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ORIGINAL ARTICLE

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

A. Infection characteristics of potential indicator species

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Abstract As part of integrated biological-effect monitoring, the parasite fauna of the flounder *Platichthys flesus* (L.) was investigated at five locations in the German Bight, with a view to using parasite species as bio-indicators. Over a period of 6 years, parasites from 30 different taxa were identified, but only 7 taxa of the parasite community occurred regularly at all locations and in sufficient abundance that they could be considered as potential indicator species. These species were the ciliophoran *Trichodina* spp., the copepods *Acanthochondria cornuta*, *Lepeophtheirus pectoralis* and *Lernaeocera branchialis*, the helminths *Zoogonoides viviparus* and *Cucullanus heterochrous* and metacercaria of an unidentified digenean species. Infection characteristics of these parasites are presented, with a comparison of the results from individual sampling periods and those of the long-term data set. Natural influences on the infection levels, such as temporal variations, habitat conditions and host-related factors, were evaluated. All of these parasite species showed significant differences in their infection levels between the Elbe estuary, as the most polluted site, and the less polluted coastal and marine locations: Helgoland, Outer Eider estuary and Spiekeroog, especially in the long-term data set. Gradual differences between the Elbe, the Outer Eider and Helgoland, which were not detected in individual sampling periods, also became evident in the pooled-data set. These were found in the prevalence of *Trichodina* spp., *A. cornuta*, *Z. viviparus* and *C. heterochrous*. Although salinity is considered as the most important natural factor, influencing the distribution pattern of the majority of the potential indicator species, infection levels of most of these species differed between locations with similar salinity conditions. Infection levels corresponded to a contamination gradient (Elbe > Inner Eider, Outer Eider > Helgoland) established across the locations. Seasonal variation in the infection parameters affected the spatial distribution of the copepod species *Lepeophtheirus pectoralis* and *Lernaeocera branchialis*. Annual variations are considered to occur in the range of natural variability, so no trend of increasing or decreasing infection levels of the parasites was found during the course of the study. This study underlined the idea that an analysis of fish-parasite fauna is very useful in ecosystem monitoring.

Keywords Parasites · Fish · *Platichthys flesus* (L.) · Biological effects monitoring · North Sea

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Introduction

The potential of fish parasites as indicators for pollution monitoring is widely and controversially discussed, because the presence and the infection levels of parasites are not only influenced by environmental contaminants but also by a variety of natural factors (MacKenzie et al. 1995; Kennedy 1997; Overstreet 1997). From previous work, reviewed by Overstreet (1997) and Kennedy (1997) for instance, it was concluded that a profound knowledge of the ecology of each parasite species and its tolerance to known pollutants would be required, in order to separate pollution effects from natural effects. Since the ecological and physiological requirements of many parasite species are still unknown, it is often recommended (Gelnar et al. 1997; Khan and Payne 1997; Overstreet 1997) that, in pollution monitoring studies, parasitological data should be accompanied by other types of data, such as biochemical bio-indicators.

This approach was followed in a study by Broeg et al. (1999) who, in the framework of biological-effects monitoring, investigated the parasites of the flounder *Platichthys flesus* (L.) at different locations in the German Bight for their potential use as bio-indicators of pollution effects and, for the first time, supplemented the parasitological data by well known biochemical and histochem-

ical biomarkers recommended by the ICES Advisory Committee on the Marine Environment (ACME) for application in biological-effects monitoring programmes (ICES 1996, 2002).

The results of this study were promising, since infection levels of several parasite species were reduced in the Elbe estuary which had a higher contamination load than the other sites considered. These parasitological findings were supported by the responses of other biomarkers, which are largely correlated with the parasitological data (Broeg et al. 1999), but the responses of very abundant Crustacea, Nematoda and Digenea to pollutants were most important, since little was known on the effects of natural influences on the distribution and infection levels of these parasites.

Therefore, in the present study, a detailed investigation of the infection characteristics of the parasite species of the flounder in the German Bight, which might be indicators of pollution effects, was conducted over a period of several years. In continuation of the work by Broeg et al. (1999), sampling was carried out at the same locations, and the two data sets were combined to obtain data for an observation period of 6 years.

Special attention was given to natural variations in the distribution and infection level of the parasites, such as spatial and temporal fluctuations or the relation of prevalence and infection intensity to host-related factors like sex, body length or condition factor. Ecological requirements and life-cycle of the parasites and their intermediate hosts were also considered, in order to evaluate their influence on the distribution of the parasites at different sampling sites.

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

Sampling

During spring and autumn of the years 1995–2000, nine sampling periods were carried out in the German Bight; 802 flounder, *Platichthys flesus* (L.), were collected. Catches were made with the research vessel "Uthörn" of the Alfred Wegner Institute, Bremerhaven, except for the site in the Inner Eider estuary, where a commercial fish trawler was used. Fishing was done with a bottom trawl; the fishing period was limited to 30 min. On board the vessel, the flounder were kept in tanks with permanent water flow-through and aeration for up to 6 h until dissection (for details see Broeg et al. 1999). At each location, 9 to 30 flounder in the size-class 18–25 cm were investigated. Fish with externally evident diseases were excluded from the investigation. Numbers of evaluated fish specimens for each sampling period and site are given in Table 1.

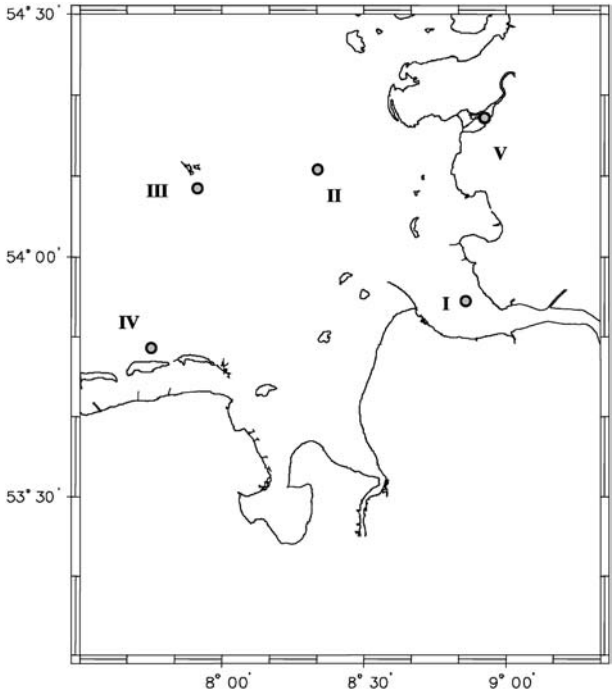

Fig. 1 Sampling locations of flounder *Platichthys flesus* (L.) in the German Bight, North Sea. *I* Elbe estuary (54°53'N, 8°47'E), *II* Outer Eider estuary (54°12'N, 8°25'E), *III* Helgoland "Tiefe Rinne" (54°06'N, 7°58'E), *IV* Spiekeroog (53°49'N, 7°44'E), *V* Inner Eider estuary (54°16'N, 7°50'E)

Study area

Five locations with differing habitats were selected for sampling (Fig. 1), four sites were identical to those sampled by Broeg et al. (1999): the Elbe estuary (considered to be a highly polluted estuarine site with fluctuating salinity and 6–10 m water depth); Helgoland "Tiefe Rinne" (considered to be a low-polluted offshore site with stable salinity and 20–40 m water depth), the Outer Eider estuary (considered to be a less polluted offshore site with stable salinity and 15–20 m water depth); and Spiekeroog (considered to be a highly polluted coastal site with stable salinity and 15–20 m water depth). As a fifth location, the Inner Eider estuary was included from April 1999 to September 2000. This station was

Table 1 Sampling of flounder parasites. Flounder were collected at five sites in the German Bight, during sampling periods 1995–1997 and 1999–2000. Given are numbers of flounder specimens evaluated at the sampling sites during individual collections. The sampling periods are identified by month and year; *Apr* April, *Sep* September, *Oct* October

Sampling period	Elbe	Helgoland	Outer Eider	Spiekeroog	Inner Eider
Sep 1995	30	9	30	–	–
Apr 1996	30	14	30	–	–
Oct 1996	30	30	30	–	–
Apr 1997	30	30	28	30	–
Sep 1997	30	30	30	30	–
Apr 1999	20	20	19	9	15
Sep 1999	20	20	21	20	0
Apr 2000	20	20	20	9	18
Sep 2000	20	20	20	20	0

considered to be a less polluted estuarine site with fluctuating salinity and 4–6 m water depth.

The sites were considered to represent a pollution gradient, with the highest pollution in the Elbe estuary, moderate pollution in the Outer Eider estuary, and the lowest pollution at Helgoland. The Inner Eider estuary and Spiekeroog were taken as "reference sites" for the Elbe and the Outer Eider estuary, representing similar salinity conditions and water depths, but differing in their pollution level. The impact of xenobiotics on the parasite community is considered in another communication.

Parasitological examination

On board, the flounder were examined for ectoparasites. Specimens were collected from the skin and stored in 70% ethanol for further counting and identification. Fresh smears were taken from skin, gills and nose cavity and immediately examined for the presence of parasites. Then the fish were killed and dissected. Gills then were fixed in 4% buffered (pH 7.2) formaldehyde solution. The gut and gall bladder were removed and opened. Fresh smears were taken directly from the gut and gall bladder epithelia and immediately examined for the presence of parasites. The gut was then transferred to saline solution (0.9% NaCl) and a drop of detergent was added. Under these conditions, parasites detached from the intestinal tissue and settled at the bottom of the vial. The supernatant fraction was discarded, the sediment resuspended in saline and again allowed to settle for a few minutes. After three washes organic waste was removed and the parasites were collected from the gut contents. The parasites were fixed in 70% ethanol for further investigation. Then gut, kidney, gall bladder and gills were fixed in 4% buffered formaldehyde solution. Transverse sections of mid- and hind-gut, as well as small parts of kidney, were used for histological investigation. Gills, gut and gut contents were examined for metazoan parasites. Parasites were collected, counted and stored separately for each fish. Sections of gut and kidney were processed by standard histological procedures (Romeis 1989), stained by Giemsa's technique and examined for tissue-invading parasites.

For identification of macroparasites, individuals were cleared in 80–90% lactic acid and mounted in glycerine jelly. Smears of *Trichodina* spp. were air dried and stained by Klein's silver impregnation method (Lom and Dyková 1992).

The parasites were identified according to standard literature (Yamaguti 1959, 1963a, 1963b, 1971; Kabata 1979) with the help of Dr. M. Køie (Marine Laboratory, Helsingør, Denmark), for digeneans, cestodes and acanthocephalans, and of Dr. F. Moravec (Institute of Parasitology, Ceské Budejovice, Czech Republic), for nematodes.

Parasite populations

Levels of parasite infections were analysed using the following ecological terms, according to the recommendations of Bush et al. (1997) and Rózsa et al. (2000):

Prevalence—the number of hosts infected with one or more individuals of a particular parasite species divided by the number of hosts examined for that parasite species, expressed as a percentage (%).

Mean infection intensity—the number of individuals of a particular parasite species found in a sample divided by the number of hosts infected by that parasite species.

Mean abundance—total number of individuals of a particular parasite species in a sample of a particular host species divided by the number of hosts of that species examined; mean abundance is equivalent to mean intensity multiplied by prevalence.

Prevalences were calculated for all parasite species, whereas intensity and abundance were calculated only for countable macroparasites. For *Trichodina* spp., a scale of abundance was used to classify infection strength: 0 absent; 1 1–3; 2 4–10; 310–20; 4 >21 individuals per slide.

Evaluation of data

Data analysis was carried out for single sampling periods as well as for the pooled data separated by season (spring or autumn). Infection levels of parasites are presented for single sampling periods using only prevalences and abundances, since the number of infected hosts was sometimes too low to compare intensities. For the long-term data set, mean values of prevalence and abundance, as well as intensities, are given, by season.

Evaluation of seasonal fluctuations in the prevalence and abundance of a particular parasite species was based on the pooled data set. The analysis of annual fluctuations that might occur between sampling periods during the course of the study was based on the abundance data of different sampling periods, separated by seasons.

The infection level of the parasites at different locations is shown for single sampling periods, as well as for the long-term data set.

Values of prevalence, intensity and abundance were compared in order to decide which of these parameters would be most suitable for identifying spatial differences.

Relations between fish parameters, such as sex and fish length and the infection levels of the parasites, were calculated using the entire data set.

Statistical analysis

Normal distribution of the data was tested using the Kolmogorov-Smirnow test. Data on intensity were normalized by logarithmic transformation [$\log_{10}(n+1)$]. The prevalence of a particular parasite at different locations or sampling periods was compared by a chi-square test. Normalized data on infection intensity were compared by Student's *t*-test and by ANOVA and Tukey's post hoc comparison of means; abundances were compared by the non-parametric Mann–Whitney's *U*-test or Kruskal–Wallis ANOVA and Dunn's post hoc test. Differences were considered to be statistically 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 to be 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

During sampling in spring and autumn of 1995–1997 and 1999–2000, 802 flounder were dissected. Parasites from 30 different taxa were identified, including 24 macroparasite and 6 microparasite taxa. A list of parasites and some of their biological characteristics are given in Table 2.

Prevalence, mean intensity and mean abundance of all parasite taxa are summarized in Tables 3 and 4, by sampling site and season.

In general, all of the fish examined were infected with one or more parasite species. The majority of parasite taxa showed an aggregated distribution pattern (data not shown).

Parasites of 17 different taxa were present at all sampling sites, but not all of them were found during both seasons or during each sampling period. Only 7 species/taxa were regularly present and sufficiently abundant over the whole sampling period to be considered as potential indicator species: the ciliophoran *Trichodina* spp., the

Table 2 List of parasite species recovered from flounder in the German Bight during nine sampling periods in spring and autumn 1995–2000 and some of their biological characteristics. For life-cycle: *mx* monoxenous, *hv* heteroxenous species. For parasite life-mode: *end* endoparasitic, *ec* ectoparasitic species. For origin: *m* marine, *e* estuarine, *l* limnetic species. For status *g* generalist, *s* specialist, *aut* autogenic. For location: *E* Elbe, *H* Helgoland, *O* Outer Eider, *S* Spiekeroog, *I* Inner Eider, *all* all locations; *u* status unknown, *?* uncertain

Taxonomic group	Parasite species	Target organ/tissue	Host	Life-cycle	Parasite life mode	Origin	Status	Location
Apicomplexa	*Epieimeria* sp.	Gut	Final	mx	end	u	u	all
Ciliophora	*Trichodina* spp.	Gills	Final	mx	ec	e	u	all
Microsporea	Microsporea sp.1	Kidney	Final	mx	end	u	u	all
	Glugea stephani	Gut	Final	mx	end	m	g, aut	H, O, I
Myxozoa	Myxozoa sp. 1	Kidney	?	hx	end	u	u	all
	Myxidium incurvatum	Gall bladder	Final	hx	end	m	?, aut	all
Monogenea	*Gyrodactylus* sp.	Gills	Final	mx	ec	m	u, aut	E, H, O
Digenea	*Derogenes varicus*	Gut	Final	hx	end	m	g, aut	all
	Brachyphallus crenatus	Gut	Final	hx	end	m	g, aut	all
	Zoogonoides viviparus	Gut	Final	hx	end	m	g, aut	all
	Lecithaster gibbosus	Gut	Final	hx	end	m	g, aut	H, S, I
	Podocotyle atomon	Gut	Final	hx	end	m/e	g, aut	all
	Metacercaria sp. 1	Gills	Intermediate	hx	end	m/e	u	all
Cestoda	*Bothriocephalus* spp.	Gut	Final	hx	end	m	g, aut	E, H, O, S
	*Proteocephalus*sp.	Gut	Final	hx	end	l/e	g, aut	E, O
	Cestoda larvae sp. 1	Gut	?	hx	end	m	u	H, S
	Cestoda larvae sp. 2	Gut	?	hx	end	l/e	u	E
Nematoda	*Paracapillaria gibsoni*	Gut	Final	hx	end	m	s, aut	all
	Cucullanus heterochrous	Gut	Final	hx	end	m/e	g, aut	all
	Dichelyne minutus	Gut	Final	hx	end	m/e	g, aut,	E, H, O, S
	Goezia sp.	Gut	Final	hx	end	m	u	E, O, S
	Hysterothylacium aduncum	Gut, liver	Final	hx	end	m/e	g, aut	all
Acanthocephala	*Corynosoma* sp.	Gut	Intermediate	hx	end	m	u	E, H, O, S
	Echinorhynchus gadi	Gut	Final	hx	end	m/e	g, aut	E, H, I
	Pomphorhynchus laevis	Gut	Final	hx	end	l	?	I
Copepoda	*Acanthochondria cornuta*	Gill cavity	Final	mx	ec	m	g, aut	all
	Caligus elongatus	Skin	Final	mx	ec	m	g, aut	E, H, O, S
	Holobomolochus confusus	Nose cavity	Final	mx	ec	m	g, aut	all
	Lernaeocera branchialis	Gills	Intermediate	hx	ec	m	g, aut	all
	Lepeophtheirus pectoralis	Skin, fins	Final	mx	ec	m	g, aut	all

copepods *Acanthochondria cornuta*, *Lepeophtheirus pectoralis* and *Lernaeocera branchialis*, the helminths *Zoogonoides viviparus* and *Cucullanus heterochrous* and the metacercaria of an unidentified digenean species.

In the following, infection characteristics of each of these species are shown for all of the sampling periods. Ecological measurements are compared with reference to their suitability for indicating differences between sites. The results of single sampling periods are compared with those obtained from the long-term data set. Seasonal and annual fluctuations are shown in order to decide whether there is a preferred season for sampling and whether a trend of increasing or decreasing infection levels of the parasites during the study can be observed. The relation of the infection levels of the parasites to such fish factors as length, sex, condition factor are evaluated, and their potential influence on the site- specific infection levels of the parasites assessed.

Trichodina spp. are monoxenous, ciliate protozoa that were found on the gills of flounder. It was not possible to identify this parasite to the species level.

During all sampling periods, prevalences and abundances of this parasite were significantly higher at the estuarine sites than at Helgoland (Fig. 2). When the spring sampling periods of the pooled data set (Table 5) were considered, gradual differences between sites were found in the prevalence and the abundance of *Trichodina* spp. in a descending order: Elbe, Inner Eider, Spiekeroog > Outer Eider > Helgoland (E, I, S>O, H, $P<0.001$; O>H, $P<0.05$). In autumn, no gradual differences were observed, but prevalence as well as abundance were significantly higher at the Elbe station than at all other sites ($P<0.001$).

A clear seasonal pattern in infection levels was not detected, but annual variation of abundance was more evident in autumn than in spring (spring: Outer Eider 97<96; autumn: Elbe 95<99; Outer Eider, all years <99; Helgoland 00<99; Spiekeroog 00<97, 99; $P<0.05$).

Abundance of trichodinids was negatively correlated with fish length ($R=-0.120$; $P<0.001$), whereas the condition factor exhibited a positive correlation ($R=0.15$; $P<0.01$). These correlations were due to differences in prevalence and not in infection intensity. Infection levels were not related to the sex of the flounder.

Table 3 Prevalence of parasites from flounder collected at five sampling sites in the German Bight in 1996–2000. Given are mean prevalence ± standard deviation calculated from the numbers of specimens collected during 2–4 sampling periods in spring and autumn. The number of specimens evaluated is given in Table 1

Species	Elbe		Outer Eider		Helgoland		Spiekeroog		Inner Eider
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
Number of sampling periods	4	4	4	4	4	4	3	3	2
Trichodina spp.	72.9±13.4	78±11.8	42.7±20.1	39.4±33.3	22.7±10.7	19.7±24.0	65.6±1.9	43.9±33.8	84.4±6.3
Acanthochondria cornuta	20.4±7.5	23.3±19.5	70.8±13.4	92.5±6.2	88.9±5.3	98.3±2.4	73.7±6.1	94.4±1.0	39.4±0.8
Lepeophtheirus pectoralis	26.5±10.4	94.7±3.8	74.5±11.7	97.3±2.8	89.2±6.4	94.9±9.7	75.9±8.5	97.2±2.5	36.1±3.9
Lernaeocera branchialis	72.5±25.0	86.3±26.0	98.8±2.5	98.7±1.8	100±0	89.1±19.3	100±0	91.1±15.4	100±0
Metacercaria sp. 1	32.5±17.5	27±15.8	68.1±16.3	42.6±24.5	63.4±22.5	46.6±22.0	49.6±14.3	82.8±7.5	85.6±11.0
Zoogonoides viviparus	5.8±5	2±3.0	27.7±16.4	28±29.3	66.0±29.0	63.1±32.7	23.7±11.4	0	3.3±4.7
Cucullanus heterochrous	23.7±14.9	24±15.2	47.4±10.4	40.6±24.0	72.3±9.2	55.8±22.5	51.9±17.0	35±13.2	22.2±15.7
Epieimeria sp.	36.3±23	7±9.7	46.5±13.7	13.8±16.2	41.9±17.9	24.1±19.1	37.8±13.5	6.7±11.5	51.6±18
Microsporea sp. 1 (kidney)	32.5±10.2	38.7±18.2	29.2±8.8	31±9.1	28.6±9.9	46.8±28.5	37±6.4	15.6±12.6	23.9±5.5
Glugea stephani	0	0	0.8±1.7	0.7±1.5	1.8±3.6	0	0	0	6.1±0.8
Myxidium incurvatum	23.9±16.4	14.7±20.2	22.2±6.8	15.7±11.3	22±18	29±22.1	26.3±6.1	15±21.8	14.9±10.3
Myxozoa sp. 1 (kidney)	7.5±9.6	5.7±10.9	10.2±10.8	1.3±3	0.8±1.7	0.7±1.5	7.4±12.8	7.2±7.5	23.9±5.5
Gyrodactylus sp.	0	2.2±3.8	3.3±4.7	0	3.6±5.1	1.1±1.9	0	0	0
Brachyphallus crenatus	4.6±4.2	1.3±3	5.1±7.1	0	2.6±3.4	1±2.2	7.4±12.8	0	9.4±5.5
Derogenes varicus	2.1±2.5	1±2.2	8.2±2.6	6.3±6.1	9.8±13.7	4.4±9.9	4.4±7.7	8.3±14.4	3.3±4.7
Lecithaster gibbosus	0	0	0	0	0	2±2.7	3.7±6.4	0	3.3±4.7
Podocotyle atomon	0	1±2.2	9.2±9.1	3±4.5	10.9±6	2.7±4.3	3.3±5.8	3.3±5.8	14.4±11
Bothriocephalus spp.	2.1±2.5	0.7±1.5	0	1.7±2.4	1.3±2.5	0	0	1.1±1.9	0
Proteocephalus sp.	4.2±5	0	0.9±1.8	1.7±2.4	0	0	0	0	3.3±4.7
Cestoda larvae sp.1	–	0	0	0	0.8±1.7	0	0	1.1±1.9	0
Cestoda larvae sp. 2	2.1±2.5	0.7±1.5	0	0	0	0	0	0	0
Dichelyne minutus	0	14.7±11	0.8±1.7	20.1±11.8	0	12.9±4.2	0	5.5±1	0
Goezia sp.	0	3.3±2.0	–	1.3±1.8	0	–	0	6.1±7.9	0
Hysterothylacium aduncum	14.2±9.2	2±3	10.4±7.6	6.2±6.4	15.3±13.9	0.7±1.5	19.3±5.1	7.8±6.9	25.6±20.4
Paracapillaria gibsoni	2.5±5	5.7±10.9	3.5±2.4	27.7±13.8	11.8±7.9	38.2±21.1	1.1±1.9	18.9±18.4	5.6±7.9
Corynosoma sp.	3.8±4.8	3±4.5	2.5±5	0	0	2±2.7	3.7±6.4	1.7±2.9	0
Echinorhynchus gadi	14.2±23.9	0	2.2±2.6	0	0	0	0	0	6.7±9.4
Pomphorhynchus laevis	–	0	0	0	0	0	0	0	–
Caligus elongatus	1.3±2.5	0	5.5±7.2	1.3±1.8	14.5±18.3	5.7±10.1	10.4±10	0	0
Holobomolochus confusus	0	3.3±4.7	4.8±8.2	5.8±9.6	9.5±12.4	7.5±15	3.3±5.8	0	5.6±7.9

Table 4 Infection parameters of parasites from flounder collected at five sampling sites in the German Bight in 1996–2000. Given are mean intensity and the corresponding 95% confidence interval and the mean abundance calculated from the number of specimens collected during 2–4 sampling periods in spring and autumn. The number of specimens evaluated is given in Table 1

Species	Elbe		Outer Eider		Helgoland		Spiekeroog		Inner Eider
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
Number of sampling points	4	4	4	4	4	4	3	3	2
Trichodina spp.	1	1.3	1	1	1.5	1.0	1	1	2
	1.1; 1.3	1.2; 1.4	1.3; 1.7	0.9; 1.1	0.9; 1.4	–	1.0; 1.3	1.0; 1.4	1.5; 2.2
	0.9	1.1	0.6	0.4	0.2	0.2	0.7	0.6	1.6
Acanthochondria cornuta	4	3	9.1	10.40	8.3	8.37	8.9	8.2	7.3
	2.0; 5.3	1.4; 3.8	6.6; 10.0	6.9; 9.8	7.7; 10.5	8.8; 12.0	6.8; 11.1	6.6; 9.9	3.3; 11.3
	0.7	0.6	6	8.5	8.2	10.2	6.7	7.7	2.9
Lepeophtheirus pectoralis	4.2	8.64	8.5	10.29	7.4	12.81	7.7	16.0	7
	2.4; 5.9	7.4; 9.9	6.1; 8.7	11.5; 14.1	6.7; 10.3	9.1; 11.5	5.9; 9.5	13.7; 18.3	2.9; 11.1
	1.1	8.6	5.6	13	7.6	10.5	6.3	15.6	2.5
Lernaeocera branchialis	34.0	25.2	85.9	18.1	76.4	39.1	114.9	46.8	90.7
	21.4; 46.5	20.2; 30.3	62.9; 89.9	29.8; 48.4	66.1; 105.6	15.4; 20.8	90.2; 139.6	32.8; 60.8	68.3; 113.0
	25.5	22.9	75.6	45.7	85.9	18.4	114.9	41.4	90.7
Metacercaria sp. 1	35.0	22.7	35.3	48.3	36.5	25.32	16.0	45.9	105.2
	11.4; 58.6	10.2; 35.2	18.1; 54.8	3.9; 46.7	14.3; 56.3	0.4; 96.1	8.5; 23.6	27.1; 64.6	26.4; 183.9
	11.2	7.3	24.1	13.2	23.5	26.5	8.7	38.7	89.2
Zoogonoides viviparus	16.5	9	69.68	46.13	18.2	30.43	30.9	–	76
	5.4; 38.4	25.4; 43.4	5.9; 30.4	17.2; 43.7	46.1; 93.7	33.8; 58.4	17.2; 27.0	–	–
	1	0.02	5.4	7.6	43.3	29.1	7.7	–	2.3
Cucullanus heterochrous	2	4.9	4.0	5	2.9	2.9	3.4	2.5	6.3
	1.5; 3.1	–1.9; 11.7	2.1; 3.6	2.1; 3.7	3.1; 4.9	3.4; 6.4	2.3; 4.5	1.3; 3.7	-5.1; 17.7
	0.6	0.4	1.4	1.2	2.9	3.2	2	0.8	1.3
Brachyphallus crenatus	12	1	3	–	2	1	3	–	2
	14.7; 37.7	–	0.9; 6.9	–	4.9; 7.9	–	22.4; 28.4	–	3.4; 8.1
	0.5	0.02	0.1	–	0.04	0.01	0.1	–	0.2
Derogenes varicus	1	2	2	2	1	2	2	1	2
	–	–	0.5; 3.3	0.3; 4.5	0.9; 1.4	4.9; 7.9	0.6; 2.4	0.7; 2.1	–
	0.02	0.02	0.2	0.2	0.1	0.2	0.1	0.1	0.1
Podocotyle atomon	–	–	3	6	5	2	4	1	1
	–	–	1.1; 5.6	7.3; 18.8	1.1; 10.7	4.9; 7.9	8.9; 16.9	–	0.7; 1.8
	–	–	0.3	0.2	0.5	0.04	0.3	0.03	0.2
Echinorhynchus gadi	1	–	2	–	–	–	–	–	2
	0.9; 1.4	–	4.9; 7.9	–	–	–	–	–	4.9; 7.9
	0.1	–	0.03	–	–	–	–	–	0.1
Caligus elongatus	1	–	2	2	2	1	2	–	–
	–	–	0.4; 3.2	10.7; 14.7	0.5; 5.2	–	0.4; 3.6	–	–
	0.01	–	0.1	0.04	0.4	0.1	0.3	–	–
Holobomolochus confusus	–	1	1	1	1	1	1	–	1
	–	–	0.5; 2.0	–	0.5; 2.2	–	–	–	–
	–	0.01	0.2	0.01	0.4	0.1	0.08	–	0.03
Gyrodactylus sp.	–	1	3	–	2	10	–	–	–
Lecithaster gibbosus	–	–	–	–	–	1	1	–	1
Bothriocephalus spp.	1	–	–	1	1	–	–	1	–
Proteocephalus sp.	5 (2–9)	–	1	2	–	–	–	–	–
Cestoda larvae sp. 1	–	–	–	–	1	–	–	2	–
Cestoda larvae sp. 2	3 (1–5)	1	–	–	–	–	–	–	–
Goezia sp.	–	1	–	1	–	–	–	3 (2–5)	–
Corynosoma sp.	1	1	1	–	–	1	6	1	–
Pomphorhynchus laevis	–	–	–	–	–	–	–	–	23
Dichelyne minutus	–	2	6	2	–	1	–	2	–
	–	1.0; 1.9	–	1.1; 3.0	–	0.9; 2.0	–	0.2; 3.3	–
	–	2.3	0.06	0.3	–	0.2	–	0.1	–
Hysterothylacium aduncum	2	1	1	1	4	1	6	2	2
	0.6; 3.4	–	0.8; 1.9	–	0.04; 7.5	–	2.8; 14.0	0.4; 2.9	1.1; 3.6
	0.3	0.03	0.1	0.07	0.5	0.01	0.9	0.1	0.6
Paracapillaria gibsoni	4	5	7	4	6	19	1	5	2
	21.4; 29.4	1.3; 10.3	8.8; 23.5	2.9; 5.9	1.4; 13.9	6.8; 30.8	–	2.7; 7.2	10.7; 14.7
	0.08	0.3	0.2	1.1	0.8	7.2	0.02	0.8	0.1

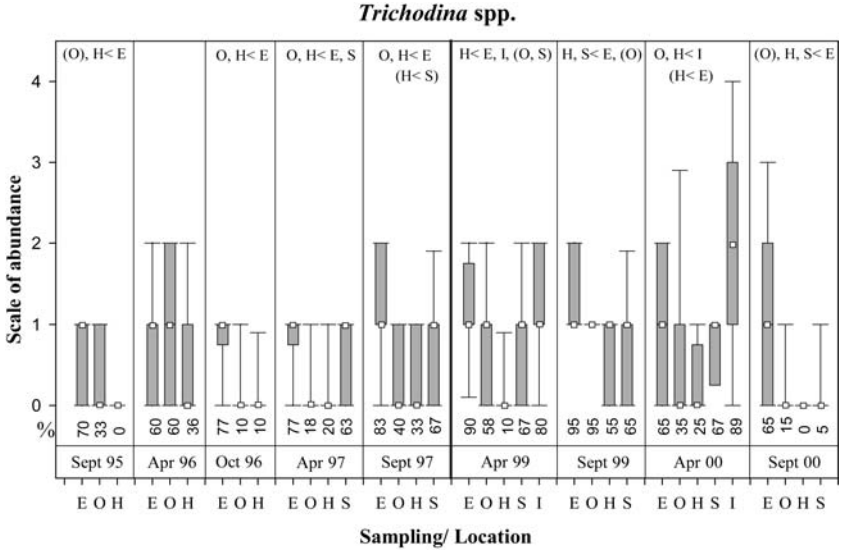

Fig. 2 Abundance of the *Trichodina* spp. on an arbitrary scale (*0* absent, *1* 1–3, *2* 4–10, *3*10–20, *4* >21 individuals per slide) at different sampling locations during the study. Given are median abundance (*squares*) with 25–75% percentiles (*boxes in grey*) and 10–90% percentiles (*whiskers*). Prevalences [%] are indicated below the box plots. The sampling periods, shown below the prevalences, are identified by month and year (*Apr* April, *Sept* September, *Oct* October). Order of abundance observed among sites is given at the top of the graph for each sampling period. In parenthesis: additional differences observed for prevalences. *E* Elbe, *O* Outer Eider, *H* Helgoland, *S* Spiekeroog, *I* Inner Eider. The numbers of flounder evaluated during the sampling periods are given in Table 1

Table 5 Spatial differences in the parasite fauna of flounder in the German Bight. Given are the statistically significant differences between sites in the infection levels of the predominant species in the parasite community during both seasons. Level of significance indicated:$P<0.05$.*E* Elbe, *O* Outer Eider,*H* Helgoland, *S* Spiekeroog,*I* Inner Eider

	Prevalence		Intensity		Abundance	
	Spring	Autumn	Spring	Autumn	Spring	Autumn
Trichodina spp.	H<O<E, S, I	H, O, S<E	–	H, O<E	H<O<E, S, I	H, O, S<E
A. cornuta	E<O, H, S; O, I<H	E<O, H, S	E<O, H, S, I	E<O, H, S	E<O, H, S; I <H	E<O<H; E<S
L. pectoralis	E, I<O, H, S	–	E<O, H, S	E<O, S	E<O, H, S; I <H	E<O, H
L. branchialis	E<O, H, S, I	E<O, H, S	E<O, H, S, I	–	E<O, H, S, I	H<O
Metacercaria sp. 1	E<O, H, S, I; S<I	E<O, H	–	O<S	E, O, S<I; E<S	–
Z. viviparus	E<O<H; S, I <H	E<O<H; S<H	E, O, S <H	E, O<H	E, O, S, I <H	E, O, S<H
C. heterochrous	E<O<H; E<S; I <H	E<O<H	–	E<H	E, O<H	E, O, S<H

Acanthochondria cornuta is a monoxenous, marine copepod, which was found on the gills and in the buccal cavity of the flounder.

The distribution of this species was constant throughout the study. In all sampling periods, prevalences and abundances were lower in the Elbe estuary than at the coastal and offshore sites, whereas prevalence and abundance in the Inner Eider estuary were only lower than those at Helgoland. These differences were found to be statistically significant in most of the sampling periods (Fig. 3). In the spring samples in the pooled data set, the prevalence of *A. cornuta* at the sample sites increased in the order Elbe < Outer Eider < Helgoland (E<O, H; $P<0.001$, O<H; $P<0.05$). Gradual differences were not found in intensities, but the number of parasite individuals was significantly lower in individuals from Elbe than in flounder from all other sites, including fish from the Inner Eider estuary. During the autumn sampling, significantly lower prevalences and intensities were found in fish from the Elbe estuary than in those at the other locations. Gradual differences were, however, found when abundance was considered (Table. 5).

Seasonal variations in prevalence and intensity of *A. cornuta* were only found at a single site, the Outer Eider estuary, where prevalence and intensity of this parasite were significantly higher in autumn ($P<0.001$) than in spring. This variation had no influence on the distribution of *A. cornuta* among the sites. Annual fluctuations were only found in autumn samplings (Elbe 95<00; Outer Eider 95<97; Helgoland 96<99; $P<0.05$), but they were not consistent between sites.

The abundance of *A. cornuta* was positively correlated with fish length ($R=0.289$; $P<0.001$), due to prevalence as well as intensity. Infection levels were not, however, related to sex and condition factor of the flounder.

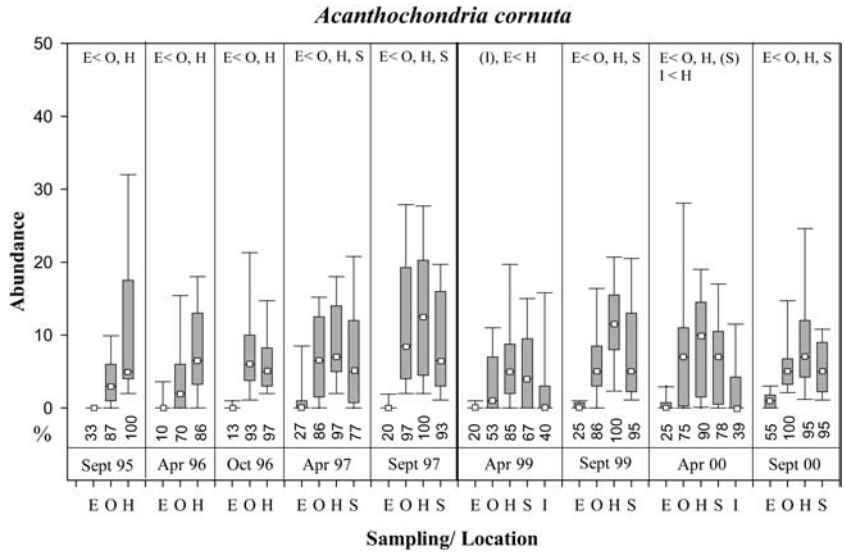

Fig. 3 Abundance of *Acanthochondria cornuta* at different sampling locations in the German Bight during the study. For key, see Fig. 2

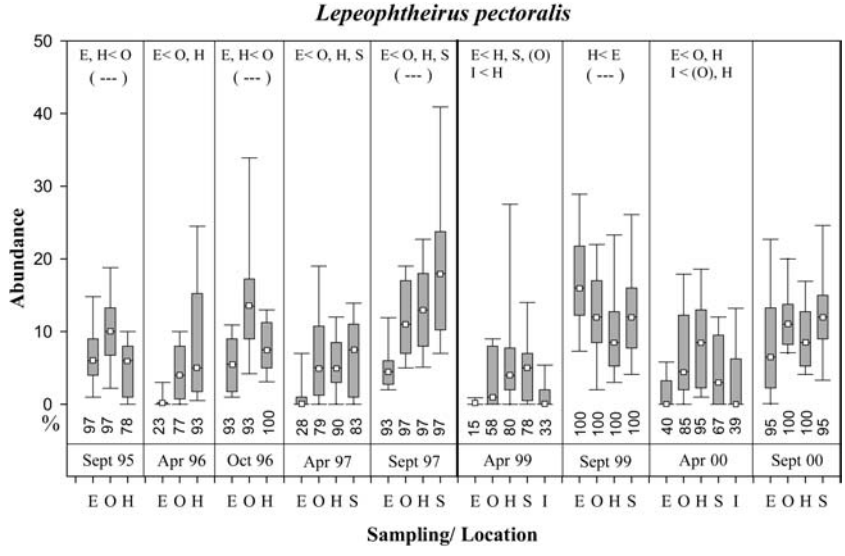

Fig. 4 Abundance of *Lepeophtheirus pectoralis* at different sampling locations in the German Bight during the study. For key, see Fig. 2

Lepeophtheirus pectoralis is a monoxenous, marine copepod that parasitises the skin and fins of the flounder.

This species exhibited a marked seasonal pattern in its infection level. Prevalence and infection intensity were significantly higher in autumn than in spring ($P<0.001$).

Strongest seasonal fluctuations were observed in prevalence at the Elbe station, ranging between 10% and 40% in spring and 90% and 100% in autumn. These changes had a clear effect on the site-specific distribution of the parasite.

During sampling periods in spring, prevalences and abundances were significantly lower at the Elbe station than at the coastal and offshore sites. Prevalence and abundance at the Inner Eider station were lower only than those at Helgoland. During sampling periods in autumn, differences between sites were only found in the abundance (Fig. 4).

The pooled data showed a more detailed picture. In spring, prevalences were significantly lower at both estuarine sites than at the coastal and offshore sites, whereas the number of parasite individuals was significantly lower in fish from the Elbe estuary than at all other sites, including the Inner Eider estuary. In autumn, differences between sites were observed only in intensities, which were significantly lower in fish from the Elbe estuary than in fish from Spiekeroog and Outer Eider (Table 5).

Annual fluctuations were only found in autumn sampling periods (Elbe, all years <99; Helgoland 95, 96<97; $P<0.05$), but did not correspond among sites.

The abundance of *L. pectoralis* was positively correlated with fish length ($R=0.213$; $P<0.001$), due to differences in prevalence as well as in intensity. The infection levels were not, however, influenced by sex and condition factor of flounder.

Lernaeocera branchialis is a heteroxenous, marine copepod. It was the most common and most numerous species in the community. Its larvae prefer flounder as

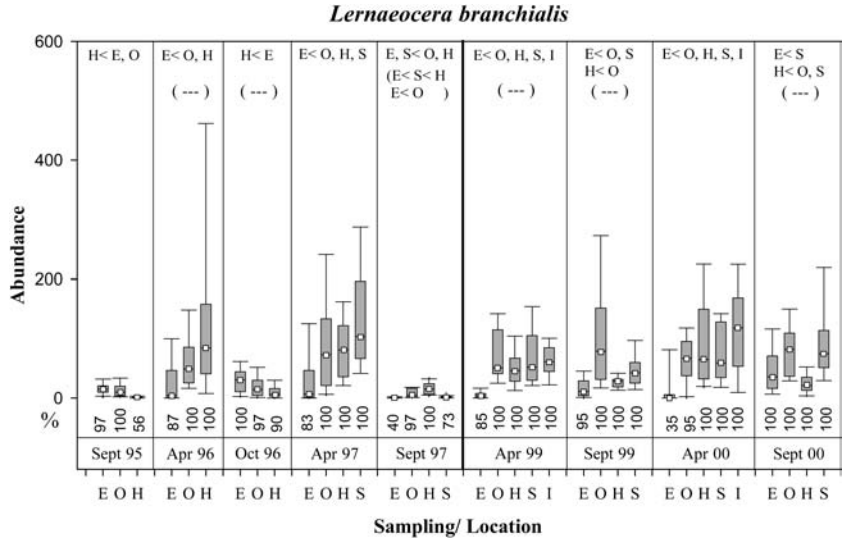

Fig. 5 Abundance of *Lernaeocera branchialis* at different sampling locations in the German Bight during the study. For key, see Fig. 2

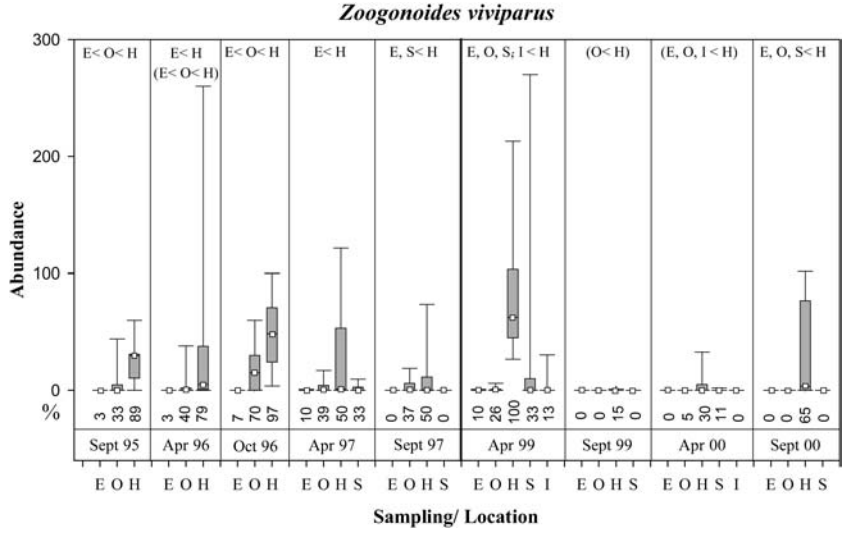

Fig. 6 Abundance of *Zoogonoides viviparus* at different sampling locations in the German Bight during the study. For key, see Fig. 2

their intermediate host and parasitise the gills. The final hosts are gadoid fishes.

In all spring sampling periods, abundances were significantly lower at the Elbe station than at all other sites, including the Inner Eider estuary (Fig. 5). Prevalences reflected this picture only in half of the sampling periods. During the autumn sampling periods, no clear pattern could be observed in the infection levels at the sites.

In the pooled data set, lowest prevalences were found at the Elbe station compared to all other sites, including Inner Eider estuary. This distribution was observed during both seasons (Table 5). In spring, the number of parasite individuals was also lowest in fish from the Elbe estuary.

No seasonal fluctuations were observed in the prevalence of *L. branchialis*, but intensity was significantly higher in spring than in autumn ($P<0.001$). These changes were responsible for the specific infection pattern at the sampling sites during the spring sampling periods.

Annual fluctuations between sampling periods were more evident in autumn than in spring (spring: Elbe 00<96, 97; autumn: Elbe 97< all years; Outer Eider 95–97<99, 00; Helgoland 95<97; 95, 96<99, 00; Spiekeroog 97<99, 00; $P<0.05$). In autumn, infection levels were significantly lower in 1997 than in 1999 and 2000 at almost all sites.

The abundance of *L. branchialis* was positively correlated with the fish length ($R=0.221$; $P<0.001$), and negatively correlated with the condition factor of the flounder ($R=–0.16$; $P<0.01$). The correlations were due to differences in intensity and not in prevalence. The infection levels were not related to the sex of the flounder.

Zoogonoides viviparus is a heteroxenous, marine digenean that inhabits the rectum of flounder. It was the most frequent and most abundant digenean species of the community. In all sampling periods, *Z. viviparus* reached highest prevalences and abundances at Helgoland (Fig. 6).

Gradual differences were found in increasing order Elbe < Outer Eider < Helgoland in the first three sampling

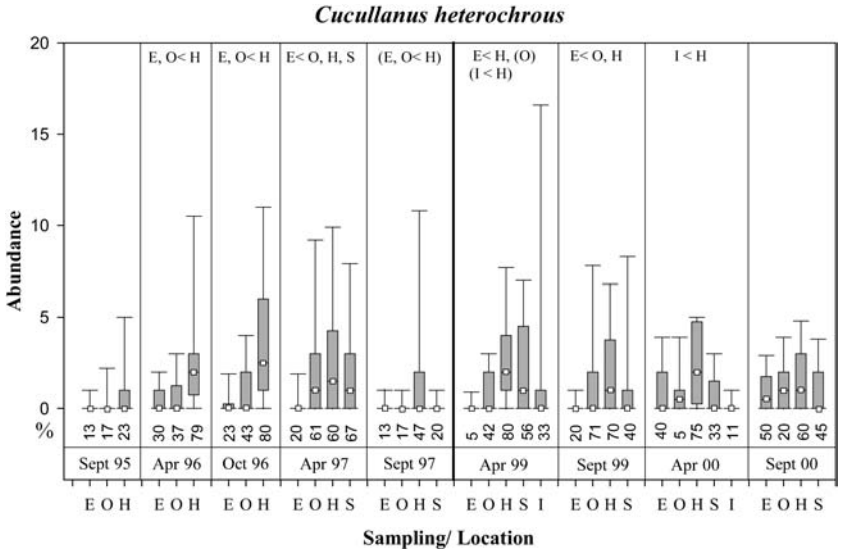

Fig. 7 Abundance of *Cucullanus heterochrous* at different sampling locations in the German Bight during the study. For key, see Fig. 2

periods (Fig. 6) and in both seasons, when data were pooled (E, O<H, P<0.001; O<H, P<0.05).

A clear seasonal infection pattern was not observed, but in the autumn sampling period of 1999, the population was dramatically reduced at all sampling sites. It slowly recovered during the subsequent sampling periods (Fig. 6).

Annual fluctuations were more pronounced in autumn than in spring sampling periods (spring: Helgoland 97, 00<99; autumn: Outer Eider 97, 99, 00<96; Helgoland 99<95, 96, 00; 97<96; P<0.05) The annual differences reflect the population collapse in autumn 1999.

The abundance of *Z. viviparus* was positively correlated with fish length (R=0.320; P<0.001), due to variations in prevalence as well as to intensity. The abundance of *Z. viviparus* was negatively correlated to the condition factor (R=–0.11; P<0.05), which was due to differences in intensity. The infection levels were not related to the sex of the flounder.

Cucullanus heterochrous is a marine nematode species that lives in the intestine of flounder. In most of the sampling periods, prevalences and abundances at the Elbe and Inner Eider stations were lower than at Helgoland, but these differences were not always significant (Fig. 7). Infection levels at the Outer Eider and Spiekeroog stations varied; in some sampling periods they were similar to those in the Elbe, and in others, similar to Helgoland.

When data were pooled, gradual differences in prevalence as well as intensity of *C. heterochrous* were observed among the sampling locations, with an increasing order Elbe < Outer Eider < Helgoland (E<H, P<0.001; E<O and O<H, P<0.05) during both seasons. Significant differences between Elbe and Spiekeroog (E<S, P<0.001) were only found in spring. In autumn, the intensity was lower in fish from Elbe estuary than in fish from Helgoland.

A seasonal pattern could not be detected. Annual variations were only found in autumn sampling periods (Outer Eider 95, 97<99; Helgoland 95, 97<96; P<0.05), but did not correspond between sites.

The abundance was positively correlated with fish length (R=0.340; P<0.001), due to differences in prevalence as well as in intensity. A negative correlation was observed between abundance and condition factor (R= –0.15; P<0.01), which was only due to prevalence. The infection levels were not related to the sex of flounder.

The metacercaria of unidentified digenean species were found encapsulated and encysted in the gill arches of the flounder. They sometimes occurred in very high numbers on a single flounder specimen. The maximum number of metacercaria found on an individual flounder was 1,237.

In half of the sampling periods, significant differences were found between sites, but the results varied between the sampling periods (Fig. 8). In the spring sampling periods, for the pooled data, significantly lower prevalences were observed at the Elbe station than at all other sites. In autumn, prevalences were significantly lower in the Elbe estuary than at Helgoland and in the Outer Eider estuary. Intensity was lower in fish from the Outer Eider estuary than in fish from Spiekeroog, but only in autumn (Table 5).

A seasonal pattern was not observed, but annual fluctuations were observed between the sampling periods of both seasons (spring: Outer Eider 96< all years; Helgoland 99<97, 00; autumn: Outer Eider 95<97, 99; Helgoland 95<00; P<0.05), without showing a clear trend.

There was no relation between fish length, sex and condition factor and the infection levels of the metacercaria.

Summary of the results

Of 30 parasite taxa found during the present study, only seven taxa were regularly present and sufficiently abundant to be considered as an indicator species. Prevalences and/or intensities of most of these taxa were significantly

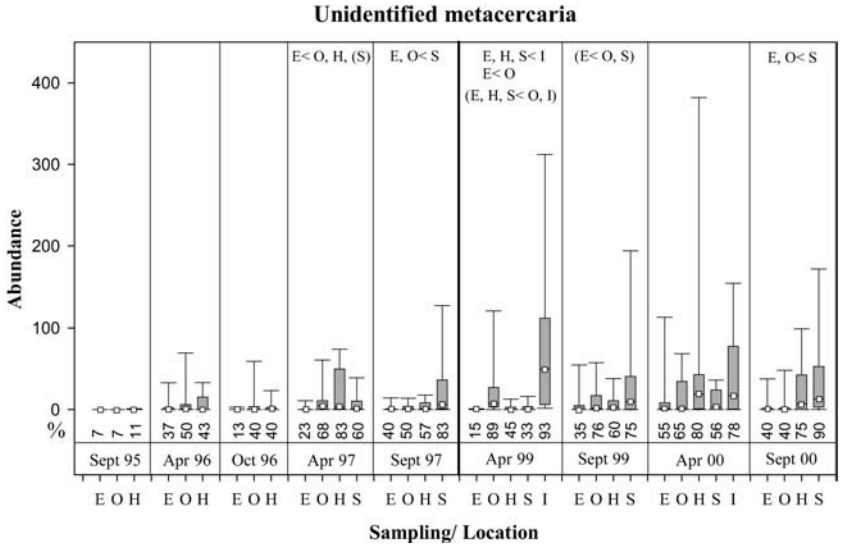

Fig. 8 Abundance of the unidentified metacercaria at different sampling locations in the German Bight during the study. For key, see Fig. 2

lower in fish from the Elbe estuary or from both of the estuarine sites than in fish from the marine and coastal sites. *Trichodina* spp. were the only species that had an opposite distribution, with highest values in the estuaries and lowest values at the coastal and marine sites.

For several species, such as *Trichodina* spp., *A. cornuta*, *C. heterochrous*, *Z. viviparus* and the metacercaria, differences between sites became evident when their prevalences were considered. This was observed for single sampling periods as well as for the long-term data. In species with strong seasonal variations in their infection levels, such as *Lernaeocera branchialis* and *Lepeophtheirus pectoralis*, differences in their abundance between sampling sites were found in almost all sampling periods, but less frequently in respect of their prevalence. In the pooled data set, differences were found in prevalence as well as in the abundance.

Gradual differences in infection levels between sites, coinciding with a known pollution gradient (Elbe < Outer Eider < Helgoland; Broeg et al. 1999; Schmolke et al. 1999), were rarely detected in single sampling periods, but in the pooled long-term data set they were found in respect of prevalence and abundance of several parasite taxa. Increasing infection levels in the order Elbe < Outer Eider < Helgoland was found for the prevalence of *A. cornuta*, *C. heterochrous* and *Z. viviparus*; and decreasing infection levels between Elbe, Spiekeroog, Inner Eider > Outer Eider > Helgoland were observed in the prevalence of *Trichodina* spp. Gradual differences were also observed in the abundance of *Trichodina* spp. and *A. cornuta*.

Only two species, *Lepeophtheirus pectoralis* and *Lernaeocera branchialis*, exhibited marked seasonal fluctuations in their infection levels, which then caused changes in the site-specific distribution of these parasites.

Annual variations were mainly found with respect to the autumn sampling periods, and were less prominent with respect to the spring sampling periods. A clear trend of increasing or decreasing infection levels with the course of the present study was not observed.

Infection levels of most of the taxa were positively correlated to fish length, except *Trichodina* spp., which were negatively correlated with fish length. Since the mean length of flounder was similar at the locations, size-related influences on the site-specific infection levels of the parasites could be neglected. For some taxa a relation with the condition factor of flounder was also found, but correlation coefficients were very low.

Discussion

Fish-parasite communities are considered to reflect water conditions. This tenet—that the biological integrity of a parasite community is a sensitive indicator of the aquatic ecosystem their host lives in—is the basis for considering that fish parasites assess environmental changes (Gelnar et al. 1997; Kennedy 1997; Overstreet 1997; Yeomans et al. 1997). The primary agents that impact on natural communities, apart from natural environmental fluctuations, are human disturbances. Because these disturbances interact in complex ways, their effects can rarely be assessed only by physical or chemical parameters as indicators of biological integrity. Thus biological integrity should be best assessed directly by measurement of aquatic biota (Fausch et al. 1990). Fish parasites are useful organisms to measure environmental deterioration, because they reflect the host-environment situation in numerous ways. Parasites can respond directly to immunosuppression of their host by a rapid multiplication. Within days an infection by a monoxenic parasite such as *Ichthyobodo necator* can rise from a moderate level to a severe level of infection (Woo and Poynton 1995). A reduction in biodiversity can result in local extinction of invertebrate species that serve as intermediate hosts for a heteroxenous parasite which then also disappears (Overstreet 1997). Thus parasites can integrate the effect of

short-term stresses as well as habitat change on a longer scale. The major disadvantage of using fish parasites to indicate biological degradation however is one of resolution: changes can be noted, but it is often difficult to determine the cause and to discriminate the human impact and the natural environmental fluctuations. In order to use parasite species or community data as bio-indicators for environmental quality assessment, long-term studies at comparable localities with known pollution levels are most desirable to distinguish between natural fluctuations and pollution-mediated effects (Kennedy 1997; Broeg et al. 1999).

When parasite species are considered as bio-indicators, it is important that they occur regularly at all locations investigated, and that differences are found in their infection levels between these sites. Additionally, they should be easy to sample, to identify and should be sensitive to environmental changes before a majority of less sensitive organisms are seriously affected. The life-cycles of the species should be known and their response to pollutants determined in laboratory studies.

In the present study on flounder from five locations in the German Bight, parasites from 30 different taxa were identified, including 24 macroparasite and 6 microparasite taxa. The findings in general correspond to the results of previous work on the same species at the same locations, where 27 macroparasite and 6 microparasite taxa were recorded (Broeg et al. 1999). After a revision of the material of that study, it was possible here to identify some of the taxa, which remained undetermined in the previous work.

Most of the recorded taxa are also known as typical flounder parasites from other regions in the North Sea and the Baltic Sea (MacKenzie and Gibson 1970; Gibson 1972; Lile 1989; Lüthen 1989; Levsen 1990; review by Fagerholm and Køie 1994; Pattipeiluhu 1996; El-Darsh and Whitfield 1999; Køie 1999; review by Palm et al. 1999).

Only seven taxa of the parasite community occurred regularly at all locations in sufficient abundances that they could be considered as potential indicator species. These species were the ciliophoran *Trichodina* spp., the copepods *A. cornuta*, *Lepeophtheirus pectoralis* and *Lernaeocera branchialis*, the helminths *Z. viviparus* and *C. heterochrous* and metacercaria of an unidentified digenean species. For most of these species, some biological prerequisites, such as different infection levels between sites, habitat requirements, or availability of information on life-cycle are known. A summary of available biological information, which is relevant for the use of these parasites in environmental monitoring programmes, is given in Table 6. The response of these parasites, except *Trichodina* spp. to selected pollutants is completely unknown, however.

Each of these parasite taxa displayed a specific infection pattern. Infection levels of some of the species varied within wide ranges during single sampling periods, but in the combined, long-term data set, all of the species showed significant differences in their infection levels between the Elbe, as the most polluted site, and the less polluted coastal and marine locations (Helgoland, Outer Eider and Spiekeroog).

Even gradual differences, which corresponded to the contamination gradient established among the sites by Broeg et al. (1999) in respect of the residues of chlorinated hydrocarbons in the muscle and the liver of flounder, and by Schmolke et al. (1999) in respect of the residues of heavy metals in sediments and blue mussel, could be confirmed. These differences were observed when the prevalence of the species *Trichodina* spp., *A. cornuta*, *Z. viviparus* and *C. heterochrous* over the 5-year sampling period was considered. Broeg et al. (1999), who studied the same fish at the same locations over a shorter period of 3 years only, were not able to establish these gradual differences among the sampling sites, which clearly underlines the necessity of prolonged observations when parasites are used in pollution monitoring.

The site-specific distribution of the species *Lepeophtheirus pectoralis* and *Lernaeocera branchialis* was strongly influenced by seasonal variations in their infection levels. These seasonal changes, observed in the German Bight, are also known from other regions in the North Sea. Van Damme and Ollevier (1996) and Van Damme et al. (1997) found an annual life-cycle of *L. branchialis* in the Oosterschelde in the southern North Sea, with highest infection intensities from spring to summer and lowest intensities in autumn. They reported that, in spring, males and females congregate for mating, until the majority of the pre-metamorphosis females detach between March and June, in order to infect whiting, their definitive host, which then enter the estuaries. Therefore, differences in infection intensities are strongly related to the transmission window, when the parasites intend to infect their final host. Boxshall (1974a), Pattipeiluhu (1996) and Van den Broek (1979) described an annual cycle for *Lepeophtheirus pectoralis* with lowest prevalences and intensities in April and highest infection levels in August/September. They noted that breeding takes place during the period of highest temperature, when population size is able to increase rapidly and considered temperature as the principal factor influencing the life-cycle of this parasite species.

The strong seasonal fluctuations in the prevalence of *L. pectoralis*, which were observed in the present study at the Elbe station, are considered to depend on seasonal changes in water salinity rather than on temperature effects. In spring, when salinity is lowest in the estuary, due to a high freshwater inflow (Möller 1990), prevalences were also significantly lower than in autumn, when salinity is highest (Möller 1990). Möller (1974) observed a similar distribution of *L. pectoralis* in the Bay of Kiel, western Baltic Sea. At this location, the parasite was only found during the winter months, when salinity reached the annual maximum. Möller (1974) considered seasonal salinity fluctuations as the principal reason for the presence or absence of *L. pectoralis* in the Bay of Kiel.

Salinity is one of the most important natural factors, which influences the distribution of parasites in coastal

waters. Möller (1978) considered stenohalinity of parasites and their hosts as the main reason for a natural reduction in the parasitic fauna in brackish water. Water salinities of about 16‰ provide unfavourable conditions for marine as well as for freshwater species. Ectoparasites and digeneans are especially affected by low salinity. Ectoparasites are directly affected by low salinities, whereas in digeneans it is the lack of molluscs that serve as intermediate hosts (Möller 1978; MacKenzie et al. 1995).

In the present study, salinity structure differed remarkably among the sampling sites in the German Bight. At the estuarine sites, salinity changed twice a day, due to the tides, whereas at the coastal and marine sites, salinity was relatively constant. Möller (1990) and Anders and Möller (1991) described considerable salinity fluctuations in the Elbe (2.4–20‰) and Inner Eider estuary (6–29‰). In the Elbe, differences up to 9.4‰ (range 2.4–13‰) were found during a single tide. Salinity fluctuations were also observed during the course of the year. In the Elbe estuary, an annual range of more than 12‰ was found downstream from km-720, close to the sampling site of the present investigation. Highest salinities were measured between October and December and lowest salinities, between April and June.

Five of the seven species considered as bio-indicators (all the copepods and the helminths *Z. viviparus* and *C. heterochrous*) are marine species that are not able to complete their life-cycles in water with low or changing salinity.

Copepods were found to be stenohaline (Kabata 1979; Knudsen and Sundnes 1998) and are quickly lost when a host individual enters the estuaries (Gibson 1972). Wichowski (1990) found that the distribution of the copepods *Lepeophtheirus pectoralis* and *Lernaeocera branchialis* in the River Elbe was limited by the 2‰ isohaline. In experimental studies, when copepods were exposed to stepwise-reduced salinities, the number of parasites decreased dramatically when salinities were between 10‰ and 16‰ (Möller 1978; Wichowski 1990). Although parasites died under those conditions, they might detach several days after death and could still be found in waters with lower salinity. Thus, marine copepods, which are found in brackish water, indicate that their hosts migrated from the sea recently.

Susceptibility to water salinity seems to vary among copepod species. Möller (1978) observed differences in the susceptibility of copepods and described a lower capacity to survive reduced salinities in *Acanthochondria depressa* than in *Lepeophtheirus pectoralis*. Kabata (1959) also suspected that abiotic factors might influence the distribution of *A. cornuta*, but the exact physiological requirements of this species remain unknown. A higher susceptibility to reduced or fluctuating salinity might be a cause of the significantly lower prevalences and intensities of *A. cornuta* at the Elbe location throughout the year.

The presence of the digenean species *Z. viviparus* depends on the distribution of its first intermediate host, *Buccinum undatum* (Gibson 1972; Køie 1976, 1983; Möller 1978). Since *B. undatum* does not enter estuaries, only low numbers of *Z. viviparus* were present in flounder collected at the estuarine sites in the German Bight. Highest infection levels of this parasite were found close to Helgoland in the "Tiefe Rinne", where *B. undatum*, polychaetes of various species and ophiurids, such as *Ophiura albida*, which act as first and second intermediate hosts, occur in high numbers and provide excellent conditions for the parasite to infect flatfishes in this area (Ibbeken and Zander 1999).

The geographical distribution of the second most abundant helminth species, *C. heterochrous*, in flatfish is almost identical with the euryhaline polychaete *Nereis diversicolor*, which is probably the most important intermediate host for this parasite (Køie 2000). Since eggs of *C. heterochrous* embryonate in only sea water, reduced prevalences of this parasite at estuarine sites in the German Bight, as observed in the present study, might be due to the susceptibility of their eggs to fluctuating salinity, rather than to the absence of the intermediate host in the estuaries. They might also sustain a lower, but more constant salinity, since they occur in the south-western Baltic Sea (Køie 1999, 2000).

As the metacercaria and the trichodinids could not be identified to the species level, distribution range and biology of these parasites cannot be discussed.

Comparison of locations with similar salinity conditions

Although salinity was similar at the estuarine sites, infection levels of the parasites differed. Similar prevalences in the Elbe and the Inner Eider estuary were only found for *Trichodina* spp., *Lepeophtheirus pectoralis* and *Z. viviparus*, when infection levels of these parasites at estuarine sites were compared to the other sites. Prevalences of *A. cornuta* and *C. heterochrous* were significantly lower at the Elbe station than at the coastal and marine sites, but in the Inner Eider estuary, the prevalences of these species were only lower compared to Helgoland. A different distribution was also observed in the prevalences of *Lernaeocera branchialis* and the unidentified metacercaria, which were significantly higher in the Inner Eider estuary than in the Elbe estuary.

Intensities of *A. cornuta* and *L. branchialis* were also significantly higher in fish from the Inner Eider estuary than in fish from the Elbe estuary, whereas no differences were found in the number of parasite individuals among fish from the Inner Eider estuary and fish from the coastal and marine sites (Table 6). Significantly elevated infection levels of some parasite species were found in fish from the Inner Eider estuary, compared to flounder from the Elbe estuary. Among these sites, a contamination gradient (Elbe > Inner Eider > Helgoland; Schmolke et al. 1999) was found in respect of residues of heavy metals in sediments and blue mussel. This gradient corresponded to the parasitological findings reported here.

Different levels of infection with parasite species were also observed in flounder collected at the offshore sites,

Table 6 Summary of biological information on predominant parasite species/taxa in the parasite community of flounder in the German Bight. *Trich Trichodina* spp., *Acan A. cornuta*, *Lep L. pectoralis*, *Lern L. branchialis*, *Zoog Z. viviparus*, *Meta* unidentified metacercaria, *Cuc C. heterochrous*, + yes or positive, *?* uncertain; References *1* Kabata (1959); *2* Boxshall (1974b, 1976); *3* Zeddam et al. (1988); *4* Kabata (1960, 1961); *5* Van Damme and Ollevier (1996); *6* Van Damme et al. (1997); *7* Knudsen and Sundnes (1998); *8* Gibson

Biological information	Trich	Acan	Lep	Lern	Zoog	Meta	Cuc
Regularly present at all sites investigated	+	+	+	+	+	+	+
Infection levels different between sites	+	+	+	+	+	+	+
Common parasite species of flounder	?	+	+	+	+	?	+
Sampling and identification easy	No	+	+	+	+	?	+
Distribution and habitat requirements known	No	+	+	+	+	No	+
Biology and life-cycle known	No	1	2, 3	4–7	8, 9	No	10
Low seasonal fluctuations	+	+	No	No	+	+	+
Low annual fluctuations: spring	+	+	+	+	No	+	+
Low annual fluctuations: autumn	No	No	No	No	No	+	No
Relation with fish length	+	+	+	+	+	No	+
Relation with sex of fish	No	No	No	No	No	No	No
Dependence on salinity	+	+	+	+	+	No	+
Response to selected contaminants known	+[a]	No	No	No	No	No	No

[a] Organic pollution

the Outer Eider estuary and Helgoland, which are characterised by relatively constant salinity levels. Prevalences of the parasite species, *Trichodina* spp., *A. cornuta*, *Z. viviparus* and *C. heterochrous*, were significantly lower in fish from the Outer Eider estuary than in flounder from Helgoland (Table 6). Between these sites, Broeg et al. (1999) found a contamination gradient (E>O>H) in respect of residues of chlorinated hydrocarbons in muscle and liver of flounder. A similar gradient could be seen in respect of the residues of heavy metals in sediments (Schmolke et al. 1999), as mentioned above. Again, these differences in contamination corresponded to the parasitological findings of the present communication.

These results indicate that salinity is most likely not the only factor that influences distribution and infection levels of parasites at the sites under study. Whether man-made influences, such as contamination-induced effects, have to be taken into account is discussed in detail in an additional communication (Schmidt et al., submitted).

The trichodinids and the metacercaria could not be identified at the species level, which did not allow us to decide whether they were of marine or estuarine origin. In general, trichodinids live on the surface of the skin and gills of fish and feed on bacteria and organic matter in the water and on the surface of the fish (Lom and Dyková 1992). Yeomans et al. (1997) discussed a possible link between increased levels of *Trichodina* infection and increased concentration of organic pollutants from sewage-treatment plant effluents. In cultured eel (*Anguilla anguilla*), the infection level of *Trichodina jadranica* was positively correlated with the content of organic matter in the water column (Madsen et al. 2000). Consequently, the availability of bacteria was regarded as a limiting factor for the presence of trichodinids in flounder and cod in the Bay of Kiel (Palm and Dobberstein 1999).

In the present study, trichodinids were predominantly found in estuarine sites with increased organic load (Möller-Buchner 1987). A possible link between man-made eutrophication and infection levels of trichodinids is discussed in an additional communication (Schmidt et al., submitted).

The annual variation in the infection levels of the parasites occurred mainly among the autumn sampling periods. Since the results were highly variable between species and sampling sites, and no trend was found the infection levels of the parasites during the study, annual variation is suggested to be within the range of natural variability.

Conclusions

From 30 parasite taxa found in flounder at different locations the German Bight, only 7 taxa were regularly present in sufficient numbers to be considered as indicator species.

Each of these parasite taxa displayed a specific infection pattern. Their infection levels differed among single sampling periods, but in the combined data set covering a period of 5 years, infection levels of all these species showed significant differences between the Elbe, as the most polluted site, and the less polluted coastal and marine locations, Helgoland, Outer Eider and Spiekeroog.

Gradual differences in the infection levels of the parasite species among flounder from the Elbe, the Outer Eider and Helgoland were not detected in individual sampling periods, but became evident in the pooled data set. These gradual differences were found in the prevalence of the species, *Trichodina* spp., *A. cornuta*, *Z. viviparus* and *C. heterochrous*, and corresponded to the contamination gradient (Elbe > Outer Eider > Helgoland) established by Broeg et al. (1999) and Schmolke et al. (1999). These results underline the need for long-term studies if parasites are to be used for pollution monitoring.

Since most of the potential indicator species were of marine origin, salinity was considered as the most important natural factor influencing the distribution of these parasites and their intermediate hosts in the area under study. The infection levels of most of the parasite taxa, however, differed between the two estuarine sites as well and also were different at the offshore sites which had similar salinities. These differences corresponded to

the contamination gradient between the sites under study (Elbe > Inner Eider > Helgoland), for sediment, mussel and fish liver (Broeg et al. 1999; Schmolke et al. 1999). Thus, in addition to salinity, man-made effects, such as pollution, most likely also had an impact on the distribution of the parasites at the locations under study.

Seasonal variations, which strongly influence the spatial distribution of the parasites, were found in the infection characteristics of two copepod species, *Lepeophtheirus pectoralis* and *Lernaeocera branchialis*. As a consequence, a direct comparison of parasite data from different seasons is not recommended, since these variations might lead to false results.

Annual variations in the infection levels of the parasites were more evident in the autumn sampling periods than in the spring sampling periods, but no trend to higher or lower infection levels was found during the study. Thus annual variations most likely remained within the range of natural variability.

Although biological and ecological information is available for most of the parasite species evaluated here, there is a lack of laboratory studies on the specific effect of known pollutants on the physiology of these parasites. From the findings presented here, it can be concluded that an analysis of the fish parasite fauna is particularly useful in ecosystem monitoring. When the abundance of *A. cornuta*, *Z. viviparus* and *C. heterochorous* were considered over the 5-year observation period, pollution-induced effects became evident over variations due to many natural factors. Thus the present analysis indicates that, besides many differences in habitat structure, the influx of pollutants generates a greater challenge to the ecosystem in the Elbe estuary than to the sampling sites near Helgoland or in the Outer Eider estuary.

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