey mullet sampled from the Israeli Mediterranean coast during 1993–1995 and 1999–2000

	Kishon Harbor		Ma'agan Michael	
	1993–1995	1999–2000	1993–1995	1999–2000
Prevalence (%)	7	0	12.7	25.4
Intensity	5	0	11	6.5
No. of species	2	0	5	6

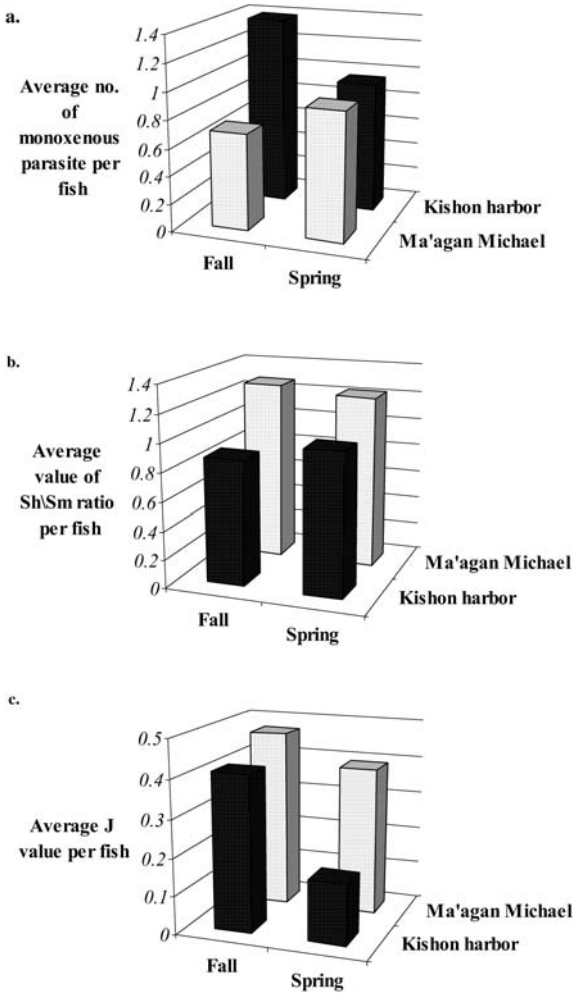
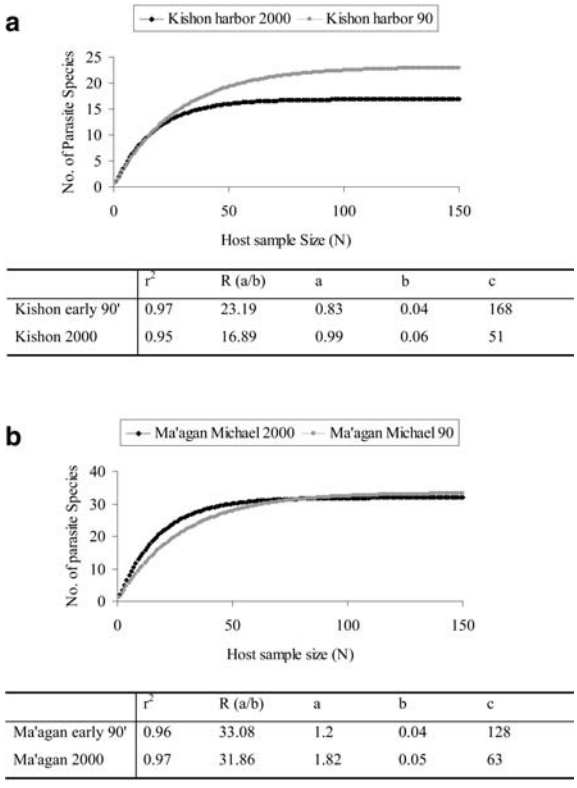


Fig. 4 Seasonal effect on: **a** monoxenous species richness; **b** heteroxenous/monoxenous ratio; and **c** J value of grey mullet parasite communities sampled from the Israeli Mediterranean coast during 1999–2000



	r^2	R (a/b)	a	b	c
Kishon early 90'	0.97	23.19	0.83	0.04	168
Kishon 2000	0.95	16.89	0.99	0.06	51

	r^2	R (a/b)	a	b	c
Ma'agan early 90'	0.96	33.08	1.2	0.04	128
Ma'agan 2000	0.97	31.86	1.82	0.05	63

Fig. 5 Long-term comparison of grey mullet (*Liza ramada*, *Liza aurata*) parasites species richness by sample size $Y=a(1-e^{-bx})/b$ sampled at the Israeli Mediterranean coast during 1993–1995 and 1999–2000. **a** Kishon Harbor**, b** Ma'agan Michael

Discussion

The characteristics of fish parasite communities as reflected by their biodiversity were found to be significantly different between reference and polluted sites. As expected, species richness and diversity indices as well as Sh/Sm were higher at the reference site compared with the polluted KH site. Kishon Harbor is known as an extremely polluted location. High levels of heavy metals and nutrients have been measured and reported, both in the water column as well as in the sediment (Herut et al. 1995, 2000).

A significant finding of this study was the complete absence of gut helminths (nematodes, trematodes, cestodes and acanthocephalans) from grey mullet sampled at the polluted site. Also, the abundance of the encysted metacercariae was significantly lower at KH in comparison to MM. At the community level, the absence of these heteroxenous parasites is mirrored by a lower mean species richness and "true" species richness of heteroxenous parasites than at the reference site. The grey mullet's heteroxenous parasites are clearly susceptible to pollution. Oil, heavy metals and anaerobic conditions have been found to be toxic to adult trematodes inside their host fish (Overstreet and Howse 1977; Kiceniuk and Khan 1983; Overstreet 1988; Khan and Thulin 1991; Valtonen et al. 1997), and lethal to free-living stages (e.g. cercariae and miracidia) as well as to mollusc intermediate hosts (Evans 1982; Munkittrick and Dixon 1988; Siddall et al. 1993). The present study lends additional support to this view that pollution compromises heterox-

enous species by blocking the completion of their life cycles.

Monoxenous parasites appear to be better adapted for survival in some polluted habitats. Various studies have demonstrated that aquatic eutrophication resulted in the proliferation of trichodinid ciliates and suggested that these protozoans may be valuable bioindicators for such contamination (Yeomans et al.1997; Broeg et al. 1999). Laboratory experiments have shown that exposure of fish to oil contamination resulted in an increase in monoxenous gill parasite prevalence (Monogenea and trichodinids) (Khan 1990; Khan and Kiceniuk 1988). The present results fit this pattern. The monoxenous parasite species richness as well as the prevalence of parasitic copepods were both higher at the polluted site than at the reference site. Copepod populations may have been enhanced by possible immune suppression of the KH grey mullet, as manifested by the decreased activity of blood phagocytic cells and increase in blood lysozyme activity (Bresler 2000). These findings are in agreement with our results that the fish sampled at KH had a significantly lower condition factor than those collected at the reference site MM.

Monogenea, on the other hand, were less abundant at the polluted site. Khan (1990) reported an increased rate of infection in Monogenea associated with oil pollution. It has been suggested that at chronic, sub-lethal levels of exposure, fish skin and gills secrete abundant mucus, which in turn may act to enhance monogenean and trichodinid rates of infection (Khan 1987; Khan and Kiceniuk 1988; MacKenzie et al. 1995). The apparent inconsistency of our findings with these reports may perhaps be explained by the variable susceptibility of parasite species, length of exposure time, concentrations and the nature of the pollutant (Lafferty 1997; Marcogliese and Cone 1997).

In this study, the exponential model that extrapolates the "true" species richness of a given habitat as a function of increasing sample size (Walther et al. 1995) was successfully implemented. The application of this approach has previously been evaluated successfully for fish parasite communities in freshwater (Gelnar et al. 1997) and with partial success also in marine environments (D'Amelio and Gerasi 1997). We employed the same approach proposed by Diamant et al. (1999) in which heteroxenous and monoxenous parasite species curves were calculated separately. Our results indicate that this approach differentiates between data and produces more accurate and significant results. Our analyses indicate that, in some cases, the heteroxenous and monoxenous curves displayed opposite trends at the same sampling site. Thus, pooled analyses of the data curves would be expected to cancel each other.

Differences between sampling sites demonstrated by "true" species richness curves of the habitat in this study agree with the results obtained by mean species richness per fish values. This was clearly demonstrated in the opposite trends of the heteroxenous and monoxenous mean species richness values per fish, which showed similar trends at both habitats with regard to "true" species richness.

A marked seasonal increase in evenness (J) was caused mainly by the increase in monoxenous species richness at the polluted site that led to a respective decrease in Sh/Sm during fall samples. At the same time, no seasonal changes in any of the indices were observed at the reference sites. It is, however, noteworthy that the copepod *C. mugilis* lodges in the opercular tissue. On the eastern Mediterranean coast of Israel, seawater surface temperature ranges between 16–17°C in the winter and 30–31°C in summer (Meteorological Services, State of Israel). During the summer months, the outflow of the Kishon River is comprised mainly of industrial and domestic effluents, unlike the winter months when this waste is diluted by freshwater (Kress and Herut 1998). We cannot, as yet, offer any explanation for the increase in monoxenous parasites generated by the increase of the copepod *C. mugilis* towards the end of the summer.

Significant changes have been noted when comparing grey mullet parasite communities at the same sampling sites over the period between 1993–1995 and 1999–2000. The decline of biodiversity at KH is clearly evident from the decline in "true" species richness as well as the disappearance of two trematode species (*H. benedenii* and *S. tensum*) found in fish sampled during 1993–1995 (Gerasi 1996). At MM, in the 1999–2000 samples, fewer fishes were found infected than in the previous year's samples, although their mean infection load, with the same parasites, was significantly higher. Differences between sampling years may be attributed to expected annual fluctuations in the infestation process and are most likely to be linked to the annual variation in overall physical and biotic conditions in the marine, coastal waters.

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