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ORIGINAL ARTICLE

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Parasites of flounder *Platichthys flesus* (L.) from the German Bight, North Sea, and their potential use in ecosystem monitoring

B. Community structure and fish parasite biodiversity

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Abstract The analysis of fish-parasite community structure and the use of ecological richness and diversity measurements are commonly used for the evaluation of environmental stress in aquatic ecosystems. As part of an integrated biological-effect monitoring, the parasite community of flounder *Platichthys flesus* (L.) was investigated for various locations in the German Bight during spring and autumn of 1995–2000, using established ecological methods. Although the parasite-community composition was very similar at the component-community level, the number of component species as well as the species accumulation curves showed clear differences among the sites. On the infra-community level, all of the ecological measurements showed significantly lower values in flounder from the Elbe estuary, the most polluted site, than in flounder from Helgoland. This was seen during a single season or during both seasons. When the data were pooled over the years, gradual differences between the sites, which were seldom detected at individual sampling periods, became evident for different measurements of species richness and species diversity and corresponded to a contamination gradient established between Elbe > Inner Eider, Outer Eider > Helgoland. Despite seasonal variations, which were observed in almost all measurements, these gradual differences were found in both seasons.

Keywords *Platichthys flesus* · Parasite community · Diversity · Heteroxenous/monoxenous species ratio · Biological effects monitoring

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Introduction

The analysis of the fish-parasite community structure and the use of ecological richness and diversity measurements are widely applied in the evaluation of environmental stress in aquatic ecosystems. Basic assumptions are that biotic diversity is highest in undisturbed environments, whereas man-made stress, as pollution, leads to a loss of species, and a reduction of diversity (Kennedy 1997).

The simplest and oldest diversity measurement is the number of species, or species richness, which only registers the number of parasite species present in a community. A more extensive approach is the calculation of species richness in relation to sampling effort, as suggested by Walther et al. (1995). This is an estimation of the true species richness at the location under study by the analysis of exponential accumulation curves, in order to determine the optimal sampling strategy. Species-diversity indices combine the information of species richness and the relative abundance of each species. Commonly used indices are the Shannon–Wiener index and the inverse Simpson index of diversity, as well as Shannon's Evenness (Magurran 1988). The problem that these indices are sensitive to the presence or absence of one or few predominant species in the community and that protistans and procaryotes must be excluded from these calculations, owing to difficulties in counting individuals of these organisms is frequently discussed (D'Amelio and Gerasi 1997). Anyway, the use of biotic indices has many advantages. Without knowledge of the identity of every single species or its susceptibility to known pollutants, biotic indices can summarise the situation in a habitat over time, or its response to changing pollution levels and it may indicate new and unexpected sources of pollution (Kennedy 1997).

A second approach to the assessment of environmental stress on parasite communities is to study the presence of heteroxenous and monoxenous parasite species in relation to different environmental conditions, as suggested by D'Amelio and Gerasi (1997). The underlying hypothesis is that, for transmission, heteroxenous parasites with complex, multi-host life-cycles depend on the presence of a variety of invertebrate and vertebrate intermediate hosts. These parasites can persist only in those environments in which all species required as intermediate and final hosts can co-exist. In disturbed habitats with a reduced overall diversity, the number of heteroxenous parasite species may also decline, owing to the lack of required hosts. In such environments, monoxenous species, which only need a single host species to reproduce, may predominate (D'Amelio and Gerasi 1997).

In the framework of biological-effects monitoring, Broeg et al. (1999) investigated the parasite community of flounder at different locations in the German Bight, by means of the two methods mentioned above and assessed their potential use as bio-indicators of pollution effects. Species richness and the ratio of heteroxenous to monoxenous species were used. In these investigations, species richness seemed to be a useful indicator: the number of parasite species was reduced at the site with the highest contamination load, the Elbe estuary, when compared to the less contaminated sites of the study. The ratio of heteroxenous to monoxenous species, however, did not lead to a clear separation of the sites. An additional problem arose from natural influences on the parasite community, which could not be separated clearly from pollution-mediated effects (Broeg et al. 1999).

The present study was a continuation of the previous work by Broeg et al. (1999). Flounders were collected at the same locations and the two data sets were combined to obtain a more extensive data base collected over a period of 6 years. This allowed a detailed investigation of the parasite community of flounders in the German Bight using common and established ecological concepts, such as species richness and diversity, as well as the alternative approach of the ratio of heteroxenous to monoxenous species and to observe natural variations such as spatial and temporal fluctuations or the relation of the ecological measurements to host-related factors like sex, body length or condition factor.

Pollution-mediated effects on the parasites and a comparison of the parasitological findings with responses of other biomarkers are presented elsewhere (Schmidt et al., submitted).

Methods

During spring and autumn of 1995–1997 and 1999–2000, individuals of European flounder *Platichthys flesus* (L.) were sampled by research-vessel trawl catches at five locations in the German Bight, North Sea.

Details of sampling, examination of flounder and parasite species have been described elsewhere (Schmidt et al. 2003). Numbers of evaluated fish specimens for each sampling campaign at each site, a list of the parasite taxa recorded during the course of the study and information about their infection levels at the sampling locations are presented by Schmidt et al. (2003).

The parasite-community structure of the flounder was examined first at the infra-community level, which is the community of parasite infrapopulations on a single host, and second at the component-community level, which is the community of parasite infrapopulations associated with a subset of a host species (Bush et al. 1997).

Measures of the component community were the total number of parasite species and the number of component and rare species per location as defined by Bush et al. (1990).

For an estimation of real species richness at a given site, depending on sample size, Walther's graph (Walther et al. 1995) was calculated according to the formula: $y=a(1-e^{-bx})/b$, where a is the increase in species richness at the beginning of sampling, b is the parameter that sets the species-richness asymptote $R=a/b$, and x is the unit of sampling effort.

Similarity in the parasite community among investigated sites was evaluated by Sorenson's index, according to Magurran (1988), which was calculated qualitatively as $C_S=2j/(a+b)$, where j is the number of species found jointly in two samples, a is the number of species in the first sample, b is the number of species in the second sample. It also was calculated quantitatively according to the formula: $C_N=2j_N/(a_N+b_N)$, where a_N is the number of individuals in sample a, b_N is the number of individuals in sample b, j_N is the sum of the lower of the two abundances of species which occur in the two samples. Sorenson's indices that exceed values of 0.6 are considered to indicate similarity, and values of more than 0.8, great similarity.

Measures of the infra-community were the mean number and range of parasite species found on individual flounders, defined as species richness (S; Bush et al. 1997), the mean number of macroparasite individuals and the following ecological indices, which were all calculated for individual fish:

the Shannon–Wiener index of diversity ($H'=-\sum(p_i \ln p_i)$, where p_i is relative intensity of parasite species i; completed by evenness ($E=H'/\ln S$, where S is total number of parasite species) and inverse Simpson index $\left(D=1/\sum p_i^2\right)$. The Shannon–Wiener index is weighted towards the richness of a community, and the Simpson index is weighted towards most abundant species (Magurran 1988).

Increasing values of the Shannon–Wiener index and of the inverse Simpson index indicate an increase in diversity. Values of evenness can range from 0 to 1. Values of 0 indicate a completely uneven distribution of parasites among hosts; values of 1, a totally even distribution. All indices were calculated according to Magurran (1988).

To evaluate the ratio of heteroxenous to monoxenous species, numbers of heteroxenous species (H_{sp}) and monoxenous species (M_{sp}) were counted and the ratio (H_{sp}/M_{sp}) was calculated according to D'Amelio and Gerasi (1999).

In the calculations presented here, all measurements of species richness (S, H_{sp}, M_{sp}, and H_{sp}/M_{sp}) were based on all parasite species of the community (micro- and macroparasites), whereas all measurements of diversity (H', E, $1/D$) were based only on countable macroparasite species.

Statistical analysis

Most of the data were not normally distributed (Kolmogorov–Smirnow test). Data of the Simpson index were normalized by logarithmic transformation [$\log_{10}(N+1)$], those of evenness, by potential transformation (x^2). These normalized data were compared by Student's *t*-test or by ANOVA and Tukey's post hoc comparison of means, whereas not normally distributed data were compared by the non-parametric Mann–Whitney *U*-test or Kruskal–Wallis ANOVA and Dunn's post hoc test. Differences between groups were considered as significant at a probability of error of $P<0.05$. Correlation coefficients were calculated with the parametric Pearson's product moment correlation or Spearman's rank correlation . Correlations were considered as significant at a probability of error of $P<0.05$. The analyses were carried out using the computer programmes SigmaStat 2.0 and STATISTICA 6 (StatSoft).

Results

Component community

During nine sampling periods in spring and autumn of 1995–1997 and 1999–2000, 802 flounders were dissected. From these fish, parasites from 30 different taxa were identified. Twenty-four species of countable macroparasites were found (1 monogenean, 6 digenean trematodes, 4 cestodes, 5 nematodes, 3 acanthocephalans and 5 copepods), comprising 77,611 individuals, and 6 species of non-countable microparasites (1 Apicomplexa, 2 Microsporea, 1 Ciliophora and 2 Myxozoa) were recorded. Nine taxa had a monoxenous development and 21 taxa a heteroxenous life-cycle.

A list of all parasite taxa and information on the relative abundance of the macroparasites and the presence of microparasites is given in Table 1 for all locations investigated.

Parasites of 17 taxa were present at all sampling sites, and 14 of these 17 species displayed prevalences of 10% or more at one or several sampling sites and thus were regarded as component species (Bush et al. 1990). Of these, seven taxa reached very high prevalences of more than 60% at one or more sites: the copepods *Lernaeocera branchialis*, *Lepeophtheirus pectoralis* and *Acanthochondria cornuta*, the helminths *Zoogonoides viviparus* and *Cucullanus heterochrous*, the metacercaria of an unidentified digenean species and the ciliate protozoan *Trichodina* spp. These taxa dominated the parasite community both by prevalence and by intensity and, except the protozoan, accounted for 96–99% of the total number of countable parasite individuals found at each of the site (Table 1). At all sampling sites, *L. branchialis* was the predominant species in prevalence and intensity, followed by the metacercaria. The infection characteristics of these seven parasite taxa, some of the natural factors influencing their infection levels, as well as their potential use as indicator species, are presented in detail elsewhere (Schmidt et al. 2003).

At all locations, the cumulative number of parasite species was similar. Flounder from the Elbe and the Outer Eider estuary harboured 26 parasite species, and individuals from Helgoland and Spiekeroog, 25 and 24 species,

Table 1 List of parasite species recovered from flounder in the German Bight during sampling periods in spring and autumn 1995–2000. For macroparasite species, the relative abundance is given as the proportion (pi) of the total number of all macroparasites of all species at the five sampling locations in the German Bight. Taxa in bold letters were the most abundant species. Life cycle: *m* monoxenous, *h* heteroxenous species, + present (for non-countable microparasites), */c* component species at the sampling location

Taxonomic group	Parasite taxa	Target organ	Life-cycle	Localities				
				Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Apicomplexa	*Epieimeria*sp.	Gut	m	+ /c	+ /c	+ /c	+ /c	+ /c
Ciliophora	*Trichodina*spp.	Gills	m	+ /c	+ /c	+ /c	+ /c	+ /c
Microsporea	Microsporea sp.1	Kidney	m	+ /c	+ /c	+ /c	+ /c	+ /c
	Glugea stephani	Gut	m	–	+	+	–	+
Myxozoa	Myxozoa sp. 1	Kidney	h	+	+ /c	+	+	+ /c
	Myxidium incurvatum	Gall bladder	h	+ /c	+ /c	+ /c	+ /c	+ /c
Monogenea	*Gyrodactylus* sp.	Gills	m	0.0002	0.0003	0.0005	–	–
Digenea	*Derogenes varicus*	Gut	h	0.0004	0.0015	0.0005	0.0009	0.0003
	Brachyphallus crenatus	Gut	h	0.0054	0.0005	0.0002	0.0004	0.0011
	Zoogonoides viviparus	Gut	h	0.0141	0.0810 /c	0.2648 /c	0.0256 /c	0.0121
	Lecithaster gibbosus	Gut	h	–	–	0.0001	0.0001	0.0002
	Podocotyle atomon	Gut	h	0.0001	0.0024	0.0018 /c	0.0010	0.0010 /c
	Metacercaria sp. 1	Gills	h	0.2088 /c	0.1648 /c	0.1812 /c	0.2152 /c	0.4674 /c
Cestoda	*Bothriocephalus* spp.	Gut	h	0.0003	0.0001	0.00004	0.0001	
	Proteocephalus sp.	Gut	h	0.0023	0.0002	–	–	–
	Cestoda larvae sp. 1	Gut	h	–	–	0.00004	0.0001	–
	Cestoda larvae sp. 2	Gut	h	0.0008	–	–	–	–
Nematoda	*Paracapillaria gibsoni*	Gut	h	0.0039	0.0078 /c	0.0311 /c	0.0041 /c	0.0006
	Cucullanus heterochrous	Gut	h	0.0115 /c	0.0122 /c	0.0220 /c	0.0105 /c	0.0070 /c
	Dichelyne minutus	Gut	h	0.0034 /c	0.0027 /c	0.0008 /c	0.0005	–
	*Goezia*sp.	Gut	h	0.0004	0.0001	–	0.0008	–
	Hysterothylacium aduncum	Gut, liver	h	0.0035 /c	0.0009 /c	0.0016 /c	0.0038 /c	0.0030 /c
Acanthocephala	*Corynosoma*sp.	Gut	h	0.0007	0.0001	0.0001	0.0005	–
	Echinorhynchus gadi	Gut	h	0.0016 /c	0.0001	–	–	0.0005
	Pomphorhynchus laevis	Gut	h	–	–	–	–	0.0037
Copepoda	*Acanthochondria cornuta*	Gill cavity	m	0.0161 /c	0.0716 /c	0.0703 /c	0.0595 /c	0.0151 /c
	Caligus elongatus	Skin	m	0.0001	0.0006	0.0016 /c	0.0009 /c	–
	Holobomolochus confusus	Nose cavity	m	0.0002	0.0006	0.0016	0.0003	0.0003
	Lepeophtheirus pectoralis	Skin, fins	m	0.1310 /c	0.0973 /c	0.0674 /c	0.0959 /c	0.0133 /c
	Lernaeocera branchialis	Gills	h	0.5951 /c	0.5552 /c	0.3544 /c	0.5799 /c	0.4748 /c

Table 2 Total number of species in the parasite component community of flounder at five sampling locations in the North Sea

	Elbe	Outer Eider	Helgoland	Spiekeroog
No. of fish evaluated	230	228	193	118
No. of species	26	26	25	24
No. of component species (≥10%)	12	14	15	13
No. of rare species (<10%)	14	12	10	11

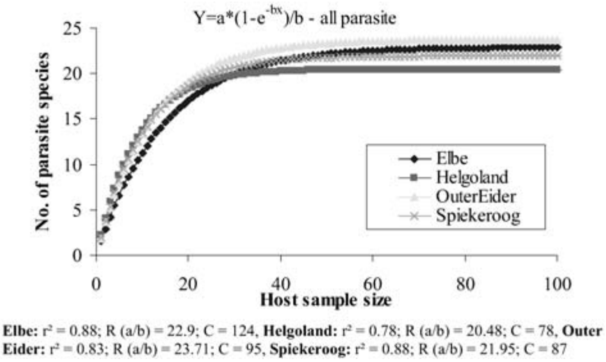

Fig. 1 Total species richness of flounder in the North Sea as a function of the number of hosts examined. Data are plotted according to the exponential species accumulation model proposed by Walther et al. (1995); r^2 regression coefficient, *R(a/b)* calculated "true" species richness, *C* capacity or number of hosts needed to reach "true" species richness

respectively. The number of component species, however, differed among sites and was lowest in the Elbe estuary, with 12, and highest at Helgoland, with 15 species, while the number of rare species was highest in the Elbe estuary, with 14, and lowest at Helgoland, with 10 species (Table 2).

For an estimation of the true parasite richness related to the sample size, a richness sampling-effort curve, according to Walther et al. (1995), was calculated. Inner Eider could not be included, because here the sample size was too small for this procedure. Figure 1 shows that a continuum maximum was reached at a sample size of about 124 individuals at Elbe and 78 individuals at Helgoland. At Outer Eider and Spiekeroog, 85 and 87 specimens were needed, respectively. Thus, at all four sites, the number of fish investigated during the present study was sufficient to detect the real species richness.

When the data were evaluated separately for the two seasons, the total number of parasite taxa was lower than in the combined data set. At Helgoland, in both seasons, 22 parasite species were present on the flounder; at other locations, such as the Elbe or the Outer Eider estuary, slight seasonal differences were recorded. At all sites, the number of species was similar during both seasons. (Table 3). At the Inner Eider location, flounder could only be collected during spring sampling periods. In this season, the number of parasite species from individuals at the Inner Eider estuary location was equal to that recorded from individuals collected in the Elbe estuary (Table 3). During both seasons, the number of component species was higher at Helgoland than in the Elbe estuary, and the number of rare species was higher in the Elbe estuary than at Helgoland. At the other sites, the number of species varied and sometimes it was similar to that of the Elbe estuary and sometimes similar to that of Helgoland (Table 3).

L. branchialis was the predominant parasite species at all sites in both seasons, except for the autumn sampling periods at Helgoland, where *Z. viviparus* was predominant.

Sorensen's qualitative and quantitative indices of similarity indicated that the composition of the parasite community was highly similar between the sampling sites (Table 4). The values for the qualitative Sorenson's index were 0.76–0.88 in spring and 0.78–0.89 in autumn. Similarity in the number of individuals, as calculated by Sorensen's quantitative index, had a greater range, of 0.45–0.94 in spring, and 0.63–0.99 in autumn. In both seasons, the lowest similarity in the number of individuals was found between the Elbe estuary and the Helgoland and Outer Eider sites offshore. The highest similarity was found between the Inner Eider estuary and Spiekeroog, as well as between Helgoland and the Outer Eider estuary.

At all locations, the composition of the parasite fauna was highly similar in spring and in autumn. This similarity between the seasons was seen in species composition as well as in numbers of parasites at all sites (Table 4).

Infra-community

All flounder individuals investigated were infected with one or more parasite species. A maximum of 11 parasite taxa was recorded from individual fish from Helgoland and the Outer Eider estuary. During single sampling periods (data not shown), the mean number of parasite taxa (species richness) ranged between 3 and 7. In almost all sampling periods, fish from the Elbe estuary harboured significantly fewer parasite species than fish from Helgoland and the Outer Eider estuary (Table 5). The mean number of macroparasite individuals per fish ranged between 5 and 180. In most of the sampling periods, flounder from the Elbe estuary were significantly less infected with macroparasite individuals than fish from Helgoland or the Outer Eider estuary (Table 5). Mean values of the Shannon–Wiener index of diversity per fish ranged between 0.12 and 1.25, mean values of the inverse Simpson index, between 1.1 and 3.1. During the sampling periods of 1995–1997, fish from the Elbe location exhibited significantly lower diversity values for both diversity measurements than fish from Helgoland or the Outer Eider. Between the latter two sites, differences in

Table 3 Seasonal changes in the total species number of the parasite component community of flounder (*Platichthys flesus*) at five sampling locations in the North Sea

	Elbe		Outer Eider		Helgoland		Spiekeroog		Inner Eider
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
No. of fish evaluated	100	100	97	101	84	100	48	70	33
No. of species	21	23	24	22	22	22	20	19	21
No. of component species (≥10%)	11	9	12	12	16	12	12	9	12
No. of rare species (<10%)	10	14	12	10	6	10	8	10	9
Predominant species	*L. branchialis*	*L. branchialis*	*L. branchialis*	*L. branchialis*	*L. branchialis*	*Z. viviparus*	*L. branchialis*	*L. branchialis*	*L. branchialis*

diversity were not found in 1995–1997, but from 1999 to 2000, diversity values were highest in fish from Helgoland than at any other site under study (Table 5). Mean values of evenness ranged between 0.2 and 0.8. Significant differences were found between the sampling sites in only half of the sampling periods. The results were highly variable, and only in two sampling periods, was evenness significantly lower in fish from the Elbe estuary than in fish from Helgoland or the Outer Eider estuary (Table 5).

Mean numbers of heteroxenous (H_{sp}) and monoxenous species (M_{sp}) ranged between 1 and 4. While H_{sp} was significantly lower in fish from Elbe estuary than in fish from the Outer Eider estuary and Helgoland, in almost all sampling periods, M_{sp} exhibited the same differences in only half of the sampling periods (Table 5). Mean values of the H_{sp}/M_{sp} ratio were in the range of 0.45 and 1.1. Significant differences between sites were only found during three sampling periods. Then fish from the Elbe had lower H_{sp}/M_{sp} values than fish from the coastal and offshore locations (Table 5).

All parasitological measurements, except for species richness, exhibited strong seasonal variations. The diversity measurements H', E and $1/D$ and the number of monoxenous species (M_{sp}) were significantly higher in autumn than in spring ($P<0.001$), whereas the number of macroparasite individuals (N), the number of heteroxenous species (H_{sp}) and the H_{sp}/M_{sp} ratio were significantly higher in spring than in autumn ($P<0.001$).

Therefore, data were summed across the sampling periods by season, in order to evaluate observations over the complete sampling period. The results are given in Table 6, by location and season.

Statistical evaluation of these data revealed that, in general, species richness, the number of macroparasite individuals and of heteroxenous species were significantly lower in fish from the Elbe estuary than in fish from all other sites, whereas the Shannon–Wiener index and the inverse Simpson index were highest in fish from Helgoland (Table 7).

For S, H', $1/D$ and H_{sp}, gradual differences were found during both seasons in an increasing order: Elbe < Outer Eider < Helgoland. For N, these differences were only found in spring (for H' and $1/D$, $P<0.001$ for all comparisons; for S, N, H_{sp},E<O, H, $P<0.001$; O<H, $P<0.05$). Gradual differences were also found: Elbe < Spiekeroog < Helgoland. In autumn these differences were evident for S, H' and $1/D$ (for $H''$and $1/D$, $P<0.001$; for S, E<S, H, $P<0.001$; S<H, $P<0.05$) and in spring they were detected for H_{sp} (S<H, $P<0.05$). In spring, additional differences were found in the order: Elbe < Inner Eider < Helgoland for the measurements of S and H_{sp} (for all comparisons $P<0.05$), and Elbe < Outer Eider < Inner Eider for N (O<I, $P<0.05$) (Table 7).

For M_{sp}, H_{sp}/M_{sp} ratio and evenness (E), differences between the sampling sites were not so marked. In spring, M_{sp} was significantly lower in fish from the Elbe estuary than in fish from Outer Eider estuary, Helgoland or Spiekeroog. In autumn, differences were only found

Table 4 Similarity of the parasite component community of flounder at five sampling locations in the German Bight of the North Sea in spring and in autumn, and a comparison between seasons at each site by qualitative Sorenson's indices and by quantitative Sorenson's indices. Upper half of spring and autumn panel: qualitative Sorenson index; lower half: quantitative index. *E* Elbe, *O* Outer Eider, *H* Helgoland, *S* Spiekeroog, *I* Inner Eider

Spring						Autumn					Comparison between seasons		
Sites	E	O	H	S	I	Sites	E	O	H	S	Sites	Qualitative	Quantitative
E	–	0.84	0.79	0.83	0.76	E	–	0.84	0.89	0.86	E	0.82	0.91
O	0.53	–	0.89	0.86	0.84	O	0.64	–	0.99	0.83	O	0.87	0.96
H	0.45	0.87	–	0.86	0.84	H	0.63	0.82	–	0.78	H	0.86	0.86
S	0.74	0.76	0.66	–	0.88	S	0.81	0.82	0.81	–	S	0.77	0.98
I	0.80	0.71	0.61	0.94	–								

Table 5 Differences in the parasite component community of flounder (*Platichthys flesus*) among five sampling sites in the German Bight, North Sea, during individual sampling campaigns. Level of significance $P<0.05$. Ecological indices: *S* species richness,*N* number of macroparasite individuals, *H′*Shannon–Wiener index of diversity, *E* evenness,*1/D* inverse Simpson index of diversity, H_{sp} number of heteroxenous species, M_{sp} number of monoxenous species, H_{sp}/M_{sp} ratio of heteroxenous to monoxenous species. The campaigns are identified by four-digit numbers: the first two digits encode the year; the last two encode the month. Sampling sites (in columns headed by campaign codes): *E* Elbe,*O* Outer Eider, *H* Helgoland,*S* Spiekeroog, *I* Inner Eider

Ecological Indices	9509	9604	9610	9704	9709	9904	9909	0004	0009
S	E<O, H	E<O, H	E<O, H	E<O, H, S	E<O, S<H	E<H	E, S<O, H	E<O, H ; I <H	–
N	E<O	E<H	E<O, H	E<O, H, S	E<O<H ; E<S	E<O, H, S, I	E, H<O, S	E<H, I	E<H
H'	E<O, H	E<O, H	E<O, H	E<O, H ; S<H	E<S<H ; E<O	E, O<H	E<S<H ; O<H	E, I <H ; I <O	E, O, S<H
E	–	E<O	–	S<H	E, S<O, H	–	O<E, H	–	E, O<H
$1/D$	E<O	E<O, H	E<O, H	E, S<O, H	E<S<O, H	E, O<H	E, O, S<H	I <H	E, O, S<H
H_{sp}	E<O, H	E<O<H	E<O, H	E<O, H, S	E<O, S<H	E<H	E<O, H	E<O, H ; S, I <H	E<H
M_{sp}	E<O	E<O, H	–	E<O, H, S	E<O<H ; S<H	–	E<O	E<H	S<E
H/Msp	–	–	E<O, H	–	E<O, H, S	–	–	–	E<O, H, S

between fish from Elbe estuary and Helgoland. No differences were found between sites when the H_{sp}/M_{sp} ratio and E were considered during the spring sampling periods, but in autumn, the H_{sp}/M_{sp} ratio was significantly lower in fish from the Elbe estuary than in fish from Helgoland, and values of E were significantly lower in fish from Elbe estuary and Spiekeroog than in fish from Helgoland (Table 7).

At individual sampling locations, the ecological measurements considered here exhibited annual variations. These variations were more evident in autumn sampling periods than in spring sampling periods. During the study, however, decreasing or increasing values of these measures could not be observed (data not shown).

Correlations of species richness and the diversity measurements with the sex of the flounder were not observed, but almost all of these measurements, except that of evenness, were correlated with fish length, (Table 8). Since the mean fish length was similar at all sampling sites (Elbe 21.2±2.3, Helgoland 23.1±1.8, Outer Eider 21.9±2.9, Spiekeroog 22.3±2.4, Inner Eider 20.8±2.8), this factor was neglected. Correlations of ecological measurements were also found with the condition factor of flounder (S: r=0.123; N: r=0.125; E: r=0.119; all $P<0.05$), but here the correlation coefficients (r) and level of significance were very low.

Discussion

The parasite community structure of flounder was investigated at the component-community and the infracommunity levels according to Bush et al. (1997), using established ecological concepts as species richness, species diversity, a species accumulation curve and the ratio of heteroxenous to monoxenous species, in order to characterise five locations in the German Bight that were considered to differ in their contamination load.

The basic hypotheses were first that species richness and species diversity are reduced in contaminated habitats and second that the ratio of heteroxenous to monoxenous species changes in favour of monoxenous species under such conditions.

Component community

In the present study, 30 parasite taxa were recorded from flounder in the German Bight, including 24 macro- and 6 microparasite species. Flounder from individual sampling sites harboured 24–26 taxa. In comparison with other regions of the North Sea and the Baltic Sea, the parasite component community of flounder in the German Bight, in general, can be considered as species-rich (Table 9).

The number of species in the component community was almost equal at all investigated sites. It is known that

Table 6 Seasonal changes in the diversity characteristics of the parasite infracommunity of flounder (*Platichthys flesus*) at five sampling locations in the North Sea. For all ecological measurements, mean values ± standard deviation are given; they were calculated from the number of specimens collected during 2–4 sampling campaigns in spring or autumn. For other explanations, see Table 5

Diversity characteristics	Elbe		Outer Eider		Helgoland		Spiekeroog		Inner Eider
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
No. of sampling campaigns	100	100	97	101	84	100	48	70	33
S (range)	4.0 (1–8)	4.5 (1–9)	5.9 (1–11)	5.9 (1–10)	6.7 (4–11)	6.8 (3–10)	6.3 (3–10)	5.3 (1–9)	5.6 (2–10)
N	43.2 ±65.2	38.6 ±38.8	121.3 ±100.1	82.9 ±78.5	174.9 ± 126.8	101.8 ±139.3	150 ±94.3	106.7 ±88.7	193.8 ±206.2
H'	0.40 ±0.39	0.63 ±0.31	0.77 ±0.37	1.03 ±0.35	0.97 ±0.35	1.21 ±0.27	0.65 ±0.36	0.92 ±0.31	0.58 ±0.35
E	0.41 ±0.38	0.58 ±0.27	0.53±0.22	0.67 ±0.21	0.57 ±0.18	0.73 ±0.16	0.41 ±0.21	0.63 ±0.20	0.47 ±0.26
$1/D$	1.48 ±0.61	1.70 ±0.51	1.89 ±0.67	2.48 ±0.93	2.22 ±0.77	2.88 ±0.87	1.62 ±0.55	2.19 ±0.70	1.67 ±0.60
H_{sp}	2.1 (0–5)	2.0 (0–5)	3.1 (1–7)	3.0 (0–6)	3.7 (1–7)	3.6 (1–6)	3.1 (1–6)	2.7 (0–6)	3.2 (1–6)
M_{sp}	1.9 (0–5)	2.5 (0–4)	2.7 (0–5)	2.8 (0–5)	2.9 (0–6)	3.2 (1–6)	3.2 (1–5)	2.6 (0–5)	2.5 (0–5)
H_{sp}/M_{sp}	0.87 ±0.65	0.56 ±0.35	0.99 ±0.86	0.83 ±0.40	1.10 ±0.69	0.95 ±0.40	0.81 ±0.45	0.80 ±0.42	1.05 ±0.64

Table 7 Spatial differences in the parasite fauna of flounder in the German Bight. Shown are the statistically significant differences in species richness and diversity measurements of the parasite infracommunity among the five sampling sites in both seasons. Level of significance: $P<0.05$. For explanations, see Table 5

Indices	Spring		Autumn
S	E<O, I<H	E<S	E<O, S<H
N	E<O<H, I	E<S	E<O, H, S
H', $1/D$	E<O<H	S, I<H	E<O, S<H
E	–	–	E, S<H
H_{sp}	E<O, I<H	S<H	E<O<H; E<S
M_{sp}	E<O, H, S	–	E<H
H_{sp}/M_{sp}	–	–	E<O, H

Table 8 Correlation coefficients (r) between fish length and ecological indices. For explanation, see Table 5

Indices	r
S	0.377 ***
N	0.350 ***
H'	0.228 ***
E	–
$1/D$	0.167 ***
H_{sp}	0.353 ***
M_{sp}	0.213 ***
H_{sp}/M_{sp}	0.200 ***

the invertebrate and parasitic fauna, in general, is reduced in the central Elbe estuary, owing to natural variations in habitat conditions (Möller 1990), but parasite species are occasionally introduced from marine as well as from limnetic environments into the estuary by invading hosts. This explains why the total number of parasite species found in the Elbe estuary was as high as at the other sites. When a calculation of the true species richness according to the procedure of Walther et al. (1995) was considered, a different picture was observed. In the Elbe estuary much more flounder individuals were needed for a good estimate of the true richness than at all other sites. This was obviously a result of the lower number of parasite species individual fish were infested with at this location. The distribution of component and rare species at the sampling sites also underline the influence of different habitat conditions. More than half of the recorded species occurred at all sites, but most of them reached highest prevalences at Helgoland, the site with the most constant habitat conditions (see also Schmidt et al. 2003). Here the highest number of component species and the lowest number of rare species were found. In the Elbe estuary, one of the sites with most varying habitat conditions, the lowest number of component species and the highest number of rare species were observed.

As the Sorenson's index indicated, the composition of the parasite component community was very similar at all locations under study, obviously attributable to the high number of species that occurred at three, four or all sampling locations. The high similarity in parasite numbers, especially between the offshore sites, the Outer Eider estuary and Helgoland, and between Spiekeroog

Table 9 Number of parasite taxa reported in studies on the parasite community of flounder (*Platichthys flesus*) from different areas in the North Sea and the Baltic Sea. *N* Number of fish

Study	Year	Area	Habitat	*N* (fish)	Parasites		Parasites[a]	
					Micro-	Macro-	Micro-	Macro-
MacKenzie and Gibson (1970)	1970	Scotland	Ythan estuary	900	–	27	–	13
Lile (1989)	1989	North Norway	Marine waters	?	–	14	–	10
Lüthen (1989)	1989	East Germany	Baltic Sea	569	6	28	2	11
Levsen (1990)	1990	West Norway	Marine waters	76	–	19	–	14
El-Darsh and Whitfield (1999)	1999	England	Thames estuary	390	1	23	1	16
Køie (1999)	1999	Transect	Baltic Sea	200	–	27	–	11
Present study	2003	Germany	German Bight	802	6	24	–	–

[a] Number of parasite species also found in the present study

and the Inner Eider estuary was mainly due to similar numbers of parasite individuals of the predominant crustacean species *L. branchialis* and of the metacercaria of an unidentified trematode species. A much lower similarity was found between the offshore sites and the Elbe estuary, where the number of macroparasite individuals was significantly reduced in comparison to the offshore sites.

Infra-community

Richness and diversity measurements of single flounder specimens also allowed a clear separation of the sites under investigation.

In both seasons, flounder from Helgoland had the richest and most diverse parasite community, when compared to the other sites. The largest infra-community consisted of 11 parasite species in hosts from Helgoland and the Outer Eider estuary; the highest mean number of 7 species was also found in fish from Helgoland. Community richness and diversity in fish from Elbe were significantly lower. At this station, the community consisted of 4 species, on average, with a maximum of 8 species, the lowest parasite number in flounder from the study area.

Studies of the parasite community of flounder at the infra-community level are lacking for other regions of the North Sea and Baltic Sea. Data are available from another flatfish species, the common dab (*Limanda limanda*), which was studied at different locations in the North Sea (Ibbeken and Zander 1999). Dab collected from a location close to the Helgoland location of the present study had a maximum of 4 parasite species, but almost half of the specimens were not infected with parasites at all. Dab from two Scottish locations harboured a maximum number of 9 parasite species, but the mean parasite number of the community was 4–5 species. Previous studies by MacKenzie and Gibson (1970), Lüthen (1989) and Levsen (1990), who compared parasite component-community richness of different flatfish species, including flounder and dab, showed that, of all flatfish species investigated, flounder exhibited the richest parasite community. Thus it is not surprising that the species richness of dab specimens was lower than of flounder specimens taken from the same location.

For the sampling locations of the present study, a contamination gradient between the sites—Elbe > Outer Eider > Helgoland and Elbe > Inner Eider > Helgoland—could be established in respect of the chlorinated-hydrocarbon residues in the muscle and the liver of flounder (Broeg et al. 1999) and in respect of heavy-metal residues in sediments and blue mussel (Schmolke et al. 1999). Corresponding gradual differences also could be observed when flounder parasites were considered. The prevalence of four of the dominant parasite species of the community showed these gradual differences when data from the 5 years of sampling were pooled (Schmidt et al. 2003) and in the present study, the measurements of species richness and diversity allowed us to establish these gradual differences even for shorter periods of 3 years in 1995–1997 and in 1997–2000. The Shannon–Wiener index of diversity displayed these differences already, when data from the April sampling periods of 1999 and 2000 were considered. These were the sampling periods that included sampling at the Inner Eider estuary location.

The evenness index was less suitable for the separation of the sampling sites. Especially in spring, it was low at all stations. Then the value range was 0.41–0.57. In autumn the values were significantly higher and then flounder from Helgoland had significantly higher values of evenness than individuals from the Elbe estuary or from Spiekeroog. In helminth communities in fish, evenness was strongly influenced by the presence or absence of a few, very abundant species (Poulin 1996). When these species were present in the community, they predominated by number and led to uneven communities. When the predominant species were absent, the other species coexisted at a lower overall abundance and no species became highly abundant. This led to more even communities (Poulin 1996). In the present study a group of seven parasite taxa dominated the community by abundance. Three of these taxa reached very high abundances at one or more sampling sites, and at all locations the copepod *L. branchialis* was the predominant species in the community. The influence of this species becomes clearly evident when seasonal variations of diversity and evenness are considered: all diversity measurements (H', E, $1/D$) exhibited higher values in autumn than in spring, although the number of component species was higher in spring than in autumn. In spring, however, the number of *L. branchialis* individuals was elevated, which most likely caused the reduced diversity and evenness indices compared to autumn, when a lower number of *L. branchialis* individuals were found on the flounder (Schmidt et al. 2003). This indicates that the parasite community was dominated by *L. branchialis*.

In the present study, all ecological measurements showed high variations between the years at all the sampling sites. Over the observation period of 5 years, a pattern or trend could not be discerned and therefore these fluctuations most likely occurred within the range of natural variability.

Despite seasonal and annual variations, a separation of the sampling locations was possible by the Shannon–Wiener index and the inverse Simpson index of diversity during both seasons. A separation of sampling sites by the calculation of H_{sp}/M_{sp} ratio, however, was not possible. In autumn, flounder from the Elbe estuary had lower values of H_{sp}/M_{sp} than individuals from the Outer Eider estuary and from Spiekeroog, but gradual differences between these sites, as described by diversity measurements, could not be detected. When numbers of heteroxenous and monoxenous species were considered separately, clear differences between the sites were found during both seasons. The values of H_{sp} exhibited gradual differences similar to species richness, and values of M_{sp} were significantly lower in fish from the Elbe estuary than in fish from Helgoland or from other sites.

Following the assumptions of D'Amelio and Gerasi (1997), monoxenous species should be accumulated under the unfavourable conditions found in the Elbe estuary, but in the German Bight, almost half of the monoxenous species were copepods, which seemed to be more vulnerable to environmental changes than their hosts. The prevalences and the individual numbers of these species did not accumulate but decreased under the habitat conditions of the Elbe estuary (Schmidt et al. 2003). Thus the values of the H_{sp}/M_{sp} ratio did not decrease in the Elbe estuary, the location considered to present a challenge.

When the two estuarine locations, the Elbe and the Inner Eider estuary, were compared, differences were detected by species richness, the number of macroparasite individuals and the number of heteroxenous species. While both species-related measurements, *S* and H_{sp}, showed gradual differences, Elbe < Inner Eider < Helgoland, individuals from the Inner Eider estuary harboured as many macroparasite individuals as flounder from Helgoland. When the biology of the parasite taxa (reviewed in Schmidt et al. 2003) that formed the community is considered, it becomes evident that the majority of the taxa are of marine origin. This means that their distribution as well as their reproductive ability is strongly influenced by hydrology, as reduced or changing salinity, since they are found in estuarine habitats. Therefore, prevalences and intensities of the most abundant species were reduced at both estuarine sites, compared to Helgoland or the Outer Eider estuary, but flounder from the Inner Eider estuary had significantly more parasites and, as confirmed by the diversity measures *S*, H_{sp} and *N*, a more complex parasite community than individuals from the Elbe location. In order to separate natural from pollution-induced effects, a comparison with the residue analysis and with the responses of several established biomarkers, which were also made in the study, is presented in a separate communication (Schmidt et al., submitted).

Conclusions

The present study showed that ecological concepts of parasite species richness and species diversity are useful indicators of changes in the parasite community structure at the sites under study. This was observed at the infra-community as well as at the component-community levels. Although the parasite community composition on the component community level was very similar at the sites, the analysis of species-accumulation curves exhibited clear differences between flounder from the Elbe estuary, the most polluted site, and the less polluted coastal and offshore sites, Spiekeroog, Helgoland and the Outer Eider. The number of component species was also lower in the Elbe estuary than at the other sites.

At the infra-community level, lowest values of parasite species richness and species diversity were found in fish from the Elbe estuary, when compared to fish from the other sites. When data were pooled, even gradual differences were observed among the sites, which corresponded to a contamination gradient (Elbe > Outer Eider, Inner Eider > Helgoland) established by Broeg et al. (1999) and Schmolke et al. (1999). Despite seasonal variation in the ecological measurements, these differences were found in both seasons.

The ratio between heteroxenous and monoxenous species was not as successful for the separation of sites, because heteroxenous as well as monoxenous species were reduced in number in fish from the Elbe estuary, so the ratio remained constant in most of the sampling periods.

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