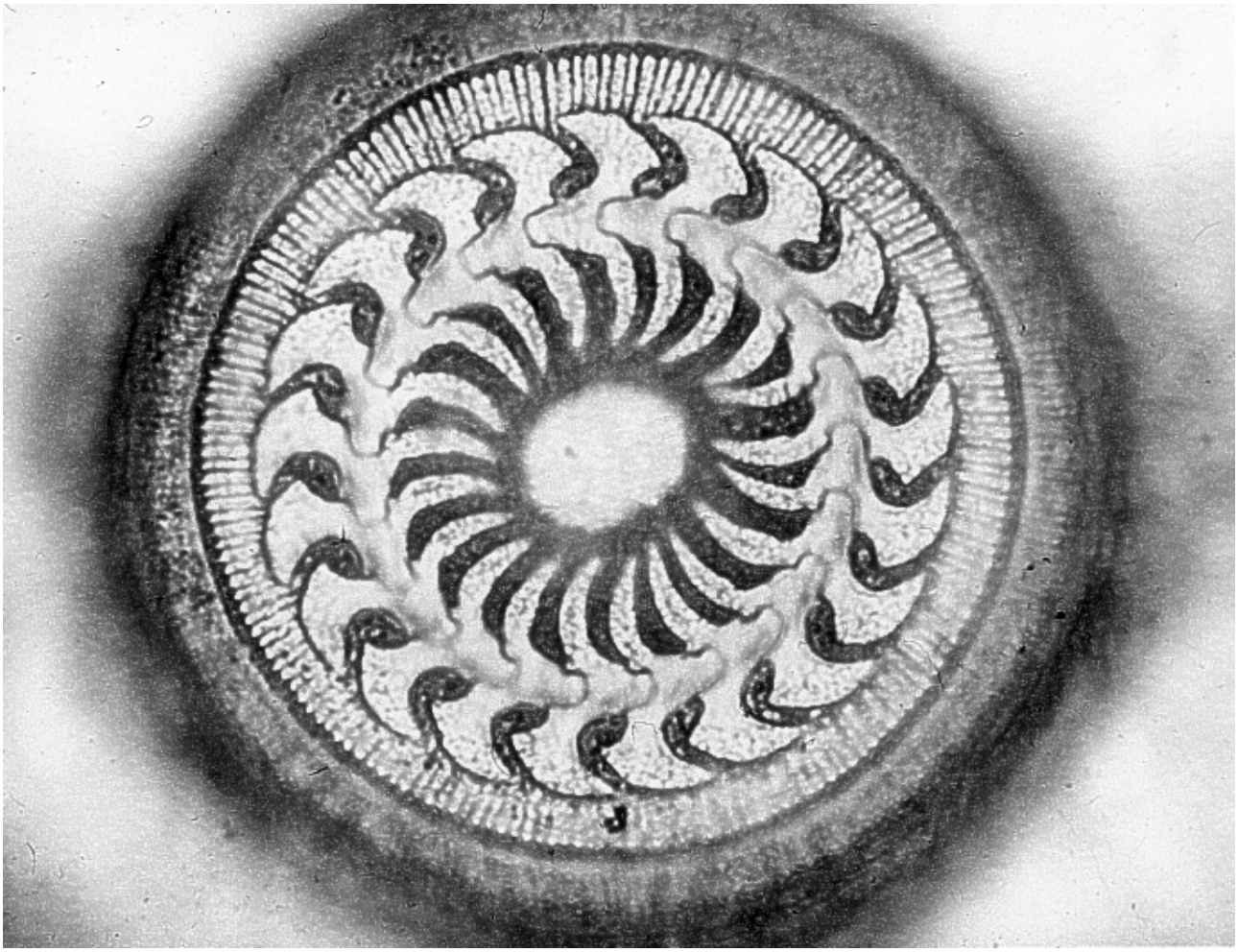
Parasites of European flounder (*Platichthys flesus* L.) from the German Bight, North Sea, and their potential use in ecosystem monitoring



Verena Schmidt 2003

Cover illustration: *Trichodina* sp. (Ciliophora) from flounder (*Platichthys flesus*) in the German Bight, North Sea

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

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Abbreviation list

ANOVA	: Analysis of variance
ChE-Br	: Cholinesterase activity in brain
ChE-f	: Cholinesterase activity in muscle
DDD	: 1,1-dichloro-2,2-bis(p-chlorophenyl) ethane
DDE	: 1,1-dichloro-2,2-bis(chlorophenyl) ethylene
DDT	: 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane
DNA-f	: Desoxyribonucleinacid unwinding
E	: Elbe estuary
EROD	: Ethoxyresorufin-O-Deethylase
et al.	: et alii (and others)
Fig.	: Figure
H	: Helgoland “Tiefe Rinne“
HCB	: Hexachlorobenzene
HCH	: Hexachlorocyclohexan
I	: Inner Eider estuary
ICES	: International Council for the Exploration of the Sea
LY1	: Lysosomal stability type 1
LY2	: Lysosomal stability type 2
Lys	: Lysozyme activity
MAA	: Macrophage aggregate area
MAM	: Macrophage aggregate activity
N	: Number of sampled individuals
No.	: Number
O	: Outer Eider estuary
OCS	: Octachlorostyrole
OSPAR	: Oslo Paris Commission
PCB	: Polychlorinated biphenyls
p	: Probability of error
pers. comm.	: Personal communication
Pin	: Pinocytosis activity
R	: Correlation coefficient
ROS	: Reactive oxygen species
ROS-PMA	: ROS, stimulated by phorbol-12-myristate-13-acetate
S	: Spiekeroog
S.D.	: Standard deviation
sp.	: species
Tab.	: Table

Abstract

The potential use of fish parasites as sensitive biological indicators for pollution monitoring has attracted increasing interest during the last 20 years. As part of an integrated biological effects monitoring, the parasite fauna of flounder, *Platichthys flesus* (L.), was investigated from 5 locations in the German Bight, North Sea, over a period of 6 years. The sampling sites included 2 estuarine locations (Elbe estuary, Inner Eider estuary) and 3 coastal and marine locations (Outer Eider estuary, Helgoland, Spiekeroog). The sites differed in hydrographical conditions and in contamination load. Parasites were evaluated on different organisation levels, using established ecological concepts. At the species level, infection characteristics of single parasite species were calculated, while concepts as species richness and species diversity were applied at the community level. Natural influences, such as host related factors, water salinity and temporal changes due to season and year, biological and ecological requirements of parasites and intermediate hosts were considered as well. The influence of anthropogenic factors on the parasite community was assessed in an integrated approach, which included information on the chemical burden in sediments and biota as well as responses of recommended biomarkers to pollution exposure. During the course of the study, 30 different parasites taxa were identified from a total of 1073 flounder individuals, including 6 microparasites (Apicomplexa, Ciliophora, Myxozoa and Microsporea) and 24 macroparasites (Monogenea, Digenea, Cestoda, Nematoda, Acanthocephala and Copepoda). Only 7 parasite taxa occurred regularly at all locations in sufficient abundances to be considered as potential indicator species. According to the working hypothesis, infection level of all of these parasite species differed significantly between Elbe, as the most polluted site, and the less polluted coastal and marine locations, Helgoland, Outer Eider and Spiekeroog. In some species even gradual differences between the sites could be observed, when the infection data were pooled over all sampling points. Similar results were found for species richness and diversity, which were significantly lower in parasite fauna of flounder from the Elbe estuary, when compared to flounder from the other sampling sites. In addition, gradual differences between the locations became evident, when the data were pooled over the years. Salinity was considered as the most important natural factor, influencing the distribution pattern of the parasite species and their intermediate hosts in estuarine and coastal waters. Anyway, infection levels of most of the potential indicator species and most of the community data differed between locations with similar salinity conditions but different contamination levels. Seasonal variations were observed in most of the parasitological measurements, but effects of these variations on spatial differences were more evident in the infection characteristics of single parasite species than in the community data, which appeared to be relatively robust against seasonal fluctuations. Results of the integrated study showed that parasitological data clearly reflected a contamination gradient, which was established between the sampling sites based on residue analysis from sediments and blue mussel (*Mytilus edulis*). Thus differences between the sites were not only due to natural factors, such as salinity, but also to pollution induced stress. The responses of the biomarker corresponded best to the residual burden of individual flounder, which did not reflect equally the site- specific pollution gradient. Correlations were also found between parasitological data and contamination load of individual fish as well as with selected biomarker responses. Short-term responses to new contamination events, found for some of the biomarkers, were not reflected by the parasitological data, but long-term data of the parasitological parameters were most appropriate to give a general characterisation of the sampling sites. A general impoverishment in the parasite community of flounder in the German Bight could not be observed over a study period of 6 years.

The present study showed that the parasite community of flounder is a valuable tool for the assessment of ecological consequences of environmental deterioration in marine and coastal waters, when accompanied by other types of data, such as biochemical biomarkers. Corresponding to the working hypothesis, infection levels of selected parasite species as well as species richness and parasite diversity of flounder in the German Bight were reduced with increasing levels of site-specific pollution and individual contamination of the host. The abundance of the protozoan taxon *Trichodina* spp., which is discussed as an indicator of organic pollution, was elevated under such conditions. Acute and chronic responses of established biomarkers confirm the impact of pollutants on the fish host and establish a link between mechanistic processes on the sub-cellular level of individual fish and the ecological significance of pollution on the population and community levels of biological organisation, represented by dynamics in the parasite community.

Key words: parasites, *Platichthys flesus*, bioindicators, integrated pollution monitoring, North Sea

Chapter 1
General introduction

Since the beginning of industrialisation, the environment is increasingly loaded with foreign anthropogenic chemicals (xenobiotics). In the 20th century, thousands of organic pollutants, such as polychlorinated biphenyls (PCB's), polycyclic aromatic hydrocarbons (PAH's), organochlorine pesticides (OCP's) as well as heavy metals have been produced and partly released to the environment by communities, agriculture and industries (Van der Oost et al. 2003) and several hundreds of new chemicals are still introduced each year (Moriarty 1993). Since the early sixties mankind has become aware of potential long-term adverse effects of these chemicals in general and their potential risk for aquatic ecosystems in particular. The ultimate sink for many of these contaminants is the aquatic environment as a result of direct discharges or due to hydrologic and atmospheric processes (Stegeman and Hahn 1994).

As a consequence, national and international monitoring programs were initiated to monitor the development of the aquatic environment with the objective to reduce the input of xenobiotic substances into the marine ecosystem (North Sea Task Force 1993).

The monitoring programs in the 1970s started with the determination of a set of well- known contaminants in abiotic environmental compartments like water and sediment (= chemical monitoring). These measurements were soon supplemented by the identification and quantification of these substances in living organisms (= bioaccumulation monitoring) (Westernhagen et al. 2001; Van den Oost et al. 2003). Both methods only allow the identification of a small number of selected contaminants, without considering complex synergetic and antagonistic reactions of the chemicals in the ecosystem. Information about the biological relevance of xenobiotics to organisms in the aquatic ecosystem is not obtained by these methods.

During the last decade, the interest in biological endpoints has been increased by looking at effects of xenobiotics on living organisms in the field (Westernhagen et al. 2001). This approach in environmental monitoring investigates the responses of biological criteria, including biomarkers (= biological effects monitoring) and bioindicators (= health and ecosystem monitoring) on different levels of biological organization (Van Gastel and Van Brummelen 1994; Adams 2002; Van den Oost et al. 2003).

Biomarkers are defined as functional measures of exposure to environmental stressors such as xenobiotic chemicals (Van Gastel and Van Brummelen 1994). These molecular, biochemical, as well as physiological responses are usually expressed at the suborganismal level of biological organization. Biomarkers are stressor sensitive and rapidly responding endpoints that help to

identify the mechanistic basis of causal relationship between stressor and its effect. They are characterized by relatively high response variability, rarely integrate effects of stressors over long periods of time and generally have a low ecological relevance (Adams 2002).

Bioindicators, on the other side, are considered as structural entities, such as sentinel species or they are viewed functionally as biological effects endpoints at higher levels of organization, such as the population and community level (Van Gastel and Van Brummelen 1994; Adams 2002). In contrast to biomarkers, they provide little information about the underlying causal mechanisms between stressors and effects due to a low sensitivity and specificity to stressors, and they tend to integrate the effects of multiple stressors over large spatial and temporal scales (Depledge and Fossi 1994; Adams 2002). Bioindicators show a relatively low degree of response variability and are characterized by a high ecological relevance (Adams 2002). Figure 1 shows different kinds of biomarkers and bioindicators at different levels of biological organization in the continuum between mechanistic understanding and ecological relevance.

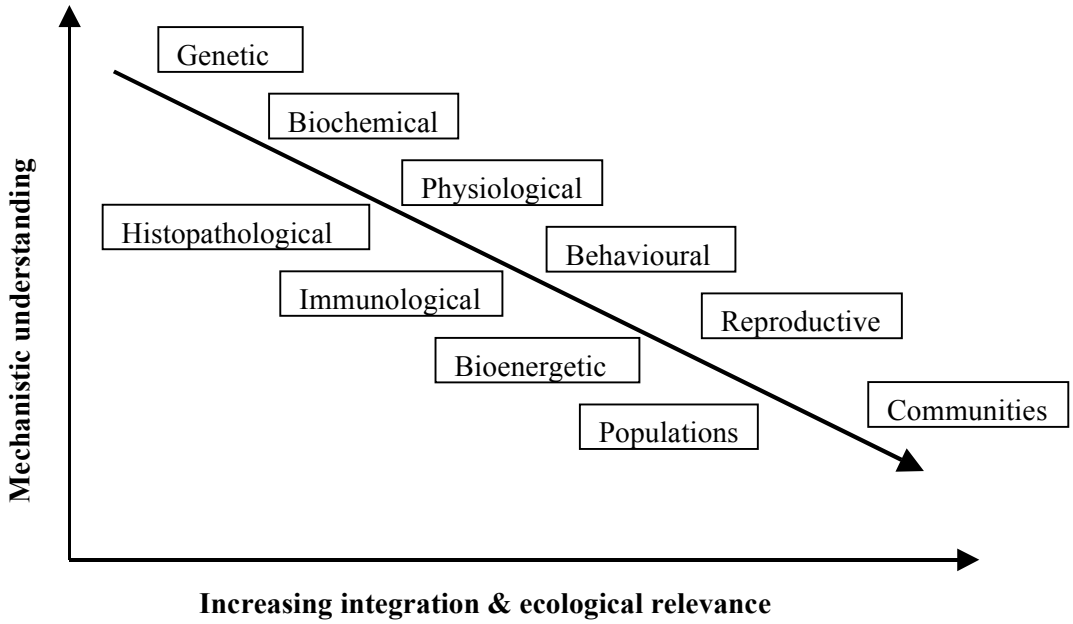


Figure 1: Increasing levels of biological organization result in decreasing mechanistic understanding but increasing levels of ecological significance. A selected suite of measures along this continuum of levels of organization is recommended in the design of bioassessment studies in aquatic ecosystems (Adams 2002).

Due to the complexity of natural systems, single parameters are not appropriate to reflect the effects of multiple stressors on the integrity of aquatic systems. An adequate set of endpoints is required for determining the biological significance of stress and the underlying cause or mechanistic basis of observed effects (Attrill and Depledge 1997). Therefore, environmental monitoring programs should include a variety of chemical, physical and biological indicators, with each being used in their respective roles as environmental stressors (i.e. xenobiotics), exposure response (i.e. biomarkers) and effects response (i.e. bioindicators) (Adams 2002).
The sequential responses to pollutant stress is visualised in figure 2.

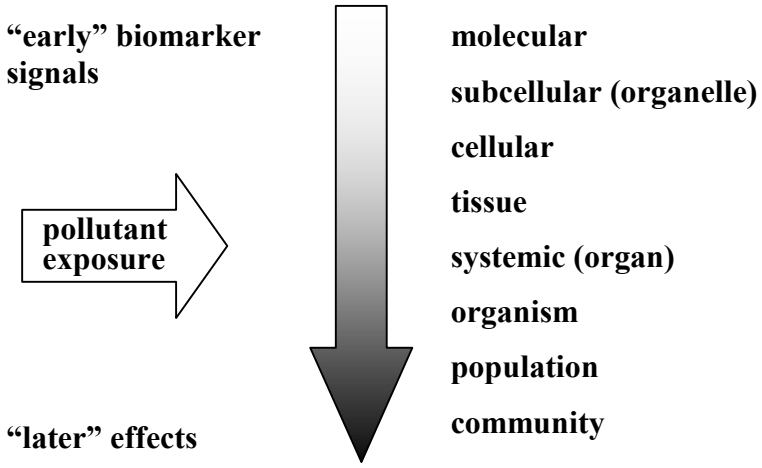


Figure 2: Schematic representation of the sequential order of responses to pollution stress within a biological system. Modified from Bayne et al. (1985) and Van den Oost et al. (2003).

In monitoring programs in aquatic environments, fishes are widely used as sentinel species to assess environmental health. Their complex biological organisation and their high position in the food chain allow to observe bioaccumulation processes of xenobiota in different tissues of the fish and integrate different kinds of biomarker responses at the suborganism and organism level of biological organisation. In addition, fishes themselves provide a habitat for other organisms, such as invertebrate parasites, which inhabit external as well as internal tissue of the host and reflect environmental health on the population and ecosystem level.
The use of fish parasites as bioindicators of environmental quality and pollution impact has received much attention during the past 15 years. A number of papers have already provided clear evidence of links between pollution and changes in parasite abundance and/ or community structure (reviews by Khan and Thulin 1991; MacKenzie et al. 1995; Arthur 1997; Kennedy 1997).

Nevertheless the use of parasites as bioindicators is widely and controversially discussed, because presence, infection characteristics and biodiversity of parasites are not only influenced by environmental contaminants but also by a variety of natural factors (Arthur 1997; Kennedy 1997; McVicar 1997; Overstreet 1997).

The applicability of parasites to biomonitoring studies is based on the observations that changes in the abundances of certain parasites may provide information about acute and chronic changes in the environment, which may eventually affect less sensitive aquatic organisms. These changes in abundance are sometimes positive, as is often the case for ectoparasitic protozoans and monogeneans (Arthur 1997; Overstreet 1997). Low levels of pollution may provide nutrients or chemicals, which can be utilized directly by the parasite or its intermediate host, or subject the host to chronic stress, impairing its immune system and allowing the parasite population to flourish. They may also be directly negative (e.g. as a result of pollution, which directly affects a sensitive free- swimming transmission stage or a parasite on skin, gills, or within the gastro- intestinal tract) or indirectly negative (e.g. through changes caused to the parasite's habitat in or on the fish, or through the reduction of the population of an intermediate host) (Arthur 1997; Overstreet 1997).

Among the studies conducted are those investigating the use of parasites as indicators of pollution due to oil spills, heavy metals, electrical generating plants, pulp mills, sewage and agricultural and industrial activities (reviewed by Khan and Thulin 1991; MacKenzie et al. 1995; Kennedy 1997). The principal problem of these studies was to separate effects of pollutants from those of numerous natural factors, both abiotic and biotic, which also influence the abundances of a given parasite species (table 1).

Table 1: Principle biotic and abiotic factors identified as affecting marine parasites. Modified from MacKenzie et al. (1995).

Abiotic	Biotic
Latitude/ longitude	Host species
Temperature	Schooling of fish
Salinity	Age, length and growth rate of host
Season	Migration of host
Year	Host diet/ feeding behaviour
Oxygen	Host hormone levels
Water mineral contents	Host pathology
PH	Host immunity responses
Light	Life history of intermediate/ definite host
Depth	Life history of parasite
Water levels	Interaction between parasites

Beside the use of parasites as bioindicators of pollution effects, an interesting issue of some adult helminths of fish, particularly intestinal acanthocephalans, is their ability to accumulate extremely high levels of heavy metals, which are orders of magnitude higher than those found in the host's tissue or in the environment (reviewed by Sures et al. 2001). As the extremely high concentrations of heavy metals in intestinal acanthocephalans of fish are not the result of a slow process of accumulation but instead a relatively rapid uptake to a steady-state level, chemical analysis of acanthocephalans tissue could provide an early warning for heavy metal pollution (Arthur 1997; Sures 2001).

There are few basic approaches employed for the use of parasites as bioindicators of environmental degradation, which are identical with those applied for free- living invertebrates (Kennedy 1997) and for fishes (Fausch et al. 1990):

a) The recognition of indicator species, whose presence or absence and abundance can be related to specific pollutants or environmental conditions. The choice of appropriate parasite species should include considerations such as ease of sampling, sorting, identifying and counting. As presence or absence of an indicator species can depend on a variety of factors (table 1), this approach requires a profound knowledge of the life cycle, the ecological and physiological requirements of each parasite species (Kennedy 1997).

b) The use of community data, especially diversity indices to assess environmental health. The underlying hypothesis is that parasite diversity is higest in unpolluted waters, whereas pollution stress leads to a loss of species, change in dominance and reduction in diversity. The calculation of diversity indices takes into account species richness and the abundance of each species. The advantage of this approach is that knowledge on the identity of a species, its biology, or susceptibility to pollutants is not required. A change in the index thus can indicate a change in water quality, but it cannot provide any informtion on the nature of the change or on the identity of the pollutant.

c) The use of multivariate analysis to assess pollution. This technique incorporates a number of factors, but has the disadvantage of requiring more expertise in calculation and especially in interpretation. At present, biotic indices are still favoured for their low cost and simplicity (Kennedy 1997).

As ecological and physiological requirements of many parasite species are still unknown, it is suggested by many authors (Gelnar et al. 1997; Kennedy 1997; Khan and Payne 1997; McVicar 1997; Overstreet 1997) that parasitological data when used in pollution monitoring as indicators should be accompanied by other data set such as biochemical biomarkers.

In the present study, which was conducted in the frame of an integrated environmental monitoring program (MARS project), the parasite fauna of flounder; *Platichthys flesus* (L.), from the German Bight of the North Sea was investigated for its potential use as bioindicators in environmental health assessment. Parasites were collected from 5 stations in the German Bight, representing a contamination gradient, over a period of six years (1995-2000). Parasitological data were backed up by chemical analyses of sediments and biota as well as by the responses of a set of biochemical, histopathological, immunological and genetic biomarkers.

The flounder from the family Pleuronectidae is a common sentinel species of various national and international monitoring programs. This euryhaline species is wide spread in western European waters and inhabits coastal areas and estuaries up to freshwater regions. It prefers fine-grained sediments, where it buries itself. Flounder is an opportunistic feeder of bentic invertebrates and small fishes. Most of the year, flounder is a rather stationary species. The juveniles live in shallow waters of coastal and estuarine areas, which are also used as feeding grounds by the adults. In winter, adult flounder migrate into deeper and warmer waters to spawn, and return in spring/ early summer to the shallow regions they left the winter before (Cooper and Chapleau 1998). Due to its life and feeding habits, flounder come in direct contact to contaminated sediment and accumulate xenobiotics as a carnivorous organism at a high position in the food chain. As biology and ecology of flounder as well as its parasite fauna are well studied and a lot of information about biomarker responses of flounder to xenobiotics is already available, flounder was chosen as sentinel species in the present study.

The aim of the study was to give detailed information about distribution, biology and ecological requirements of all parasite species of flounder from the German Bight reported during the course of the study as well as the investigation of their infection characteristics, parasite community structure, natural influences and the impact of xenobiotics on the parasite fauna of flounder.

A brief literature review about the most important aspects of the biology and the ecology of each of the parasite species as well as their infection levels at all sampling locations during the course of the study are presented in chapter 2. Chapter 3 deals with the use of potential indicator species, focusing mainly on selected natural factors that may influence infection levels of these parasites. Aspects on the community structure and diversity indices are presented in chapter 4. An integrated correlation analysis that combines information obtained from parasitological data at the population and ecosystem level with those obtained from responses of established biomarkers at the suborganismal level is shown in chapter 5. These calculations include residual data from sediments, blue mussel tissue (*Mytilus edulis*) and muscle tissue of flounder as well as biochemical, genetic, immunological and histopathological biomarker data of flounder, recommended by the International Council for the Exploration of the Sea (ICES 1996, 2002), which were provided by cooperating working groups of the MARS project.

Chapter 2
The parasite fauna of flounder *Platichthys flesus* (L.) from the German Bight

Introduction

Many authors emphasize the lack of a comprehensive knowledge of the biology of parasites as the most critical point in the interpretation of parasitological data in environmental monitoring (MacKenzie 1995; Kennedy 1997; Overstreet 1997). This chapter therefore has two focal points: first a literature overview on the systematic, biology and ecology of each of the parasite taxa/ species recorded in flounder, *Platichthys flesus* (L.), during the present study and second a detailed presentation of infection characteristics of the parasites over a period of six years, in order to amplify the knowledge about the parasite fauna of flounder from this geographical region of the North Sea.

Materials and methods

Sampling and study area

From 1995 to 2000 thirteen sampling points were carried out in the North Sea, where a total of 1073 flounder were collected from five selected sampling locations in the German Bight. The stations were determined by their geographical position (figure 1) and differed in their habitat conditions:

a) **Elbe estuary**: considered to be a highly polluted estuarine site with fluctuating salinity and 6-10 m water depth
b) **Helgoland "Tiefe Rinne"**: considered to be a low polluted offshore site with stable salinity and 20-40 m water depth
c) **Outer Eider estuary**: considered to be a less polluted offshore site with stable salinity and 15-20 m of depth
d) **Spiekeroog**: considered to be a highly polluted coastal site with stable salinity and 15-20 m of depth
e) **Inner Eider estuary**: considered to be a less polluted estuarine site with fluctuating salinity and 4-6 m of depth

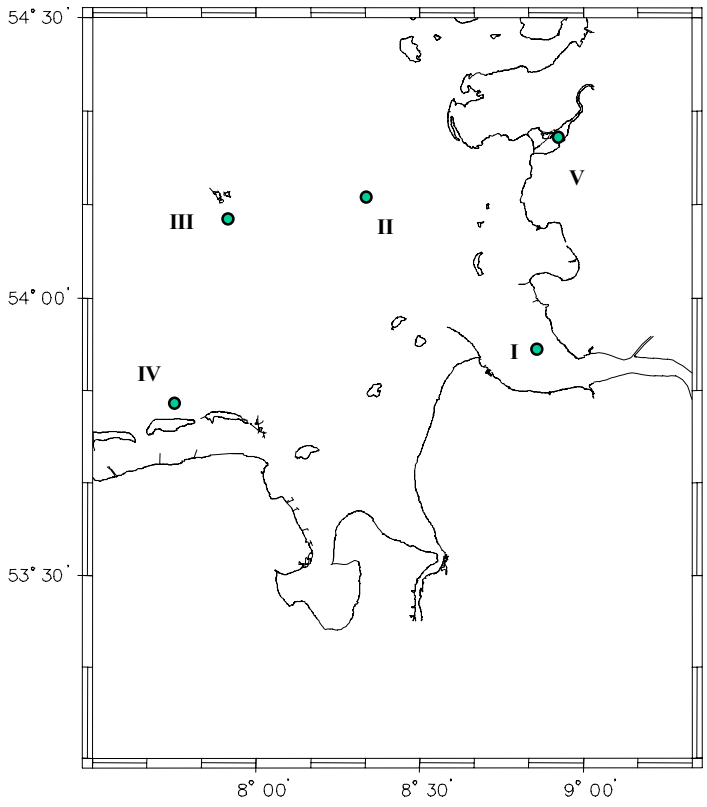


Figure 1: Locations of sampling of flounder *Platichthys flesus* (L.) in the German Bight, North Sea. **I**. Elbe estuary (54°53' N, 8°47' E), **II**. Outer Eider estuary (54°12' N, 8°25' E), **III**. Helgoland „Tiefe Rinne" (54°06' N, 7°58' E), **IV**. Spiekeroog (53°49' N, 7°44' E), **V**. Inner Eider estuary (54°16' N, 7°50' E). A characterization of the sampling sites is given below in the paragraph about the study area.

The sites were considered to represent a pollution gradient with the highest pollution at Elbe estuary, a moderate pollution at Outer Eider estuary and the lowest pollution at Helgoland. Inner Eider estuary and Spiekeroog were taken as "reference sites" for Elbe and Outer Eider estuary representing similar salinity conditions and water depths, but differing in their pollution level. Elbe estuary, Outer Eider estuary and Helgoland were sampled during all sampling points, while Spiekeroog was included in April 1997 and Inner Eider estuary from April 1999.

Catches were made with the research vessel "Uthörn" of the Alfred-Wegner Institute of Bremerhaven, except of the location Inner Eider estuary, where a commercial fish trawler was used. Fishing was done with a bottom trawl, fishing period was limited to 30 minutes. On board of the vessel, the flounder were kept in tanks with permanent water flow-through and aeration for up to 6h until dissection (for details see Broeg et al. 1999). At each location 3 to 32 flounder of the size class 18-25 cm were investigated. Fish with externally evident diseases were excluded from the investigation. Numbers of evaluated fish specimen for each sampling points at each site are given in table 1.

Table 1: Sampling program on flounder parasites. Flounder were collected at five different locations in the German Bight, during sampling points from 1995 to 1997 and 1999 to 2000. Given are numbers of flounder specimen evaluated for parasitological investigation at the sampling sites during individual collections. The sampling periods are identified by month and year.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider	
Jun 95	32	29	30	-	-	
Sept 95	30	30	9	-	-	
Jan 96	29	31	30	-	-	
Apr 96	30	30	14	-	-	
Jul 96	30	9	30	-	-	
Oct 96	30	30	30	-	-	
Jan 97	0	18	3	-	-	
Apr 97	30	28	30	30	-	
Sep 97	30	30	30	30	-	
Apr 99	20	19	20	9	15	
Sep 99	20	21	20	20	0	
Apr 00	20	20	20	9	18	
Sept 00	20	20	20	20	0	
Total	321	315	286	118	33	1073

Parasitological examination

On board, the flounder were examined for ectoparasites. Specimen 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 by light microscopy. Then the fish were killed and dissected. Gills were fixed in 4% buffered (pH 7.2) formaldehyde solution. The gut and gall bladder were removed and opened. Fresh smears were taken from gut and from gall bladder epithelium and 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. Then 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 using a compound microscope. 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 taken for histological investigation. Gills, gut and gut contents were examined for metazoan parasites with a dissection microscope. Parasites were collected, counted and stored separately for individual fish. Sections of gut and kidney were processed by standard histological procedures (Romeis 1989), stained by Giemsa's technique and examined with a light microscope for tissue invading parasites.

For identification of macroparasites, individuals were cleared in 80-90% lactic acid, mounted in glycerine-jelly and observed with a microscope (Berland 1984). Smears of *Trichodina* spp. were air dried and stained by Klein's silver impregnation method (Lom and Dyková 1992).
The identification of parasites was done using standard literature, which is indicated in the chapters, where the parasites are described. In addition, support was given by Dr. M. Køie (Marine Laboratory, Helsingør, Denmark) for the identification of trematodes, cestodes and acanthocephalans and by Dr. F. Moravec (Institute of Parasitology, Ceské Budejovice, Cezechian Republic) for the identification of nematodes.

Parasite populations

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

a) Prevalence: the number of hosts infected with 1 or more individuals of a particular parasite species divided by the number of hosts examined for that parasite species. It is expressed as a percentage (%).
b) Mean infection intensity (I): the number of individuals of a particular parasite species found in a sample divided by the number of hosts infected by that parasite species.
c) Mean abundance (x): 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 intensity was used to classify infection level, 0= absent, 1= 1-3, 2= 4-10, 3=10-20, 4= > 21 individuals per slide.

Statistical analysis

Normal distribution of the data was tested using the Kolmogorov- Smirnow test. The prevalence of a particular parasite at different locations or sampling points was compared by a chi-square test. Normalized data of infection intensity were compared by the Student's t-test and by ANOVA and Tukey's post hoc comparison of means, abundances by the non-parametric Mann-Whitney's U-test and Kruskal-Wallis ANOVA and Dunn's post hoc test. Differences were considered to be statistically significant a probability of error $p < 0.05$. Correlation coefficients were calculated with the parametric Pearson's Product Moment Correlation or Spearman's

correlation on ranks. Correlations were considered to be significant at a probability of error $p < 0.05$.

The analyses were carried out using the computer programs SigmaStat® 2.0 and STATISTICA 6 (StatSoft).

Results and Discussion

During 13 sampling points in the period of 1995-1997 and 1999-2000, a total of 1073 flounder were dissected. All flounder examined were infected with at least one parasite species. In total, 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.

In the following, biology and infection characteristics of the parasite taxa/ species reported in the present study are presented according to their systematic order.

Table 2: List of parasites species recovered from flounder in the German Bight during sampling points in spring and autumn from 1995-2000 and some of their biological characteristics. [1]) m= monoxenous, h= heteroxenous species, [2]) end= endoparasitic, ec= ectoparasitic species, [3]) m= marine, e= estuarine, l= limnetic species, [4]) G= Generalist, S= Specialist, aut= autogenic, [5]) E= Elbe, H= Helgoland, O= Outer Eider, S= Spiekeroog, I= Inner Eider, all= all locations, *= status unknown

Taxonomic group	***Parasite species***	Target organ/ tissue	Host	Life cycle [1]	Ecto-/ endoparasitic [2]	Origin [3]	Status [4]	Location [5]
Apicomplexa	*Epieimeria* sp.	gut	final	m	end	*	*	all
Ciliophora	*Trichodina* spp.	gills	final	m	ec	e	*	all
Microsporea	*Glugea stephani*	gut	final	m	end	m	G, aut	H, O, I
	Microsporea sp.1	kidney	final	m	end	*	*	all
Myxozoa	*Myxidium incurvatum*	gall bladder	final	h	end	m	?, aut	
	Myxozoa sp. 1	kidney	?	h	end	*	*	all
Monogenea	*Gyrodactylus* sp.	gills	final	m	ec	m	*, aut	E, H, O
Digenea	*Podocotyle atomon*	gut	final	h	end	m/e	G, aut	all
	Zoogonoides viviparus	gut	final	h	end	m	G, aut	all
	Derogenes varicus	gut	final	h	end	m	G, aut	all
	Lecithaster gibbosus	gut	final	h	end	m	G, aut	H, S, I
	Brachyphallus crenatus	gut	final	h	end	m	G, aut	all
	Metacercaria sp. 1	gills	intermediate	h	end	m/e	*	all
Cestoda	*Bothriocephalus* spp.	gut	final	h	end	m	G, aut	E, H, O, S
	Proteocephalus sp.	gut	final	h	end	l/e	G, aut	E, O
	Cestoda larvae sp. 1	gut	?	h	end	m	*	H, S
	Cestoda larvae sp. 2	gut	?	h	end	l/e	*	E
Nematoda	*Paracapillaria gibsoni*	gut	final	h	end	m	S, aut	all
	Hysterothylacium aduncum	gut, liver	final	h	end	m/e	G, aut	all
	Goezia sp.	gut	final	h	end	m	*	E, O, S
	Cucullanus heterochrous	gut	final	h	end	m/e	G, aut	all
	Dichelyne minutus	gut	final	h	end	m/e	G, aut,	E, H, O, S
Acanthocephala	*Echinorhynchus gadi*	gut	final	h	end	m/e	G, aut	E, A, I
	Pomphorhynchus laevis	gut	final	h	end	l	?	I
	Corynosoma sp.	gut	intermediate	h	end	m	*	E, H, O, S
Copepoda	*Holobomolochus confusus*	nose cavity	final	m	ec	m	G, aut	all
	Acanthochondria cornuta	gill cavity	final	m	ec	m	G, aut	all
	Caligus elongatus	skin	final	m	ec	m	G, aut	E, H, O, S
	Lepeophtheirus pectoralis	skin, fins	final	m	ec	m	G, aut	all
	Lernaeocera branchialis	gills	intermediate	h	ec	m	G, aut	all

Table 3: Parasites recorded in flounder from the German Bight, North Sea in the years 1995-2000. N = total number of flounder examined, N (infected) = total number of flounder infected with a parasite species, P [%] = Prevalence in %, I = total number of parasite individuals

Parasite species	N	N (infected)	P [%]	I
Epieimeria sp.	1067	292	27,37	-
Trichodina spp.	1072	568	52,99	-
Microsporea sp.1	1070	319	29,81	-
Glugea stephani	1073	7	0,65	-
Myxozoa sp. 1	1073	40	3,73	-
Myxidium incurvatum	1056	197	18,66	-
Gyrodactylus sp.	742	8	1,08	22
Podocotyle atomon	1073	52	4,85	178
Zoogonoides viviparus	1073	308	28,70	1 791
Derogenes varicus	1073	61	5,68	115
Lecithaster gibbosus	1073	10	0,93	13
Brachyphallus crenatus	1073	29	2,70	106
Metacercaria sp. 1	1073	486	45,29	20966
Bothriocephalus spp.	1073	8	0,75	8
Proteocephalus sp.	1073	12	1,12	35
Cestoda larvae sp. 1	1073	3	0,28	4
Cestoda larvae sp. 2	1073	4	0,37	9
Paracapillaria gibsoni	1073	127	11,84	1102
Hysterothylacium aduncum	1073	83	7,74	192
Goezia sp.	1073	17	1,58	34
Cucullanus heterochrous	1073	436	40,63	1504
Dichelyne minutus	1073	93	8,67	594
Corynosoma sp.	1073	14	1,30	19
Echinorhynchus gadi	1073	18	1,68	22
Pomphorhynchus laevis	1073	1	0,09	23
Holobomolochus confusus	680	32	4,71	35
Acanthochondria cornuta	1072	683	63,71	5495
Caligus elongatus	1073	35	3,26	69
Lepeophtheirus pectoralis	1071	840	78,43	8045
Lernaeocera branchialis	1073	994	92,64	74708

Phylum: Apicomplexa Levine 1970

Class: Sporozoa Leuckart, 1879

Subclass: Coccidia Leuckart, 1879

Order: Eimeriida Léger and Dubosq, 1911 (“true coccidia”)

Family: Eimeriidae Minchin, 1903

Genus: ***Epieimeria*** Dyková and Lom, 1981

Organisms of the phylum Apicomplexa were classified according to Lom and Dyková (1992). Eimeriidae are endoparasitic, mostly monoxenous protozoans, which spread by spores. Their life cycles include sexual and asexual phases. In the basic life cycle, a zygote, formed by fusion of gametes, divides by multiple fission or sporogony to form a spore with different numbers of

sporozoites. In the host, sporozoites hatch from sporocysts and infect target cells such as intestinal enterocytes. Within the host cell, sporozoites differentiate to trophozoites. Each sporozoite grows to a meront, which then divides by multiple fission or merogony to form merozoites which infect additional host cells. After 2-3 merogonic cycles merozoites finally differentiate into macrogamonts, which transform into macrogametes, and into microgamonts, which transform by binary or multiple fission into microgametes. A microgamete fuses with a macrogamete to form a zygote. Thus there are three schizogonies in the basic life cycle. These are merogony, gametogony and sporogony (Perkins et al. 2000). In a typical eimerid life cycle, the macrogamont produces an oocyte-like macrogamete. Sporogony produces the infective stages, the oocysts, which contain sporocysts with sporozoites (Lom and Dyková 1992). Detailed descriptions of the morphology and the life cycle of Eimeriidae are given by Lom and Dyková (1992), Molnár (1995) and Perkins et al. (2000).

Parasites of the genus *Epieimeria* are typically found in the epithelial cells of the intestine. They are characterized by the formation of 4 sporocysts each containing 2 sporozoites. Sporocysts bear a special structure on one pole, the Stieda body. Merogony and gametogony occur in a submembrane ("epicellular") position, while sporogony takes place deep in the cytoplasm (Lom and Dyková 1992).

In the present study 27.4% of the flounder examined were infected with *Epieimeria* sp. (table 3). Different developmental stages were found in submembrane position in the intestinal epithelium of flounder (plate 1). The parasite was regularly present in flounder from all sampling sites. During single sampling periods prevalences ranged between 3-79% (table 4). Prevalences did not differ significantly between the sampling sites, but a marked seasonal pattern was observed during the course of the study. From June 1995 to April 1997, when samples were taken in all four seasons, prevalences were lowest in winter, increased during spring and reached a maximum in summer, decreased again during autumn and fell to a minimum in winter (figure 2). Thus life cycle appeared to be annual. When infectivity data were pooled over all sampling points, prevalences of *Epieimeria* sp. were always significantly higher in spring than in autumn ($p < 0.001$). These differences were observed only in flounder from the Elbe estuary, Outer Eider estuary and Spiekeroog, while fish from Helgoland did not show a clear seasonal pattern.

The only record of eimerian parasites in European flounder was given by Möller (1974), who found sporocycsts in fresh intestinal smears of flounder from the Baltic Sea. Information about prevalence or seasonality of the parasite was not presented.

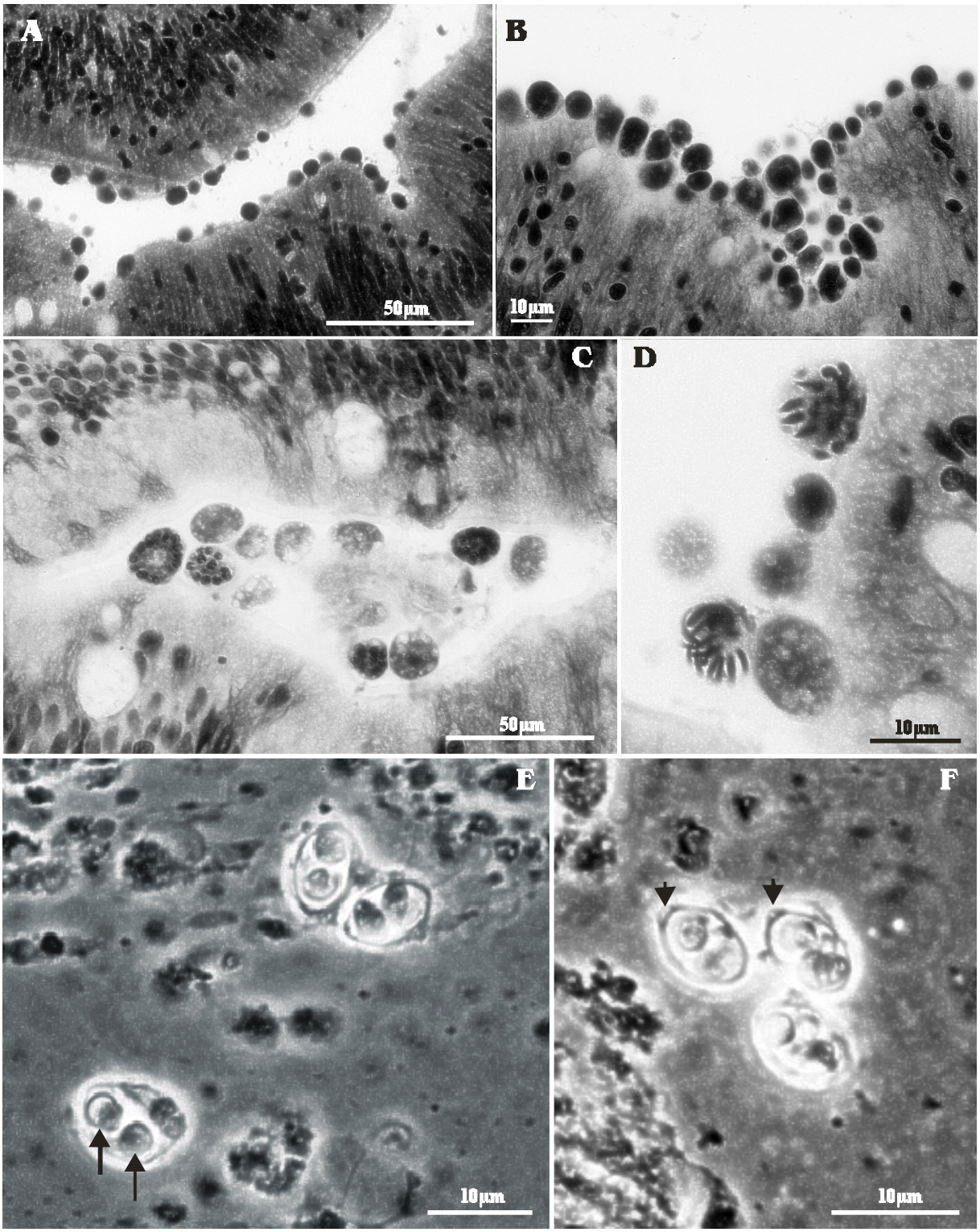


Plate 1: Developmental stages of *Epieimeria* sp. from the intestine of flounder. **A-D**: Histological sections, **A-B**: Meronts and gamonts in "epicellular" position in the intestinal epithelium, **C-D**: Merogonic and gametogonic stages (macrogamonts indicated by an arrow), **E-F**: Fresh mounts of sporocysts, each with two sporozoites (long arrows) and Stieda body (short arrows), observed by phase contrast microscopy

Table 4: Prevalence [%] of ***Epieimeria* sp.** at five locations in the German Bight, North Sea, during sampling points from 1995-1997 and 1999-2000. The sampling periods are identified by month and year, the collection was done. Cells marked with an "nd" indicate that the sample was not done, while cells marked with an "-" indicate that no fish was available from that location. Empty cells indicate that no parasite was found at the station during the particular sampling period. Calculation of mean values and S.D. were based on all fish examined during the course of the study. For numbers of flounder evaluated per sampling site and sampling period see table 1.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95	44	35	3	nd	nd
Sep 95		7	22	nd	nd
Jan 96	3	6	3	nd	nd
Apr 96	43	63	50	nd	nd
Jul 96	67	44	79	nd	nd
Oct 96	20	41	20	nd	nd
Jan 97	-	6		nd	nd
Apr 97	37	48	63	47	nd
Sep 97		7	53		nd
Apr 99	60	44	25	22	64
Sep 99	15	14	25	20	-
Apr 00	5	30	25	44	39
Sep 00					-
Mean	24.5	26.5	28.3	22.2	51.5
S.D.	24.7	20.7	25.6	20.4	17.7

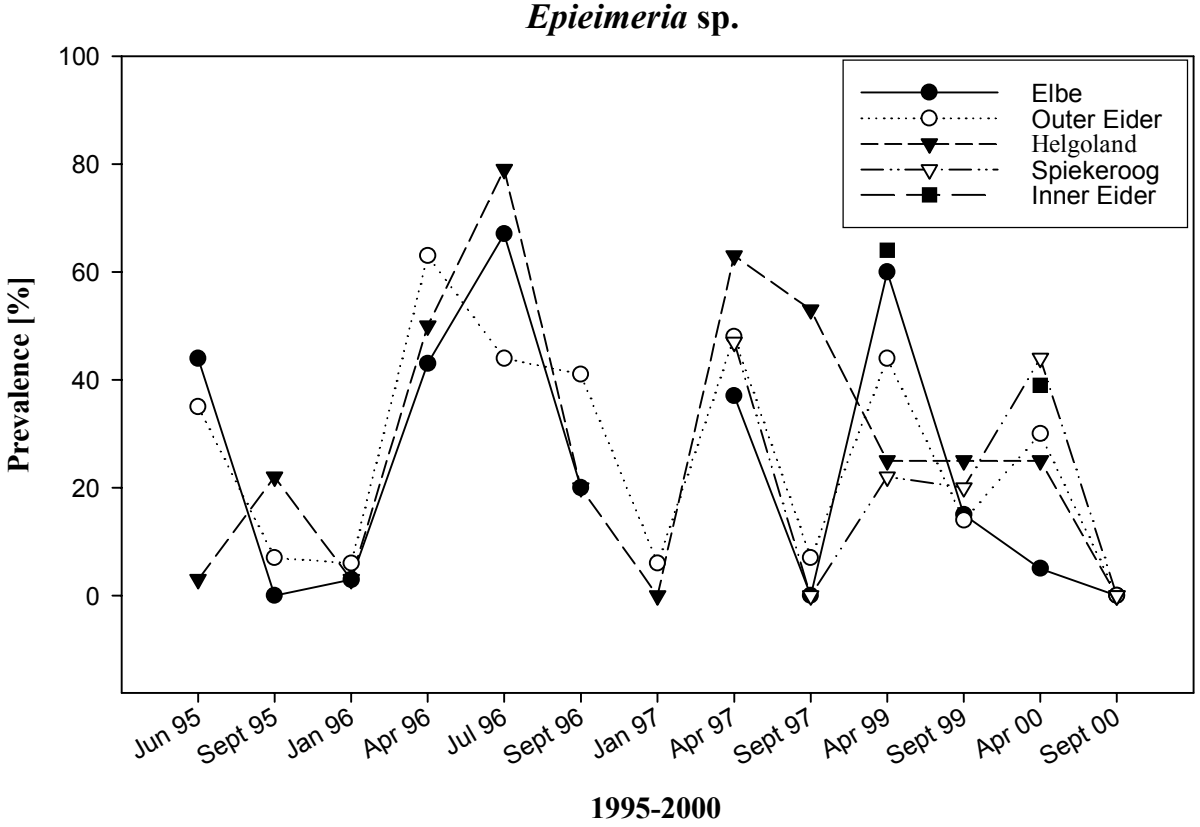


Figure 2: Prevalence of *Epieimeria* sp. at different sampling locations in the German Bight from 1995-2000. For numbers of flounder evaluated during sampling points, see table 1.

Phylum: Ciliophora Doflein, 1901

Class: Oligohymenophorea de Puytorac et al., 1974

Subclass : Peritrichia Stein 1859

Order: Mobilina Kahl, 1933

Family: Trichodinidae Raabe, 1959

Genus: ***Trichodina*** Ehrenberg, 1838

Trichodinids were classified according to Lom and Dyková (1992) and Lynn and Small (2000). Trichodinid ciliates are among the most common ectoparasites of both freshwater and marine fishes (Lom 1995). They are widely distributed over all parts of the world. Some species, like *T. jadranica*, occur on both freshwater and marine fishes, while other are restricted to marine or freshwater environments (Lom and Dyková 1992). Very few species of *Trichodina* are host specific, while most species can infect several to many fish species (Lom and Dyková 1992; Lom 1995).

Trichodinid taxonomy is based on the structure of the buccal ciliature, the configuration of nuclei and the morphology of the adhesive disc, which can only be studied adequately using the silver impregnation technique of Klein (Lom 1958; Welborn 1967). Unfortunately a great number of species were described without the results of this technique and therefore are not valuable. About 190 species of *Trichodina* were described from fishes, but only a part of them were adequately described (Lom 1995). Especially marine trichodinids remain insufficiently studied (Grupcheva et al 1989). Some of the important taxonomic papers on this group are cited by Lom (1995). On flounder, several species of *Trichodina* were reported from individuals collected in the Baltic Sea, including *T. jadranica*, *T. claviformis*, *T. borealis*, *T raabei*, *T. domerguei* (reviewed by Fagerholm and Køie 1994; Palm and Dobberstein 1999; Dobberstein and Palm 2000). Six trichodinid species, *T. borealis*, *T. cottidarum*, *T. frequentis*, *T. jadranica*, *T. nigra* and *T. raabei*) were found in pleuronectid fishes from other localities in the northern hemisphere (Zhukov 1964; Shtein 1969, 1979; MacKenzie 1969; Lom 1970; MacKenzie et al 1976).

Trichodinids live as commensals mostly on the gills or the skin of their hosts and feed on water-born particles and bacteria as well as detritus from the fish surface. On healthy fish they occur in low numbers, but under unfavourable conditions, which lead to a decrease in host resistance, they rapidly proliferate (Lom and Dyková 1992; Lom 1995). In heavy infected fish an epithelial hyperplasia may occur between the gill lamellae. Although this is a protective reaction by the host, trichodinids take advantage of this by feeding newly produced cells and cell debris (Lom 1995).

In recent studies, a close correlation between increasing content of organic matter in the water and the number of trichodinids on a host species was observed, therefore *Trichodina* is discussed as a potential indicator of eutrophication (Yeomans et al. 1997; Palm and Dobberstein 1999; Madsen et al. 2000).

In the present study, *Trichodina* spp. was one of the most prevalent parasites of the community. It was found mainly on the gills of flounder, rather than on the skin (plate 2). *Trichodina* spp. could not be determined up to the species level. When the silver impregnation method of Klein was applied, silver ions formed precipitates with chloride from the seawater (Lom and Dyková 1992). Thus the number of individuals which allowed to analyse the structure of the adhesive disc after the impregnation was not sufficient for a reliable determination of the species.

Trichodina spp. were found at 53% of all flounder examined during this study (table 3). It was regularly present in flounder from all sampling sites. During single sampling periods, the prevalence ranged between 5-100% (table 5). Highest prevalences were found in fish from estuarine sites, while lowest values were found at Helgoland. Detailed information about the infection characteristics at the sampling sites during the course of the study are given in chapter 3.

Table 5: Prevalence [%] of ***Trichodina* spp.** at five locations in the German Bight, North Sea, during sampling points from 1995-1997 and 1999-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95	44	93	60	nd	nd
Sep 95	70	33		nd	nd
Jan 96	48	52	40	nd	nd
Apr 96	60	60	36	nd	nd
Jul 96	100	67	20	nd	nd
Oct 96	77	13	10	nd	nd
Jan 97	-	67	33	nd	nd
Apr 97	77	18	20	63	nd
Sep 97	83	40	33	67	nd
Apr 99	90	57	10	67	80
Sep 99	95	95	55	60	-
Apr 00	65	35	21	67	89
Sep 00	65	15		5	-
Mean	72.8	49.6	26.0	54.8	84.5
S.D.	17.6	27.1	19.0	24.6	6.4

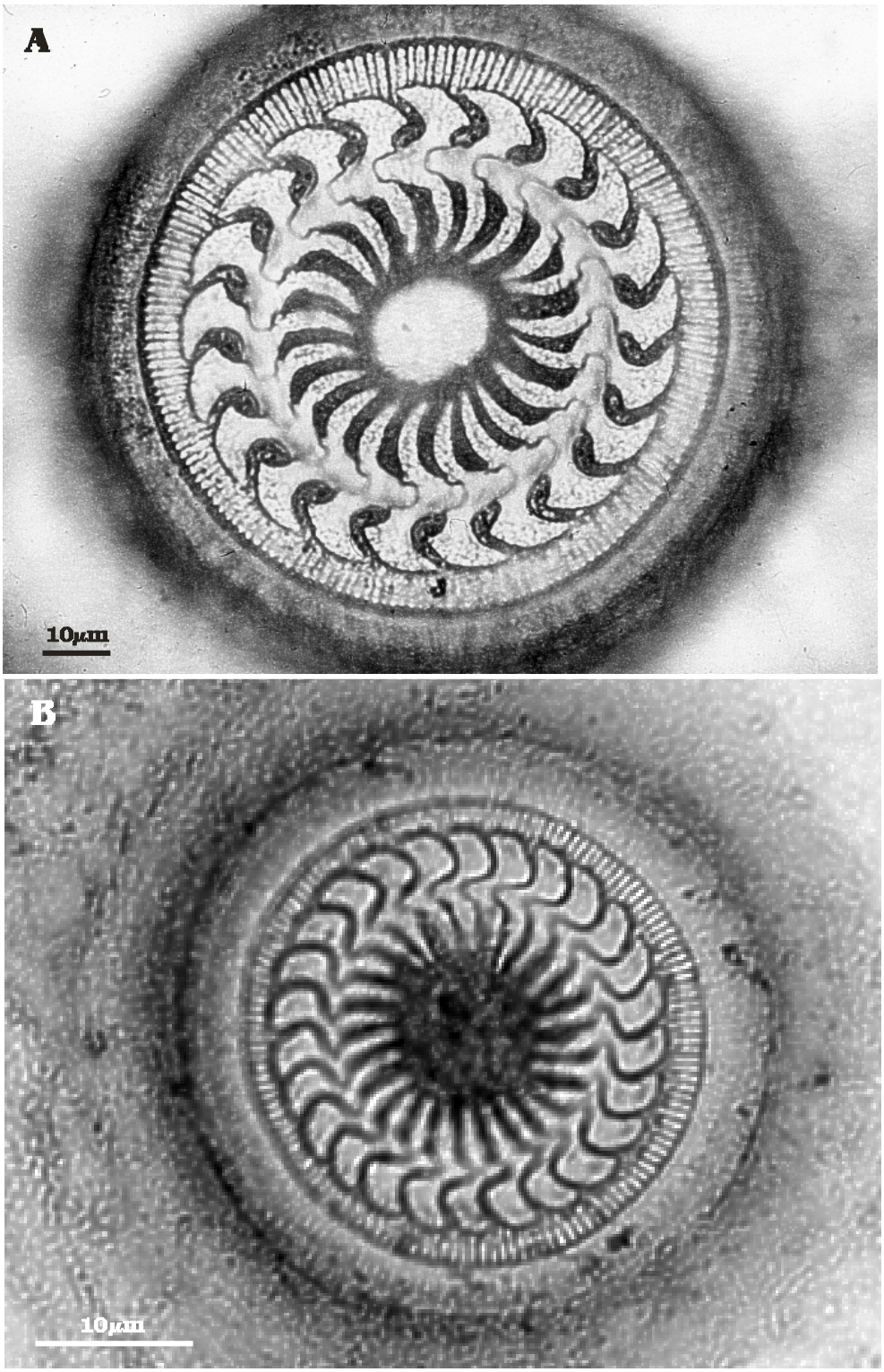


Plate 2: Adhesive discs of trichodinids impregnated with Klein's dry silver impregnation method. A-B: *Trichodina* spp. from the gills of flounder

Phylum: Microspora Sprague, 1977

The microsporidians were classified according to Lom and Dyková (1992).

Microsporida are strictly intracellular parasites, which are characterized by the production of spores (Dyková 1995). They can infect cells from all major tissues and also occur in the majority of the host's organ systems (Bush et al. 2002). Recent taxonomic research suggests that Microsporida (Microspora or Archezoa) are very primitive eukaryotic organisms related to fungi (Canning and Vavra 2000). They contain a membrane-bound nucleus but lack centrioles or mitochondria and have a prokaryotic 70 S ribosome. Current taxonomy is based on the morphology of the spore. The taxonomic status of the prokaryotes and the primitive eukaryotes currently is uncertain but evidence suggests that Microsporida belong to a separate kingdom (Bush et al. 2001).

Morphology and life cycle of microsporidians are described in detail by Vávra and Sprague (1976), Larsson (1986), Lom and Dyková (1992) and Dyková (1995). Life cycle of microsporidians is monoxenous. Spores are ingested by the host. The infective sporoplasm is then extruded from the spore through the polar tube and invades the host cell. Inside the cell it passes through a proliferation phase, a merogony, which results in a large number of parasites. In a second phase, the sporogony, mature spores are formed (Lom and Dyková 1992). Studies on the ultrastructure of *G. stephani* showed that microsporidians form a functional and structural unit with the host cell, a xenoma (Takvorian and Cali 1983; Bekhti and Bouix 1985).

Class: Microsporea Delphy, 1963

Order: Microsporidia Balbiani, 1892

Undetermined species sp. 1 (kidney)

Spores and developmental stages of an undetermined microsporidian organisms were frequently found in different parts of the renal system of flounder, including interstitial tissue, renal tubules and renal corpuscles (plate 3). The species was present in 29.8% of all flounder individuals investigated and occurred regularly at all sampling sites (table 3). During single sampling periods, the prevalence ranged between 6-90% (table 6). Regional or seasonal differences in the prevalences of this species were not observed. This organism was only found in histological sections.

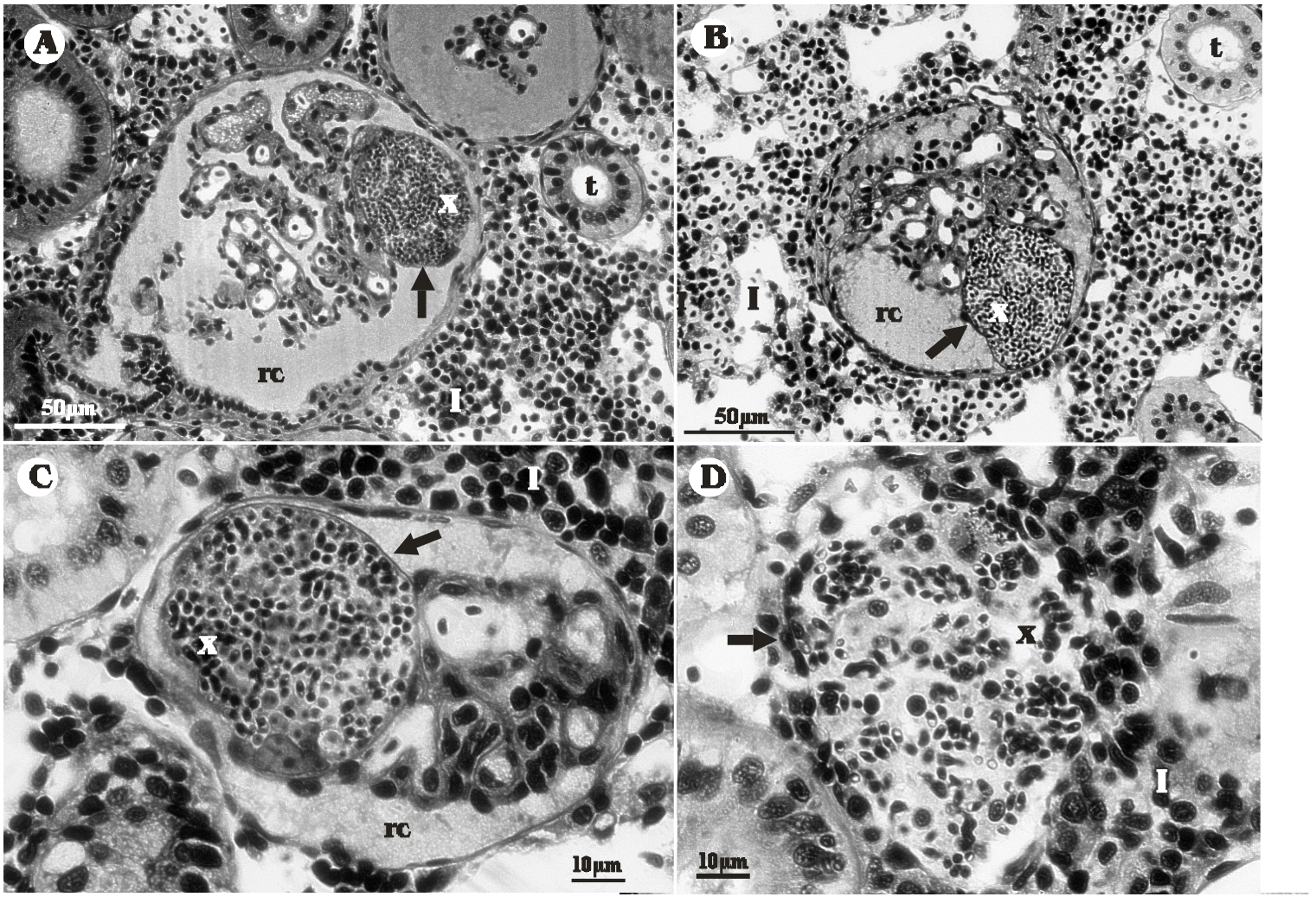


Plate 3: Histological sections of Microsporea sp. 1 from kidney of flounder. **A-C**: Xenomas (x) with spores (indicated by an arrow), located in renal corpuscles (rc); interstitial tissue (I), renal tubules (t). **D**: Xenoma (x) with spores located in the interstitial tissue (I).

Table 6: Prevalence [%] of **Microsporidia sp. 1** at five locations in the German Bight, North Sea, during sampling points from 1995-1997 and 1999-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95	16	17	17	nd	nd
Sep 95	33	37	56	nd	nd
Jan 96	7	32	37	nd	nd
Apr 96	23	17	14	nd	nd
Jul 96	23	11	27	nd	nd
Oct 96	63	27	43	nd	nd
Jan 97	-	6		nd	nd
Apr 97	37	33	30	33	nd
Sep 97	17	43	90	7	nd
Apr 99	45	37	35	44	20
Sep 99	20	24	20	25	-
Apr 00	25	30	30	33	28
Sep 00	50	20	15	10	-
Mean	29.9	25.7	31.8	25.3	24
S.D.	16.2	11.0	22.6	14.4	5.7

Order: Microsporidia Balbiani, 1892

Suborder: Pansporoblastina Tuzet, Maurand, Fizel, Michel and Fenwick, 1971

Family: Glugeidae Thélohan, 1892

Genus: *Glugea* Thélohan, 1891

***Glugea stephani* (**Hagenmüller, 1899) Woodcock, 1904

This microsporidian is a common parasite in the intestine of different flatfish species in European seas, in the Atlantic and Pacific Ocean and the Mediterranean (Lom 1984; Lom and Dyková 1992; Dyková 1995). The development of *G. stephani,* however, depends on warmer waters. Temperatures up to 11°C inhibit its development (McVicar 1975; Olson 1976; Overstreet 1982; Lom and Dyková 1992). During heavy infections the intestine is pervaded by large xenomas up to 0.5 mm in size and their aggregates (Lom 1984). Juvenile fish individuals are particularly susceptible to infection and individuals with heavy infections may not survive their first year (Lom 1984). Flounder (*Platichthys flesus*) is one of the common host species of *G. stephani*. Möller (1974) reported a prevalence of 9% and Lüthen (1989) 9-20% of *G. stephani* in flounder form the German Baltic Sea, while El-Darsh and Whitfield (1999) found prevalences of 3-16% of this parasite in flounder from the tidal Thames, North Sea.

In the present study, only 7 individuals (<1%) of the flounder were found to be infected with spores of this microsporidian species (table 3). One infected individual was found in the Elbe estuary, and two each at Helgoland, Outer Eider and Inner Eider estuary. Tissue damage due to the infection was not observed.

Phylum : Myxozoa Grassé 1970

The myxozoan parasites were classified according to Lom and Dyková (1992) and Kent et al. (2000). Historically the myxozoans were classified as the phylum Myxozoa among the unicellular protozoans (Levine et al. 1980). Recent phylogenetic studies on the molecular and genetic level indicated that myxozoans in fact are true metazoans (Smothers et al. 1994; Siddall et al. 1995; Schlegel et al. 1996). The definite position of myxozoans among the metazoa, however, is not clear. A close relationship between myxozoans and cnidarians is discussed (Bush et al. 2002).

Myxozoans are extracellular parasites of vertebrates and invertebrates. In the vertebrate hosts, mainly fishes rarely amphibians and reptiles, they inhabit the gall bladder, urinary bladder, and ureters, or they are found in tissue such as cartilage, muscle, gills and skin. In the invertebrate hosts, mostly annelids, they occur in the intestinal epithelium (Bush et al. 2002). The complete heteroxenous life cycle of a myxozoan, *Myxobolus cerebralis*, was described for the first time by Markiw and Wolf (1983). *M. cerebralis* affects the cartilage and nervous system of many salmonids worldwide, and causes significant losses in salmonid hatcheries. The life cycle of most of the myxozoans follows the same pattern. Spores released by the fish host are ingested by a tubificid oligochaete, where spores release the infective sporoplasma. The development in the intermediate host culminates in the production of infective triactinomyxon spores, after a form of sexual reproduction. These spores are released into the water with the feces. Spores are then ingested by a salmonid host, where the infective sporoblasm emerges and migrates to the site of infection. In the tissue, the sporoblast develops into a multinucleate trophozoite that feeds on surrounding tissue. Some of the nuclei within the trophozoite become surrounded by cytoplasm and form a pansporoblast, which produces spores (Kent et al. 2000). In former studies developmental stages of myxozoans from the intermediate and from the final host were regarded as different species (Weiser 1985).

Order: Bivalvulida Shulman 1959

Suborder: Variisporina Lom and Noble 1984

Family: Myxidiidae Thélohan 1892

Genus: *Myxidium* Bütschli 1882

Myxidium incurvatum Thélohan, 1892

M. incurvatum is one of the most widespread marine myxosporean parasites, which occurs in the gall bladder of about 50 different hosts species of various orders from pelagic or littoral

habitats in seas of all geographic areas (Lom and Dyková 1992). Shulman (1966, cited by Lüthen) and Lüthen (1989) reported *M. incurvatum* in a prevalence of about 0.85% in flounder from the Baltic Sea.

In the present study, trophozoites and spores of *M. incurvatum* were found attached to the wall of the gall bladder or freely floating in the biliary fluid of 18.7% of flounder individuals (plate 4B-D) examined (table 3). The parasite was present in inividuals from all sampling sites. During single sampling points the prevalence ranged between 3-50% (table 7). Regional or seasonal differences were not observed during the course of the study.

Table 7: Prevalence [%] of ***Myxidium incurvatum*** at five locations in the German Bight, North Sea, during sampling points from 1995-1997 and 1999-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95	16	4	3	nd	nd
Sep 95	10	10	11	nd	nd
Jan 96	3	3	17	nd	nd
Apr 96	3	17	14	nd	nd
Jul 96	31	11	27	nd	nd
Oct 96	10	20	43	nd	nd
Jan 97	-	39		nd	nd
Apr 97	30	30	21	23	nd
Sep 97	3	10	41		nd
Apr 99	42	17	5	22	8
Sep 99	50	33	50	40	-
Apr 00	20	25	45	33	22
Sep 00		5		5	-
Mean	18.2	17.2	21.3	20.5	15.0
S.D.	16.6	11.6	18.2	15.5	9.9

Order: Bivalvulida Shulman 1959

Undetermined species sp. 1 (kidney)

Trophozoites of an undetermined species of Myxosporea were found in the tubules and renal corpuscles of the renal system (plate 4A). Spores were not found during this study, thus a classification of the species was not possible. Only 3.8% of all flounder examined were infected by this species (table 3). Infected fish were only found from autumn 1996 to spring 2000. Prevalences ranged between 3-28% during single sampling points (table 8). Lowest prevalences were found in flounder from Helgoland. Seasonal fluctuations were not observed.

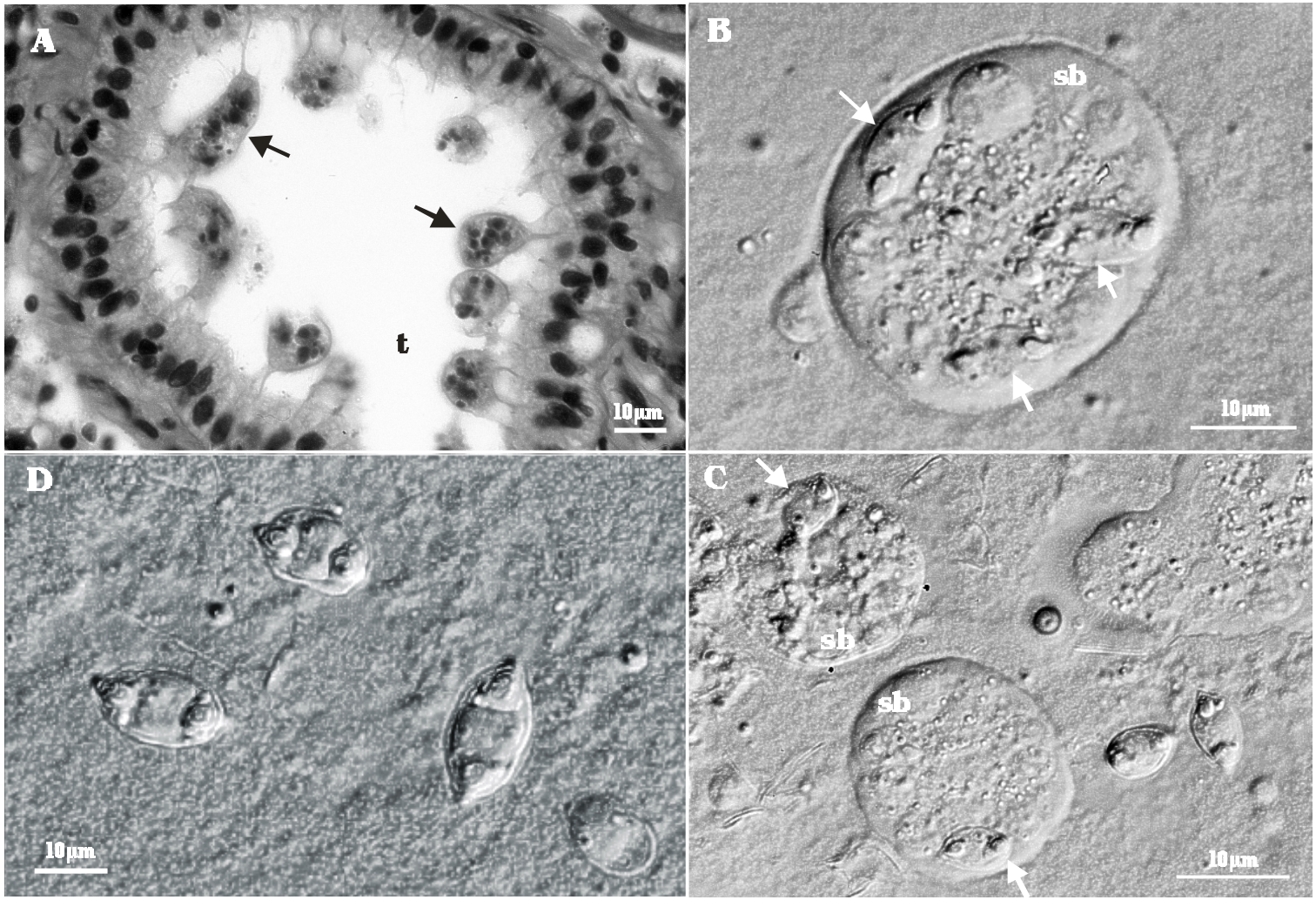


Plate 4: Developmental stages of Myxozoa in flounder. **A**: Histological sections of trophonts (arrows) of Myxozoa sp. 1, attached to the epithelium of a renal tubule (t). **B-D**: Fresh mounts of *Myxidium incurvatum* from the gall bladder; observed by Normaski optics, **B-C**: immature spores (arrows) in sporoblasts (sb), and **C-D**: mature spores, freely floating in the biliary fluid

Table 8: Prevalence [%] of **Myxozoa sp. 1** at five locations in the German Bight, North Sea, during sampling points from 1995-1997 and 1999-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Oct 96	3			nd	nd
Apr 97		11	3		nd
Sep 97		7	3	7	nd
Apr 99	10	5		22	20
Sep 99	25	15			-
Apr 00	20	25			28
Mean	4,8	4,0	0,5	8,8	16,0
S.D.	8,8	7,6	1,1	9,6	14,4

Phylum: Plathyhelminthes

Superclass**:** Cercomeria Brooks, 1982;

Sub-superclass: Neodermata Ehlers, 1984;

Class: Cercomeridae Brooks, O'Grady & Glen, 1985;

Subclass: Trematoda Rudolphi, 1808;

Infraclass **Digenea** Van Beneden, 1858

The digenean parasites were classified according to Yamaguti (1971) and Brooks and McLennan (1993). The life cycles of most of the digeneans are among the most complex in nature, and are usually linked to the feeding strategies of their definite hosts. Almost all life cycles include both free-living and parasitic stages (Bush et al. 2002).

Order: Plagiorchiformes LaRue, 1957 ;

Family: Opecoelidae Ozaki, 1925;

Genus: *Podocotyle* Dujardun, 1845

Podocotyle atomon (Rudolphi, 1802) Odhner, 1905

P. atomon occurs in several fish families and seems to have a circumpolar distribution (Køie 1994). It is common in European flounder and was reported from the North Sea (Ronald 1958; MacKenzie and Gibson 1970, as *Podocotyle* sp.; Gibson 1972, as *Podocotyle* sp.; Broeg et al 1999; El-Darsh and Whitfield 1999) as well as from the Baltic Sea (Möller 1974, 1978; Lüthen 1988, 1989; reviewed by Fagerholm and Køie 1994; Køie 1999). *P. atomon* is known for its great morphological intraspecific variation (Shulman-Albova 1966; Køie 1981, 1983).

The life cycle was described by Hunninen and Cable (1943) and substanciated experimentally by Køie (1981). The most important first intermediate hosts of this parasite are gastropods of the

genus *Littorina,* such as *L. saxatilis* and *L. littorea*. Second intermediate hosts are crustaceans such as amphipods, isopods and mysids. In fish, the definite host, *P. atomon* parasitizes the intestine.

All developmental stages of *P. atomon* show high tolerance to salinity changes. Miracidia of *P. atomon* for instance are able to develop even at a salinity of 4‰ within the egg (Möller 1978). The distribution of *P. atomon* is restricted predominantly by the geographical distribution of its first intermediate host *Littorina*. According to Ankel (1936) the eastern limit of *Littorina* in the Baltic is the waters of the isle of Bornholm and *P. atomon* was found in the Baltic up to a line, which joins southern Sweden with the isle of Rügen (Reimer 1970). *Littorina* also occurs in estuarine waters, in the North Sea as well as in the Baltic Sea (Køie 1983).

In the present study *P. atomon* was found in flounder from all sampling sites in the German Bight. Infection levels were low. Only 5% of all flounder examined were infected with a total of 178 specimens of *P. atomon* (table 3). In single sampling points prevalences ranged between 3-20% at individual sites, intensity between 1-5 parasite individuals per host, although single flounder individuals harboured up to 24 specimens of *P. atomon* (table 9). When the data were pooled over all sampling points, prevalences of *P. atomon* were significantly lower in flounder from Elbe estuary than in flounder from Outer Eider estuary and Helgoland ($E < O$; $p < 0.01$ and $E < H$; $p < 0.05$). In addition, a seasonal trend was observed in the prevalence of this trematode, which was significantly higher in spring than in autumn ($p < 0.05$).

Table 9: Prevalence [%] and range of intensity (in brackets) of ***Podocotyle atomon*** at five locations in the German Bight, North Sea, during sampling points from 1995-1997 and 1999-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95		7 (1-2)	10 (1)	nd	nd
Sep 95				nd	nd
Jan 96		13 (1-16)	10 (1-8)	nd	nd
Apr 96		13 (1-5)	14 (1)	nd	nd
Jul 96	7 (1)			nd	nd
Oct 96		10 (1-18)		nd	nd
Jan 97	-	11 (1-4)		nd	nd
Apr 97		4 (2)	7 (1-4)	10 (1-10)	nd
Sep 97			3 (1)		nd
Apr 99			5 (1)		
Sep 99					-
Apr 00		20 (1-8)	20 (1-24)		22 (1-2)
Sep 00	5 (1)	5 (2)	10 (1-2)	10 (1)	-
Mean [%]	1.0	6.4	6.1	3.3	11.0
S.D.	2.4	6.6	6.4	5.2	15.6

The low infection level of *P. atomon* in flounder from the German Bight corresponded to the results of El-Darsh and Whitefield (1999), who found only 3 flounder individuals infected with a total of 12 specimens of *Podocotyle* sp. from the brackish middle tideway of the river Thames. Køie (1999) also found only few flounder infected with *P. atomon* from the south- western German Baltic Sea. In contrast, Möller (1974) and Lüthen (1989) reported very high abundances and intensity of *P. atomon* in flounder from the same area. High infection levels were also described by MacKenzie and Gibson (1970) and Gibson (1972), who reported *Podocotyle* sp. (classified as *P. atomon* by Køie (1983)) from a marine and two estuarine sites at the east coast of Scotland. In the Scottish studies, highest infection levels were found in flounder from the Ythan estuary, while those observed in flounder from the Dee estuary or a marine site near Aberdeen were much lower. A close regional and temporal relation between the presence of *P. atomon* and the importance of the amphipod *Corophium volutator* in flounder diet was considered as a principal reason for the differences in prevanlence of *P. atomon* observed in this area (MacKenzie and Gibson 1970; Gibson 1972). *C. volutator* acts as a second intermediate host of *P. atomon* and was found in large numbers in the Ythan estuary, forming obviously the most important food source for flounder in this area, while it was absent at the marine site and not as numerous in the Dee estuary.

The composition of the invertebrate fauna and the food spectrum is one of the principal reasons for the presence of *P. atomon* in a geographic area. As the invertebrate fauna in the German Bight is diverse and flounder is an opportunistic feeder, the probability that flounder become infected with *P. atomon* appears to be lower than in the Ythan estuary, where a single copepod species, which acts as the most important intermediate host for *P. atomon* also provided the principal food source for flounder. Unfortunately, details about the specific food spectrum of flounder in the study areas investigated by Möller (1974) and Lüthen (1989) are not presented.

A seasonal trend in the infection characteristics of *P. atomon* was also reported by MacKenzie and Gibson (1970), who observed a winter maximum in the infection intensity of the parasite in flounder from the Ythan River, which was closely related to the migration activity of flounder and its food source as described above. In contrast, a summer maximum in numbers of the parasite was found in flounder from the west coast of Scotland (MacKenzie and Gibson 1970).

Order: Plagiorchiformes LaRue, 1957
Family: Zoogonidae Odhner, 1911
Subfamily: Zoogoninae Odhner, 1902
Genus: *Zoogonoides* Odhner, 1902

Zoogonoides viviparus (Olsson, 1868) Odhner, 1902

Z. viviparus is known from the rectum of a large number of fishes, especially flatfish species (MacKenzie and Gibson 1970; Køie 1976). It is one of the most abundant piscine digenean in North Atlantic waters (Køie 1976). Its geographic distribution includes the North Atlantic, Barents- and White Sea, Mediterranean and the northern Pacific (Bray and Gibson 1986). In European flounder, *Z. viviparus* was recorded only from marine areas of the North Sea (MacKenzie and Gibson1970; Gibson 1972; Lile 1989; Levsen 1990; El-Darsh and Whitfield 1999; Broeg et al. 1999), while it is not common in flounder from the Baltic Sea (Möller 1974; Lüthen 1988, 1989; reviewed by Fagerholm and Køie 1994; Køie 1999).

Morphology, life- history and general biology of *Z. viviparus* were described by Orrhage (1973), Køie (1976) and Bray and Gibson (1986). In general, the prosobranch gastropod *Buccinum undatum* L. acts as the first intermediate host of *Z. viviparus*. The tail-less cercaria develops in sporocysts in this gastropod. Ophiuroid echinoderms as *Ophiura albida* are the most important second intermediate host, while polychaetes serve as second intermediate hosts, where ophiuroids are scarce (Køie 1976).

The distribution of *Z. viviparus* coincides with the distribution of the first intermediate host, *B. undatum*. This gastropod occurs only in areas with high water salinity and avoids brackish waters such as estuaries and eastern parts of the Baltic Sea, but it survives salinities of less than 14‰ (Køie 1969).

In the present study, *Z. viviparus* was one of the most abundant parasite species of flounder in the German Bight. A detailed presentation of the infection characteristics of *Z. viviparus* at each of the sampling sites in the German Bight during the course of the study is given in chapter 3. In general, 28.7% of all flounder examined were infected, representing a total of 13 791 individuals of *Z. viviparus* (table 3). Highest prevalences and intensities were found especially in flounder from Helgoland, while lowest infection levels were observed in the estuaries (table 10).

Table 10: Prevalence [%] and range of intensity (in brackets) of ***Zoogonoides viviparus*** at five locations in the German Bight, North Sea, during sampling points from 1995-1997 and 1999-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95	9 (10-200)	10 (1-3)	83 (2-250)	nd	nd
Sep 95	3 ((25)	33 (1-220)	89 (1-60)	nd	nd
Jan 96	14 (1-50)	42 (1-100)	23 (1-100)	nd	nd
Apr 96	3 (50)	40 (1-140)	79 (1-410)	nd	nd
Jul 96	20 (1-100)	22 (6-75)	93 (3-440)	nd	nd
Oct 96	7 (1)	70 (2-100)	97 (1-270)	nd	nd
Jan 97	-	17 (2-40)	67 (15-20)	nd	nd
Apr 97	10 (1-12)	39 (1-90)	50 (2-330)	33 (2-30)	nd
Sep 97		37 (1-54)	50 (1-128)		nd
Apr 99	10 (1-34)	26 (1-72)	100 (23-292)	33 (3-270)	13 (1-76)
Sep 99			15 (1-3)		-
Apr 00		5 (1)	30 (2-70)	11 (2)	
Sep 00			65 (1-250)		-
Mean	6.3 (29.1)	26.2 (24.8)	64.7 (57.6)	12.8 (30.9)	6.5 (76)
S.D.	6.5 (45.3)	20.2 (36.4)	28.9 (74.3)	16.2 (75.7)	9.2 (0)

These findings coincide with the results of Gibson (1972), who reported *Z. viviparus* in flounder from estuarine and offshore locations at the Scottish west coast. Gibson (1972) observed highest prevalences and intensities in flounder from the marine area, while infection levels in fish from the estuaries were much lower. He considered the absence of *Buccinum undatum* L., the first intermediate host, as the main reason for the low infection levels of flounder in the estuaries and suggested *Z. viviparus* as an indicator species for migration activity of flounder from marine waters into an estuary. The results of studies by El-Darsh and Whitfield (1999) on flounder in the tidal River Thames in Great Britain and of Køie (1983) on common dab (*Limanda limanda*) in Danish and adjacent waters, support this hypothesis proposed by Gibson (1972). While El-Darsh and Whitfield (1999) found only few flounder infected with a few specimens of *Z. viviparus* in the brackish waters of the River Thames, where *B. undatum* does not occur, Køie (1983) observed prevalences of 100% in common dab throughout all size classes, especially in the North Sea and the Kattegat, where *B. undatum* is common.

Order: Hemiuriformes Travassos et al., 1969
Family: Hemiuridae Looss, 1899
Subfamily: Derogeninae Nicoll, 1910
Genus: *Derogenes* Lühe, 1900

Derogenes varicus (Müller 1784) Looss, 1901

D. varicus is probably the most widely distributed digenean. It has a world- wide distribution with a preference for subarctic and temperate areas and is known from more than one hundred species of teleosts (Køie 1979, 1983). It is a common parasite of flounder (Ronald 1959) and was reported by several authors from the North Sea and adjacent waters (MacKenzie and Gibson 1970; Gibson 1972; Broeg et al. 1999; El-Darsh and Whitfield 1999).

Morphology and life- cycle were described in detail by Køie (1979). In general, the gastropod *Natica* spp. acts as the first intermediate host. Second intermediate hosts are crustaceans, mainly copepods. Free-swimming cercaria are ingested by these copepods and develop into metacercaria. Additionally the metacercaria can be found in *Pleurobrachia pileus* (Müller) (Ctenophora) and *Sagitta* spp. (Chaetognatha). Fish, the definite hosts, become infected through ingestion of bentic or plantonic invertebrates, or through preying on other fish species, which may act as transport hosts (Gibson 1972; Køie 1979, 1983). Adult *D. varicus* parasitize in the stomach of fish (MacKenzie and Gibson 1970).

The distribution of *D. varicus* is restricted to marine environments, due to the geographical distribution of its first intermediate host, *Natica* spp. These gastropods are very susceptible to low water salinities and therefore absent from brackish waters (Ankel 1936; Möller 1994b; Køie 1983).

In the present study, 5.7% of all flounder investigated were infected and harboured a total of 115 individuals of *D. varicus* (table 3). The parasite reached prevalences of 3-25% in single sampling points. In general, it was most prevalent in flounder from the Outer Eider estuary, Helgoland and Spiekeroog and occurred sporadically in fish from the Elbe and Inner Eider estuary (table 11). Intensity ranged on average between one or two specimens of *D. varicus*, but single flounder individuals from Elbe and Outer Eider estuary harboured up to 16 parasites per fish (table 11). When the data were pooled over all sampling points, prevalences of *D. varicus* were significantly lower in flounder from Elbe and Inner Eider estuary than in flounder from Helgoland ($E < H$; $p < 0.01$ and $I < H$; $p < 0.05$). In addition to regional differences, a seasonal pattern was also observed in the prevalences of *D. varicus*, which were significantly higher in spring than in autumn ($p < 0.05$).

Table 11: Prevalence [%] and range of intensity (in brackets) of ***Derogenes varicus*** at five locations in the German Bight, North Sea, during sampling points from 1995 to 1997 and 1999 to 2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95	6 (1-16)	14 (1-9)	3 (1)	nd	nd
Sep 95		10 (1-2)	22 (1-2)	nd	nd
Jan 96	3 (1)	16 (1-2)	17 (1)	nd	nd
Apr 96		10 (1-5)		nd	nd
Jul 96			7 (1)	nd	nd
Oct 96		3 (1)		nd	nd
Jan 97	-	6 (1)		nd	nd
Apr 97	3 (1)	7 (1)	3 (1)	13 (1-2)	nd
Sep 97		3 (8)			nd
Apr 99	5 (1)	7 (1)	11 (1-2)		7 (2)
Sep 99					-
Apr 00		5 (4)	10 (1)		
Sep 00	5 (2)	15 (1-4)		25 (1-2)	-
Mean [%]	1.8	7.4	5.6	6.3	3.5
S.D.	2.4	5.4	7.4	10.5	4.9

As flounder is a migrating species, it is not surprising to find *D. varicus* in flounder from stations, such as Elbe and Inner Eider estuary, where the snail host *Natica* spp. does not occur. El-Darsh and Whitfield (1999) also found a single flounder individual with 4 specimens of *D. varicus* in the brackish waters of the middle Thames tideway, which obviously had migrated to this station from the sea.

The distribution of *D. varicus* in the German Bight corresponds to the results of Gibson (1972), who reported highest prevalences and mean intensities of *D. varicus* in flounder from a marine site (about 30% and 12 individuals per fish), and much lower infection levels in two estuarine habitats (about 10-15% and 1-2 individuals per fish) at the east coast of Scotland. MacKenzie and Gibson (1970) reported 832 individuals of *D. varicus* out of a total of 785 flounder from the same Scottish sites. Thus, prevalences and intensities were higher at the Scottish than at the German locations.

Order: Hemiuriformes Travassos et al., 1969
Family: Hemiuridae Looss, 1899
Subfamily: Lecithasterinae Odhner1905
Genus: *Lecithaster* Lühe, 1901

Lecithaster gibbosus (Rudolphi, 1802) Lühe, 1901

L. gibbosus is one of the most widely distributed digeneans in the North Atlantic Ocean and adjacent waters (Køie 1983, 1984, 1989). It is a common digenean in the intestine of various marine teleost families including Clupeidae, Salmonidae, Gasterosteidae and Pleuronectidae (Køie 1984, 1989). *L. gibbosus* was reported from flounder only from the North Sea (Ronald 1959; MacKenzie and Gibson 1970; Gibson 1972; El-Darsh and Whitfield 1999), while it seems to be absent from the Baltic.

The life cycle was described in detail by Køie (1989). Following these descriptions, *L. gibbosus* uses opistobranch gastropods of the genus *Odostomia* as the first intermediate host. The second intermediate host are calanoid copepods, such as *Artica* sp., which feed on the immotile cercaria. The metacercaria develops in the haemocoel of the crustacen hosts. Fishes, the definite hosts, become infected by ingesting infected copepods or small planktophagous fish, which suggests that *L. gibbosus* may be transferred from one fish to another (Køie 1989).

L. gibbosus was very scarce in flounder from the German Bight. Out of 1073 fish examined, only 10 individuals were infected with a total of 13 specimens of *L. gibbosus* (table 3). Mean intensity of this trematode ranged between 1-2 specimens per host (table 12). While most of the infected fish were found at Helgoland and Outer Eider estuary, only a single infected fish was found at Spiekeroog and Inner Eider estuary. In the Elbe estuary *L. gibbosus* was absent.

El-Darsh and Whitfield (1999) also reported low infection levels of *L. gibbosus* in flounder from the tidal River Thames. Prevalences ranged between 1.3 and 6.7%, mean intensities between 1-6 individuals per host. Køie (1983) described comparable low prevalences (1-10%) of *L. gibbosus* in common dab (*Limanda limanda*) from Danish and adjacent waters.

In contrast to these studies, Gibson (1972) reported prevalences up to 60% of *L. gibbosus* in flounder from a marine site and between 7-17% at two estuarine sites at the east coast of Scotland. Mean intensities ranged between 15 individuals at the marine site and 3-25 at the estuarine sites. He concluded that estuarine flounders became infected in the sea during the spawning migration.

Table 12: Number of infected flounder and range of intensity (in brackets) of ***Lecithaster gibbosus*** at four locations in the German Bight, North Sea, during sampling points from 1995-1997 and 1999-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95		4 (1-2)	2 (1-2)	nd	nd
Apr 99				1 (1)	1 (1)
Sep 99			1 (1)		
Sep 00			1 (1)		

The differences in the infection levels of *L. gibbosus* in flounder from the marine sites in the German Bight and the Scottish localities cannot be explained satisfactory. The studies in Scotland were carried out 25-30 years ago. A direct comparison of the parasite community of flounder from both geographic regions at the same time scale was not possible as studies on parasites of flounder from the German Bight conducted in the early seventies were not available and no such studies were performed in Scottish waters in recent years. In addition it is not known to the author whether environmental conditions at the Scottish west coast changed during the last decades in a way as reported from the German Bight (Schmolke et al. 1999).

Order: Hemiuriformes Travassos et al., 1969

Family: Hemiuridae Looss, 1899

Subfamily: Hemiurinae Looss, 1899

Genus: *Brachyphallus* Odhner, 1905

Brachyphallus crenatus (Rudolphi, 1802) Odhner, 1905

The digenean *Brachyphallus crenatus* has an Arctic-boreal distribution and is known from the North Atlantic, the North Pacific and adjacent seas, from shallow waters down to several hundered meters in depth (Gibson and Bray 1986; Køie 1992). It parasitizes the stomach of various marine teleosts families, but apparently has a preference for salmonids, clupeids, gasterosteids and pleuronectids (Gibson and Bray 1986). *B. crenatus* was reported in flounder from the Baltic Sea (Lüthen 1988, 1989; reviewed by Fagerholm and Køie 1994; Koçoglu 1998; Køie 1999), while it was not mentioned in studies from the North Sea (Ronald 1959; MacKenzie and Gibson 1970; Gibson 1972; Lile 1989; Levsen 1990; El-Darsh and Whitfield 1999).

The life cycle of *B. crenatus* was described by Køie (1992). The opistobranch gastropod *Retusa obtusa* (Montagu) and the prosobranch gastropod *Rissoa* spp. act as its first intermediate host (Køie 1983, 1992). The second intermediate hosts are calanoid copepods, which ingest the free-

swimming cercaria. *Sagitta* spp., *Pleurobrachia pileus* [Müller] or small fishes can act as transport hosts. The definite hosts become infected by ingesting infected copepods or small fishes. Thus, pisciphagus fish species in general have higher infection levels of *B. crenatus* than planctiphagus fish, due to an accumulation effect of parasites in the fish (Lüthen 1989).

The geographical distribution of *B. crenatus* in its fish host coincides with that of *R. obtusa*, which has a unique wide distribution and occurs around the British Isles, from Greenland and Iceland to Scandinavia, around Nova Scotia, and around the Aleutian Islands, from muddy beaches down to 300m in depth (for details see Thompson 1988). Highest infections occur in areas with high salinities (Lüthen 1989; Køie 1999). As *B. crenatus* survives in anadromous fish as salmonids during their migration into fresh water, it may act as a biological tag, which indicates a previous stay of the specimen in an marine environment (Køie 1992).

In the present study, infected flounder were found at all sampling sites, but prevalences of *B. crenatus* were low. Only 3% of all flounder examined were infected with *B. crenatus*, which harboured a total of 106 parasite specimens (table 3). During single sampling periods, only 1-2 flounder were infected per location, with an intensity of 1-6 parasite individuals per fish (table 13). An exception was found in flounder from the Outer Eider estuary during the first sampling points, when 7 flounder individuals were infected with the parasite. *B. crenatus* occurred more frequently in the spring and summer sampling periods than during autumn and winter ($p < 0.05$). Differences in the infection levels of *B. crenatus* between the sampling sites were not evident.

Table 13: Number of infected flounder and range of intensity (in brackets) of ***Brachyphallus crenatus*** at five locations in the German Bight. North Sea, during sampling points from 1995-1997 and 1999-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95	2 (1-12)	7 (1-5)	1 (1)	nd	nd
Apr 96			1 (1)	nd	nd
Jul 96	1 (2)			nd	nd
Oct 96	2 (1)			nd	nd
Apr 97	1 (6)		2 (1)		nd
Apr 99	1 (1)	1 (4)		2 (1-5)	2 (1-5)
Sep 99			1 (1)		-
Apr 00	2 (3-36)	3 (1-6)			

The results of the present study correspond to reports of *B. crenatus* in flounder from the Baltic Sea, where prevalences and intensities also were low (Lüthen 1988, 1989; reviewed by Fagerholm and Køie 1994; Koçoglu 1998; Køie 1999). Only 4-13% of flounder from locations in the western Baltic were infected with *B. crenatus*, infected fish carried only a single parasite individual per fish. Rare records of *B. crenatus* were also made for dab (*Limanda limanda*) from

Danish and adjacent waters (Køie 1983), while prevalences in cod (*Gadus morhua*) from the same area were higher, ranging between 10-60% (Køie 1984). The author concluded that planctonic invertebrates and fish obviously might have a low importance as food items for dab, but play a mayor role in the food composition of cod, as the prevalence of *B. crenatus* was positively related with fish size. The prey spectrum with a low uptake of planctonic invertebrates might also be a reason for low prevalences of *B. crenatus* in flounder.

Metacercaria sp. 1

The metacercaria of an undetermined trematode species was found encysted in the gills of flounder, which most likely act as intermediate or transport hosts. Final hosts might be piscivorous birds or aquatic mammals. Larvae were "u"- shaped inside of the cysts, which was observed in histological sections.

This trematode larva was one of the most abundant parasite species of flounder in the German Bight. 45.3% of all flounder examined were infected with this parasite, accounting for 20 966 individuals in total from 1073 flounder investigated (table 3). During single sampling points, prevalences of the metacercaria ranged between 7-93% at individual sites, intensity between 1-1270 individuals per host (table 14). In general, prevalences were lowest in flounder from the Elbe estuary when compared to flounder from the other sites. Detailed information about the infection characteristics of the metacercaria at each of the sampling sites in the German Bight during the course of the study are given in chapter 3.

Table 14: Prevalences [%] and range of intensity (in brackets) of the **metacercaria sp. 1** at five locations in the German Bight, North Sea, during sampling points from 1995-1997 and 1999-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95	9 (10-40)	3 (10)	17 (1-20)	nd	nd
Sep 95	7 (10)	7 (1-10)	11 (1)	nd	nd
Jan 96	14 (5-20)	32 (1-92)	57 (1-1150)	nd	nd
Apr 96	37 (1-228)	50 (1-426)	43 (1-35)	nd	nd
Jul 96	23 (1-200)	33 (2-11)	70 (1-263)	nd	nd
Oct 96	13 (1-83)	40 (1-548)	40 (1-58)	nd	nd
Jan 97	-	44 (1-1270)	67 (1-16)	nd	nd
Apr 97	23 (1-103)	68 (1-115)	83 (1-90)	60 (1-75)	nd
Sep 97	40 (1-125)	50 (1-35)	57 (1-171)	83 (1-265)	nd
Apr 99	15 (1-233)	89 (1-147)	45 (1-19)	33 (1-16)	93 (1-479)
Sep 99	35 (2-55)	76 (1-94)	60 (1-72)	75 (1-200)	-
Apr 00	55 (1-170)	65 (1-136)	80 (1-422)	56 (3-36)	78 (1-1012)
Sep 00	40 (1-123)	40 (1-110)	65 (2-1237)	90 (1-335)	-
Mean	25.9 (28.3)	45.9 (38.8)	53.5 (46.6)	66.2 (37.2)	85.5 (105.2)
S.D.	15.2 (51.8)	24.9 (126.4)	21.9 (149.0)	20.8 (62.5)	10.6 (203.1)

Trematodes of the genus *Cryptocotyle*, such as *C. lingua* Creplin, 1825 and *C. concavum* Creplin, 1825, are often reported as common parasites of flounder, which in the life cycle of these parasites is used as an intermediate host. First intermediate hosts are gastropods of the genus *Littorina* and *Hydrobia* (Køie 1999), while the metacercaria of these parasites are encysted in gill arches, fins and skin of fishes. The definite hosts are aquatic birds of the genus *Larus* (Køie 1977). The life cycle of *C. lingua* was elucidated by Stunkhard (1930), that of *C. concavum* by Wooton (1957).

C. lingua and *C. concavum* were repeatedly reported in flounder from the Baltic Sea (Möller 1974, 1978; Lüthen 1989; review of Fagerholm and Køie 1994; Køie 1999) as well as from the North Sea and the North Atlantic (Ronald 1959; MacKenzie and Gibson 1970; Van den Broek 1979; El-Darsh and Whitfield 1999).

Möller (1974) and Lüthen (1989) reported high infection levels throughout the year. In the Bay of Kiel prevalences of *Cryptocotyle* ranged between 70-90%, intensities between 10-28 individuals per fish with a total 36 327 individuals out of 2 183 flounder investigated (Möller 1974), while prevalences of 40-80% and intensities of 1-734 were found in the more eastern parts of the German Baltic (Lüthen 1989). Gill filaments were more heavily infected with both *C. lingua* and *C. concavum* than the fins or the skin of flounder (Van den Broek 1979; Lüthen 1989). These finding coincide with the results of the present study on flounder from the German Bight and suggest that the undetermined metacercaria may be a member of the genus *Cryptocotyle*. Anyway, this assumption has to be proved in future studies.

Subclass: Cercomeromorphae Bychowsky, 1937
Infraclass: **Monogenea** Van Beneden, 1858
Order: Gyrodactyliformes Bychowsky, 1937
Family: Gyrodactylidae Van Beneden and Hesse, 1863
Genus: ***Gyrodactylus*** Nordman, 1832

The monogenean parasites were classified according to Yamaguti (1963a) and Brooks and McLennan (1993). Gyrodactylids are very small, measuring 1mm or less in length. They are parasitic on the gills and skin of marine and freshwater fishes. Life cycle is direct and they are the only group of monogeneans that are viviparous. Its dispersal occurs directly by physical contact between host individuals and via water that is circulated through the host's gills, to which gyrodactylids are attached (Bush et al. 2002). In fish hatcheries, where high fish densities

facilitate parasite dispersal, gyrodactylids are known to cause severe damage of the gill epithelium, which leads to an increased mortality of hosts (Malmberg 1993).

In the present study, specimen of *Gyrodactylus* sp. occurred very sporadically on the gills of flounder from the Elbe estuary, Outer Eider estuary and Helgoland. From a total of 742 fish examined only 8 individuals were infected with a total of 22 individuals (table 3). Intensity ranged from 1-10 individuals per flounder. Pathological damage of gill tissue was not observed.

Due to the low number of specimen found during the study, it was not possible to identify the parasite at the species level. Malmberg (1970) points out that there exsists a pronounced host specificity in *Gyrodactylus*. Members of the *Gyrodactylus unicopula*- group parasitize different flatfish species from the northeast Atlantic waters including the Barents- and White Sea as well as the Baltic Sea. For flounder there are reports of *G. unicopula* from the Baltic Sea (Gluhova 1955; Lüthen 1988) and of *G. flexibiliradix* from the West coast of Sweden (Malmberg 1970). *Gyrodactylus* sp. was found in flounder from the tidal Thames (El-Darsh and Whitfield 1999) from West Norway (Levsen 1990), the Swedish West coast (Thulin et al. 1987) and the Baltic Sea (Køie 1999).

Infraclass: **Cestodaria** Monticelli 1891
Cohort: Cestoidea Rudolphi 1808
Subcohort: **Eucestoda** Southwell 1930
Order: Pseudophylliformes Carus 1863
Family: Bothriocephalidae Blanchard 1849
Genus: ***Bothriocephalus*** Rudolphi 1808

The Eucestoda were classified according to Yamaguti (1959), Brooks and McLennan (1993) and Khalil et al. (1994). *Bothriocephalus scorpii* (Müller 1776) was described as a common bothriocephalid species of flatfish in boreal waters. General studies on its morphology and ecology were made by various authors (Cooper 1918; Hilmy 1929; Rees 1958; Jones 1975). The heteroxenous life cycle is characterized by a free-living coracidium and by larval stages that need different intermediate hosts. The procercoids develop in small crustaceans like copepods, which act as the first intermediate host (Markowski 1935; Yamaguti 1959; Solončenco 1979, cited by Lüthen 1989), while plerocercoids and adults use different fish species as second intermediate and final hosts respectively (Markowski 1935; Reimer 1970; de Groot 1971a; Möller 1974b; Solončenco 1985 (cited by Lüthen 1989)).

Most immature pseudophyllids in flounder have been reported as *Bothriocephalus scorpii* (Müller 1776) in previous studies (MacKenzie and Gibson 1970; review of Fagerholm and Køie 1994). Renaud et al. (1983, 1984) could differenciate *B. scorpii* from turbot (*Psetta maxima*) and brill (*Scophthalmus rhombus*) as two distinct species, *B. gregarious* and *B. barbatus* respectively, using electrophoretic and biochemical tests. It appears that *B. scorpii*, believed to be a non- specific parasite with a wide distribution area and a wide variety of potential host species (Cooper 1918), in fact is a collective name including several species, each displaying a strict host specificity (Renaud et al. 1986, Roberts et al. 1990; Køie 1999).

In the present study plerocercoids of *Bothriocephalus* were found in very low abundance and intensity in the intestine of flounder from Elbe estuary, Outer Eider estuary, Helgoland and Spiekeroog. From a total of 1 073 flounder investigated, only 8 founder individuals were infected, each with a single plerocercoid (table 3). Flounder act as paratenic hosts only (Levsen 1990; Køie 1999). This is probably due to the specific morphology and topography of the intestinal mucosa, which may not present an adequate surface for attachment of the scolex. Specific bothriocephalid scolices appear to be closely associated with specific mucosa types (Rees 1958; Davey and Peachey 1968).

Order: Proteocephaliformes Mola 1928

Family: Proteocephalidae La Rue 1911

Genus: *Proteocephalus* Weinland 1858

The life cycle of several species of *Proteocephalus* in the Palearctic region was reviewed by Scholz (1999). The life cycle is heteroxenous and includes a single intermediate host. A floating egg is ingested by planctonic crustaceans, mostly copepods, which serve as intermediate hosts. The metacestode, or procercoid, develops in the body cavity of these planctonic crustaceans, and the definitive hosts, mainly freshwater fishes, rarely amphibians and reptiles, become infected after consuming these crustaceans. In general, species of *Proteocephalus* show a high degree of host specificity. Infections with alien host were never successful (Willemse 1969; Chubb et al. 1987), but it seems that some species are able to adapt to unsuitable host species under particular ecological conditions (Scholz 1999).

In the present study, only juvenile specimens of *Proteocephalus* were found in the intestine of flounder, exclusively from the Elbe and Outer Eider estuaries. Abundance and intensity, observed in the present study were low. From a total of 1 073 flounder examined, only 12 flounder individuals were infected with a total of 35 juvenile *Proteocephalus* (table 3). Intensity

ranged between 1-9 individuals per fish. Comparable low infections were reported from flounder by Janiszewska (1938) and Willemse (1968, 1969). As a reliable classification is obtained only from gravid adult individuals (Priemer 1982), an identification of *Proteocephalus* up to the species level was not assessed. Juvenile *Proteocephalus* were reported from flounder of the North Sea (Wichowski 1990) and the Baltic Sea (review of Fagerholm and Køie 1994). These host individuals most probably had migrated from freshwater environments into the sea.

Cestoda larvae

The larvae of two undetermined cestode species were rarely found in a few flounder individuals (table 15). While larvae of sp. 1 was found only at the coastal and offshore sites, larvae of sp. 2 were mainly found in the Elbe estuary.

Table 15: Number of flounder infected with cestode larvae at four locations in the German Bight, during sampling points from 1995-1997 and 1999-2000. For key, see table 4.

Larvae sp. 1

	Outer Eider	Helgoland	Spiekeroog
Sep 95	1 (1)		nd
Apr 97		1 (1)	
Sep 97			1 (2)

Larvae sp. 2

	Elbe	Outer Eider
Jan 96		1 (2)
Oct 96	1 (1)	
Apr 97	1 (1)	
Apr 99	1 (5)	

Phylum: Nemathelminthes

Class: Nematoda

The Nematoda were classified according to Yamaguti (1962), Moravec (1994) and Anderson (2000).

Family: Capillariidae Railliet, 1915

Genus: *Paracapillaria* Mendonça, 1963

Paracapillaria gibsoni Moravec, 1987

P. gibsoni is distributed in the North Sea and in brackish waters at river estuaries near the coast of Scotland (MacKenzie and Gibson 1970). It is known to infect the rectum and the pyloric caeca of European flounder (Moravec 1994), and was also reported from two other flatfish species, witch (*Glyptocephalus cynoglossus*) and long rough dab (*Hippoglossoides platessoides*) from the west coast of Norway (Levsen 1990). *P. gibsoni* is probably a marine species, which may be

introduced into freshwater areas during the migration of its hosts (Moravec 1987, 1994). The life cycle of *P. gibsoni* is still unknown (Moravec 1994).

In the present study 11.9% of all flounder examined, were infected with a total of 1102 specimens of *P. gibsoni* (table 3). This nematode was found in flounder from all sampling sites. During single sampling periods, prevalences ranged between 3-55% at individual sampling sites, intensities between 1-12 parasite specimens per fish, only in autumn of 2000, very high intensities of 2-170 were found in flounder especially from Helgoland (table 16).

Table 16: Prevalence [%] and range of intensity (in brackets) of ***Paracapillaria gibsoni*** at five locations in the German Bight, North Sea. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95	10 (1-6)	7 (1)	7 (1-3)	nd	nd
Sep 95	3 (1)	40 (1-12)	44 (1-10)	nd	nd
Jan 96		10 (1-3)	20 (1-4)	nd	nd
Apr 96			7 (1)	nd	nd
Jul 96				nd	nd
Oct 96		23 (1-10)	53 (1-10)	nd	nd
Jan 97	-	6 (1)		nd	nd
Apr 97		4 (14)	7 (3-9)	3 (1)	nd
Sep 97		7 (2-5)	3 (1)	7 (1-10)	nd
Apr 99		5 (1)	10 (1-2)		
Sep 99		29 (1-7)	35 (1-9)	10 (1)	-
Apr 00	10 (2-6)	5 (7)	24 (3-36)		11 (1-3)
Sep 00	25 (1-15)	40 (1-15)	55 (2-170)	40 (1-8)	-
Mean [%]	4 (4)	13.5 (4.2)	20.4 (14.2)	10 (4.6)	5.5 (2)
S.D.	7.6 (4.2)	14.4 (4.3)	20.1 (31.5)	15.2 (3.6)	7.8 (1.4)

When the data were pooled over all sampling points, highest prevalences were found at Helgoland, followed by Outer Eider and Spiekeroog, while lowest prevalences were observed at the estuarine sites Elbe and Inner Eider (table 16). Differences were significant between Elbe < Outer Eider, Helgoland ($p< 0.001$) and between Outer Eider < Helgoland ($p< 0.05$).

Seasonal variations were observed in the prevalence of *P. gibsoni*. In autumn, prevalences were significantly higher than in spring ($p< 0.001$).

Order: Ascaridida Skrjabin & Schulz, 1940
Superfamily: Ascaridoidea Railliet & Henry, 1915
Family: Anisarkidae Railliet & Henry, 1912
Genus: *Hysterothylacium* Ward & Magath, 1917

Hysterothylacium aduncum (Rudolphi, 1802) Deardorff & Overstreet, 1981

This nematode is a very common parasite of many marine teleosts in the North Atlantic and adjacent seas. Life cycle of *H. aduncum* was shown experimentally by Køie (1993). The first two moults occur in the egg. The third stage larvae develop in invertebrate crustaceans such as copepods, amphipods, isopods and mysids as well as in fishes. In the latter they are usually encapsulated in the viscera. The further development in fish depends on the length of the larva. Sexually mature adults inhabit the digestive tract of marine teleosts. Ctenophores, chaetognaths, polychaetes and ophiuroids, which become infected by ingesting infected crustaceans, may act as obligate intermediate hosts or transport hosts (Køie 1993). *H. aduncum* is able to leave the intestine of the host via mouth or anus after the fish had died (Möller 1978).

H. aduncum was reported by various authors in flounder from the North Sea (Ronald 1959; MacKenzie and Gibson 1970; Gibson 1972; Lile 1989; Levsen 1990; Wichowski 1990; Pattipeiluhu 1996; Broeg et al. 1999; El-Darsh and Whitfield 1999) as well as from the Baltic Sea (Möller 1974, 1978; Lüthen 1988, 1989; review of Fagerholm and Køie 1994; Køie 1999).

In the present study 7.7% of all flounder examined were infected with a total of 192 specimens of *H. aduncum* (table 3). The nematode occurred in the intestine or encysted in liver and mesenteries of flounder from all sampling sites. Prevalences ranged between 3-40%, intensities between 1-30 parasite individuals per host during single sampling points (table 17). When the data were pooled over all sampling points, no significant differences could be found in the infection level of *H. aduncum* at the sampling sites, but seasonal differences were observed with significantly higher prevalences in spring than in autumn ($p < 0.001$).

Möller (1974 b) found comparable prevalences and intensities in flounder from the Bay of Kiel, but a clear seasonal trend was not observed in the infection levels of *H. aduncum*. Möller (1974 b) concluded that the great variety of developmental possibilities due to the wide host spectrum probably was the main cause for a lack of seasonal differences in the infection levels of this parasite.

Table 17: Prevalence [%] and range of intensity (in brackets) of ***Hysterothylacium aduncum*** at five locations in the German Bight, North Sea. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95	3 (1)	10 (1-6)		nd	nd
Sep 95				nd	nd
Jan 96	10 (1)	6 (1-5)		nd	nd
Apr 96	3 (1)	3 (1)	14 (1)	nd	nd
Jul 96	17 (1)		7 (1)	nd	nd
Oct 96	7 (1)	7 (1)		nd	nd
Jan 97	-			nd	nd
Apr 97	23 (1-10)	7 (1)	7 (3-6)	13 (1-7)	nd
Sep 97	3 (1)		3 (1)	3 (1-2)	nd
Apr 99	20 (1-3)	21 (1)	5 (1)	22 (1-30)	40 (1-5)
Sep 99		10 (1)			-
Apr 00	10 (1)	10 (1-3)	35 (1-20)	22 (1-2)	11
Sep 00		15 (1)		10 (1-4)	-
Mean [%]	8.0	6.8	5.5	11.7	25.5
S.D.	8.1	6.5	9.9	9.3	20.5

H. aduncum is susceptible to changing water salinity and not able to stabilise its water balance when osmolarity of the surrounding medium changes (Möller 1978). This would explain the rare records of *H. aduncum* in the tidal River Thames (El-Darsh and Whitfield 1999), and the regular presence and high infection levels of the larvae of *H. aduncum* in flounder from the German Baltic Sea, where salinity is more stable. In this area the nematode reached prevalences of up to 70% and intensities of 1-40 individuals per host (Lüthen 1989; Køie 1999).

Subclass: Secernentea

Order: Ascaridida Skrjabin & Schulz, 1940

Superfamily: Ascaridoidea Railliet & Henry, 1915

Family: Anisarkidae Railliet & Henry, 1912

Genus: ***Goezia*** Zeder, 1800

Goezia sp. is parasitic in the alimentary tract of freshwater and migratory fishes as well as of aquatic reptiles (Moravec 1994).

Life cycle is known from *Goezia ascaroides*, which is parasitic in *Silurus*, *Salmo* and *Trachinus* (Moravec 1994), was studied by Mozgovoy et al. (1971). *G. ascaroides* occurs only locally in the North, Adriatic, Black, Azov and Caspian Sea basins and additionally to freshwater, in brackish waters of the Caspian Sea. Gravid females are localized in the stomach of catfishes (*Silurus glanis*). Eggs are released with the host's faeces into the water. The eggs develop to a second- stage larvae which hatch from the egg shell. The free-swimming larvae are ingested by

copepods, such as *Diaptomus castori*, which probably act as the only intermediate host. The larvae develop to the third-stage larva in the body cavity of the host. This stage is infective for fish. Several species of fishes may act as paratenic hosts of *G. ascaroides*, acquiring the infection by ingesting the intermediate host copepods.

In the present study only 17 fish individuals (1.6%) out of all fish examined were infected with a total of 34 specimens of *Goezia* sp. (table 3). This parasite occurred only at three of the sampling sites, Elbe estuary, Outer Eider estuary and Spiekeroog, and only during summer and autumn sampling periods (table18). Prevalence as well as intensity were low during single sampling points. Infected flounder were more frequent in the Elbe estuary than in the Outer Eider estuary (table 18).

Table 18: Number of infected flounder and range of intensity (in brackets) of ***Goezia* sp.** at three locations in the German Bight. North Sea. For key, see table 4.

Sampling period	Elbe	Outer Eider	Spiekeroog
Jun 95	6 (1)		nd
Sep 95	3 (1)		nd
Jul 96	17 (1-3)		nd
Oct 96		3 (1)	nd
Sep 97	3 (1)	3 (1)	3 (5)
Sep 99	5 (1)		
Sep 00	5 (1)		15 (2-3)
Mean [%]	3.3	0.5	3.0
S.D.	4.9	1.1	6.0

El-Darsh and Whitfield (1999) recorded *Goezia* sp. in flounder from the tidal River Thames. Infected fish were found in the middle as well as in the upper tideway, but only from April to July. Prevalences ranged between 12-67%, mean intensities between 1.7-8 individuals per host, thus infection levels in flounder from the Thames were higher than in flounder from the German Bight.

Wichowski (1990) reported *Goezia ascaroides* (Goeze, 1782) in flounder from the River Elbe in the North Sea, but no information was given on prevalence or intensity of this parasite. As in the present study most individuals of *Goezia* sp. were also found in the Elbe estuary, it might be concluded that they belong to the same species, which was identified as *Goezia ascaroides* by Wichowski (1990).

Order: Ascaridida Skrjabin & Schulz, 1940
Superfamily: Seuratoidea
Family: Cucullanidae Cobbold, 1864
Genus: *Cucullanus* Müller, 1777

Cucullanus heterochrous Rudolphi, 1802

This species is a common parasite of pleuronectids and has been recorded from the North Sea, the Russian Arctic, the Siberian coast, the Atlantic and Pacific coasts of North America and the Far East and the Mediterranean (Törnquist 1931; MacKenzie and Gibson 1970; Gibson 1972; Lile 1989; Levsen 1990; Wichowski 1990; Moravec 1994; Pattipeiluhu 1996; Broeg et al. 1999; El-Darsh and Whitfield 1999) as well as from the Baltic Sea (Möller 1974; Lüthen 1989; review of Fagerholm and Køie 1994; Køie 1999). Descriptions of adult specimen are given by Törnquist (1931), Berland (1970), MacKenzie and Gibson (1970), larval stages were studied by Gibson (1972). Contributions to the life history of *C. heterochrous* were given by Janiszewska (1939), MacKenzie and Gibson (1970) and Gibson (1972). The entire life cycle of *C. heterochrous* was experimentally shown by Køie (2000). In general, eggs embryonate in seawater but do not hatch. The infective larvae in eggs are believed to represent an L3-stage. Polychaetes mainly *Nereis diversicolor* and *N. virens* may act as intermediate hosts. The main definite host, the flounder, acquires the infection by ingesting infected polychaetes. In the fish the larvae encyst in the gut wall and moult into fourth-stage larvae. Adult nematodes are moving in the gut content, and if no food is present, they feed on the host's gut- wall (Moravec 1994).

The geographical distributions of *C. heterochrous* in flatfish and *N. diversicolor* are nearly identical, thus *N. diversicolor* is considered as the most important intermediate host (Køie (2000).

C. heterochrous was one of the most abundant parasite species of flounder in the German Bight. 40.6% of all flounder investigated were infected with a total of 1504 individuals (table 3). The parasite was regularly present at all sampling sites. During single sampling periods, prevalences ranged between 5-100%, intensities between 1-33 individuals per flounder (table 19). Details of its distribution and infection characteristics are presented in chapter 3.

Table 19: Prevalence [%] and range of intensity (in brackets) of ***Cucullanus heterochrous*** at four locations in the German Bight. North Sea. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95	19 (1-20)	38 (1-10)	34 (1-7)	nd	nd
Sep 95	13 (1-3)	17 (1-12)	22 (2-5)	nd	nd
Jan 96	21 (1)	42 (1-23)	67 (1-12)	nd	nd
Apr 96	30 (1-3)	37 (1-9)	79 (1-12)	nd	nd
Jul 96	23 (1-37)	67 (1-6)	50 (1-4)	nd	nd
Oct 96	23 (1-4)	43 (1-10)	80 (1-21)	nd	nd
Jan 97	-	39 (1-14)	100 (2-5)	nd	nd
Apr 97	20 (1-8)	61 (1-12)	60 (1-15)	67 (1-11)	nd
Sep 97	13 (1)	17 (1-2)	47 (1-25)	20 (1-4)	nd
Apr 99	5 (2)	42 (1-4)	80 (1-13)	56 (1-7)	33 (1-4)
Sep 99	20 (1-3)	71 (1-12)	70 (1-33)	40 (1-11)	-
Apr 00	40 (1-5)	5 (1-5)	75 (1-12)	33 (1-3)	11 (1)
Sep 00	50 (1-4)	20 (1-5)	60 (1-7)	45 (1-6)	-
Mean	23.1 (2.8)	38.4 (3.1)	63.4 (4.1)	43.5 (3.0)	22 (6.3)
S.D.	12.2 (5.0)	20.0 (3.3)	21.2 (4.5)	16.7 (2.8)	15.6 (12.3)

Order: Ascaridida Skrjabin & Schulz, 1940

Superfamily: Seuratoidea

Family: Cucullanidae Cobbold, 1864

Genus: *Dichelyne* Jägerskiöld, 1902

Dichelyne* (*Cucullanellus*) *minutus Rudolphi, 1819

This species is a common parasite of various teleost families. In Europe it occurs in northeast Atlantic waters, with an apparent preference for more temperate areas like the Black Sea, Mediterranean and southern Baltic Sea (Fagerholm 1982; Moravec 1994). *D. minutus* occurs most frequently in brackish water near river mouths, is often found upstream in freshwater or, and in the sea as well (Moravec 1994). In Scandinavian waters and the Baltic Sea the flounder (*Platichthys flesus*) is the main final host (Køie 2001).

D. minutus was repeatedly recorded from flounder in the North Sea (Ronald 1959; MacKenzie and Gibson 1970; Gibson 1972; Levsen 1990; Pattipeiluhu 1996; Broeg et al. 1999; El-Darsh and Whitfield 1999) and from the Baltic (review of Fagerholm and Køie 1994; Køie 1999). The Baltic Sea and the North Sea near Scotland were supposed to be the northern limit of this species (Moravec 1994), but Levsen (1990) recorded low infection levels of *D. minutus* even in flounder from the west coast of Norway, which is the northern most record for this parasite species.

The morphology of the adult worm was described by Törnquist (1931), Berland (1970), MacKenzie and Gibson (1970) and Fagerholm (1982), larval stages by Janiszewska (1939) and Gibson (1972). The entire life cycle of *D. minutus* was experimentally shown by Køie (2001). The eggs embryonate on the sea bottom. Larvae, which hatch from the eggs, are probably in their third stage. These larvae are not directly infective to flounder, but use the polychaete *Nereis diversicolor* as obligate intermediate host. Fish become infected by ingesting infected polychaetes. The third stage larvae encyst in the gut wall and develop to fourth stage larvae. Mature adults occur in the anterior part of the intestine.

In the present study, 8.7% of all flounder examined were infected with a total of 594 specimens of *D. minutus* (table 3). The nematode occurred in the intestine of flounder from all locations except of the Inner Eider estuary. It was mainly found during summer and autumn sampling periods, only single infected flounder were observed in one winter and one spring sampling period (table 20). During single sampling points, prevalences of *D. minutus* ranged between 3-35%, intensities between 1-158 parasite individuals in summer and 1-11 individuals in autumn (table 20).

When the data were pooled over all sampling points, no significant differences were found between the sites, but a seasonal pattern was clearly evident in the prevalences of *D. minutus*, which were significantly higher in autumn than in spring ($p < 0.001$).

Table 20: Prevalence [%] and range of intensity (in brackets) of ***Dichelyne minutus*** at four locations in the German Bight. North Sea. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog
Jun 95	25 (1-158)	38 (1-20)	14 (1-11)	nd
Sep 95	10 (1)	27 (1-8)	11 (1)	nd
Jan 96			3 (1)	nd
Apr 96		3 (6)		nd
Jul 96	3 (2)	33 (2-42)		nd
Oct 96	33 (1-4)	30 (1-11)	13 (1-4)	nd
Sep 97	10 (1-3)		10 (1-3)	7 (1-3)
Sep 99		24 (1)	20 (1-2)	5 (1)
Sep 00	15 (1-2)	20 (1)	10 (2)	5 (2)
Mean [%]	8.0	13.5	6.2	2.8
S.D.	11.2	15.3	7.0	3.2

These seasonal variations corresponded to observations of Janiszewska (1939), Gibson (1972), Möller (1974) and Fagerholm (1982) from flounder in the Baltic Sea and of El-Darsh and Whitfield (1999) from the River Thames. Adult individuals of *D. minutus* in flounder were only found in summer and autumn. Third stage larvae were found throughout the year (Gibson 1972).

Flounder may become infected in autumn (Janiszewska 1939), but most flounder are infected in spring, when they return to shallow waters from deeper waters (Gibson 1972). Eggs, which are released in late summer may infect flounder during autumn, while those released in late autumn may survive unhatched on the sea bottom, hatch and infect *N. diversicolor* and are ingested by flounder in the following spring (Køie (2001).

Möller (1974) described an opposite seasonal pattern for the prevalence of *D. minutus* and *C. heterochrous*, with highest prevalences of *D. minutus* and lowest prevalences of *C. heterochrous* during summer and early autumn. This distribution pattern could not be observed in the present study.

Phylum: Acanthocephala

Class: Palaeacanthocephala Meyer, 1931

The acanthocephalans were classified according to Van Cleave (1948), Yamaguti (1963 b) and Crompton and Nickol (1985).

Order: Echinorhynchidea Southwell & Macfie, 1925

Family: Echinorhynchidae Cobbold, 1879

Genus: *Echinorhynchus* Zoega in Müller, 1776

Echinorhynchus gadi Zoega in Müller, 1776

This species is the most occurring acanthocephalan of marine fish from North Atlantic and North Pacific waters (Möller and Anders 1986). It was found in the intestine of more than 60 species of fish (Arai 1989). The acanthor larvae embryonate inside of the egg. Mature eggs are released with the faeces of the host into the water, and become ingested by amphipods, which act as intermediate hosts. A list of amphipod species that were reported as intermediate hosts of *E. gadi* in the Palearctic and the Nearctic is given by Yamaguti (1963 b) and Marcogliese (1994). The acanthor hatches from the egg, migrates to the haemocoel of the crustaceans, where it grows into the acanthella stage. The end point of this growth is the cystacanth, which is infective for the definite fish host. This stage encysts attached to the wall of the intestine and matures sexually. Small fishes might act as transport hosts (Möller and Anders 1986; Bush et al. 2002).

In European flounder this acanthocephalan was reported from the North Sea (Ronald 1959; MacKenzie and Gibson 1970; Lile 1989; Levsen 1990; Broeg et al. 1999) and the Baltic Sea (Möller 1974; Lüthen 1989; review of Fagerholm and Køie 1994; Køie 1999).

In the present study, *E. gadi* occurred only sporadically in flounder and was restricted to individuals from the Elbe, Inner Eider and Outer Eider estuary. 1.7% of flounder were infected during the course of the study, representing a total of 22 individuals (table 3). The highest prevalence of *E. gadi* was found in flounder from the Elbe estuary in April of 1999, when 50% of the fish examined harboured individuals of *E. gadi*. Intensity ranged between 1-2 individuals per fish (table 21).

Table 21: Number of infected fish and range of intensity (in brackets) of ***Echinorhynchus gadi*** in flounder at three sampling sites in the German Bight from 1995-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Inner Eider
Jun 95	1 (1)		nd
Jan 96	1 (1)		nd
Apr 96	1 (1)		nd
Apr 97	1 (1)	1 (1)	nd
Apr 99	10 (1-2)	1 (2)	2 (1-2)

Möller (1974, 1975) reported a clear seasonal pattern for *E. gadi* in flounder (*Platichthys flesus*) and cod (*Gadus morhua*) from coastal waters of the south- western Baltic Sea. Infection levels were low in winter and spring, and reached a maximum in summer months. These variations were not reflected in flounder from the German Bight in the present study.

In experimental studies *E. gadi* was susceptible to changes in water salinity. It was not able to adapt to changing osmolarity in the surrounding medium (Möller 1978). In the present study, however, most of the infected fish were found at the Elbe estuary, where salinity regularly changes twice a day.

Order: Echinorhynchidea Southwell & Macfie, 1925

Family: Pomphorhynchidae Yamaguti, 1939

Genus: *Pomphorhynchus* Monticelli, 1905

Pomphorhynchus laevis (Zoega in Müller, 1776) Van Cleave 1924

P. laevis is distributed in freshwater as well as in brackish and marine environments. It is common in a variety of fish hosts in freshwater, while flounder is the preferred host in the sea (Kennedy 1984; Möller and Anders 1986). *P. laevis* was recorded in flounder from the Baltic Sea (Möller 1974; Lüthen 1989; review of Fagerholm and Køie 1994; Køie 1999), and from

estuarine sites in the North Sea (Wichowski 1990; Pattipeiluhu 1996). *Pomphorhynchus* sp. was reported in flounder from North Sea estuaries of the coast of Aberdeen (MacKenzie and Gibson 1970; Gibson 1972), from the Black, Caspian and Aral Sea (Shulman 1961) as well as sporadically in other species of fish in the English Channel. It was also found in flounders from fjords of the Norwegian Sea (Kennedy 1984).

Kennedy (1984) suggested that populations from freshwater and from marine habitats and the Baltic Sea constitute different strains of *P. laevis*: one strain which completes its entire life-cycle in the marine habitat and one strain which is restricted to freshwater.

Kennedy (1984) assumed that the freshwater strain might grow and survive in estuarine waters, but does not reach sexually maturity in flounder or eels. This only occurs in chub, barbel or trout. Thus the sea is a natural barrier to the dispersal of the freshwater strain of *P. laevis* (Kennedy 1984). This assumption might explain, why *P. laevis* is present in high abundances in one river system, but rare in or even absent in adjacent waters or nearby river systems, as it was observed for several river systems in England (Gibson 1972; Kennedy et al. 1978; Poynton and O'Rourke 1982; Pattipeiluhu 1996; El-Darsh and Whitfield 1999). For details see Kennedy (1984).

Life cycle of *P. laevis* is similar to that of *E. gadi*. The most important intermediate host in freshwater is the amphipod *Gammarus pulex*, while *G. locusta* and *G. zaddachi* appear to be the most appropriate intermediate hosts in the marine environment (Kennedy 1994).

Experimental studies on the susceptibility of *P. laevis* to heavy metal contamination showed that the adults of this species accumulate extremely high burdens of cadmium and lead inside the fish gut. Concentrations of these heavy metals were several times higher than in the tissues of their fish host (Sures and Taraschewski 1995; Sures et al. 1997). Therefore, adult *P. laevis* may be used as highly sensitive bioindicators of monitoring heavy metal contamination in the aquatic environment (Sures et al. 1997).

In the present study, only a single flounder individual from the Inner Eider estuary was infected with 23 adults of *P. laevis* in April of 1999. The parasites were located in the rectum of the host, which is the typical position for the marine strain of *P. laevis*.

P. laevis was also reported from the Elbe estuary (Wichowski 1990), but at such low abundances that it was not suitable as indicator species for migration activity of flounder in this area. *P. laevis* appears to occur only sporadically in the German Bight.

Order: Polymorphida Petrochenko, 1956
Family: Polymorphidae, Meyer 1931
Subfamily: Corynosomatinae Petrotschenko, 1956
Genus: ***Corynosoma*** Lühe, 1904

Two species of this genus were reported for flounder, *C. semere* and *C. strumosum* (Ronald (1959). *C. semere* was found in flounder from the Baltic Sea (Janiszewska 1939; review of Fagerholm and Køie 1994), while *C. strumosum* was recorded from the North Sea (MacKenzie and Gibson 1970; Gibson 1972).

Life cycle of *Corynosoma* is comparable to *E. gadi*, but the genus needs a second intermediate host to complete its development (Möller and Anders 1986). Crustaceans act as first intermediate hosts, while fishes such as flounder are the second intermediate hosts. Definite hosts are seals and piscivorous fishes (Bush et al 2002). In flounder, the encysted larvae (cystacanth) of *Corynosoma* occur in the body cavity or on the surface of the organs (Janiszewska 1939).

In the present study, cystacanths of *Corynosoma* occurred only sporadically in the intestine of flounder from the German Bight. The larvae were attached to the inner intestinal wall. 1.3% of all flounder examined were infected with *Corynosoma* sp., representing a total of 19 individuals (table 3).

Table 22: Number of infected fish and range of intensity (in brackets) of ***Corynosoma*** **sp**. in flounder at three sampling sites in the German Bight from 1995-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog
Jun 95	1 (1)			nd
Jan 96	1 (1)			nd
Apr 99	1 (1)			1 (6)
Sep 99	2 (1)		1 (1)	
Apr 00	2 (1)	2 (1)		
Sep 00	1 (1)		1 (1)	1 (1)

The larvae were present in flounder from all sampling sites, except of the Inner Eider estuary. Most of the infected flounder were found in the Elbe estuary. During individual sampling points, 1-2 flounder individuals were infected with a single parasite, only a flounder from Spiekeroog harboured a total of 6 cystacanths (table 22).

Gibson (1972) found cystacanths of *C. strumosum* in low abundances only in flounder from a marine location under study, while it was absent from two estuarine sites. Køie (1999) reported cystacants of *C. strumosum* and *C. semere* also in low abundances in flounder from the north-eastern Baltic Sea, while Janiszewska (1939) found abundances of cystacants of *C. semere*

between 1-10% in small fish groups and between 10-50% in older fish from the brackish Bight of Puck, Baltic Sea. *C. strumosum* and *C. semere* are mostly marine species (Hoffman 1999).

Crustacea "Maxillopoda"

Copepoda Edwards, 1840

The copepods were classified according to Yamaguti (1963 c) and Kabata (1979).

Suborder: Poecilostomatoida

Family: Bomolochidae Sumpf, 1871

Genus: *Holobomolochus* Vervoort, 1969

Holobomolochus confusus Stock, 1953

This copepod appears to be distributed on both sides of the North Atlantic (Höglund and Thulin 1988). It is a common species of the nasal cavities of cod (*Gadus morhua*) (Kabata 1979), but has been additionally recorded from several other fish species (Boxshall 1974a).

Records of this copepod in flounder (*Platichthys flesus*) are still scarce. The first record of *Holobomolochus* sp. in flounder was given by Thulin et al. (1989) in flounder from the Swedish West coast. Levsen (1990) found three adult females of *H. confusus* in flounder from West Norway, while Køie (1999) reported *H. confusus* for the first time in flounder from the German Baltic.

In the present study, 4.7% of all flounder specimen examined were infected with a total of 35 individuals of *H. confusus* (table 3), which were located in the nostrils of the host. The copepod was present at all sites, but it was most prevalent in flounder from Helgoland and Outer Eider estuary (table 23). During single sampling points, prevalences ranged between 5-30% at individual locations, intensities between 1-3 individuals per fish (table 23).

Table 23: Prevalence [%] and range of intensity (in brackets) of ***Holobomolochus confusus*** at five locations in the German Bight, North Sea, during sampling points from 1995-1997 and 1999-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Oct 96	3 (1)	3 (1)	30 (1)	nd	nd
Apr 97		14 (1-2)		10 (1)	nd
Apr 99			5 (1)		
Apr 00			25 (1-3)		11 (1)
Sep 00	10 (1)	20 (1)			-
Mean [%]	1.1	2.8	4.6	1.7	5.5
S.D.	2.9	6.5	10.3	4.1	7.8

This is the first record of *H. confusus* in flounder from the southern North Sea. A clear seasonal pattern was not observed in the infection levels of *H. confusus*, but in April of 2000, females of *H. confusus*, which were found in flounder from Helgoland, were full of mature eggs containing fully developed nauplius larvae.

Suborder: Poecilostomatoida

Family: Chondracanthidae Edwards, 1840

Genus: *Acanthochondria* Oakley, 1927

Acanthochondria cornuta Müller, 1776

This copepod species is a common parasite of flatfish in the North Atlantic, known from both the European (Reichenbach-Klinke 1980) as well as from the American side of the Atlantic Ocean (Kabata 1979). The morphological variability in *A. cornuta* led to a great number of synonyms and erroneously reported host species (Kabata 1979). In previous studies, *A. cornuta*, *A. flurae*, *A. solae*, *A. depressa* and *A. limandae* were reported for flounder (see review of Kabata 1959). Now it is generally accepted that *A. flurae* and *A. depressa* are synonyms to *A. cornuta* (Heegaard 1947; Ho 1970; Kabata 1979). Ho (1970) suggested a trinomial nomenclature for *A. cornuta* in order to retain the name *flurae*: *A. cornuta* f. *cornuta* and *A. cornuta* f. *flurae* (Kabata 1979). In the eastern North Atlantic, including the northern North Sea and the seas around the Faroe Islands and Iceland, *A. cornuta* f. *cornuta* occurs mainly on witch (*Glyptocephalus cynoglossus*) and, rather rarely, on plaice (*Pleuronectes platessa*), while *A. cornuta* f. *flurae* was found mainly on long rough dab (*Hippoglossoides platessoides*) and flounder (*Platichthys flesus*) (Kabata 1959, 1979).

A. cornuta was not only reported in flounder from the North Sea (Schuurmans Stekhoven 1935, as *A. solea*; Ronald 1959; MacKenzie and Gibson 1970, as *A. depressa*; Gibson 1972, as *A. depressa*; Van den Broek 1979, as *A. depressa*; Lile 1989; Levsen 1990; Wichowski 1990; Broeg et al. 1999; El-Darsh and Whitfield 1999, as *Acanthochondria* sp.), but also from the Baltic Sea (Möller 1978, as *A. depressa*; Lüthen 1989). As the genus *Acanthochondria* is of marine origin (Kabata 1979), records of *A. cornuta* in the central Baltic Sea indicate a migration activity of flounder from more saline waters into this area of the Baltic Sea.

Information on the life cycle was given only by Heegaard (1947). The life cycle is direct and consist of 7 developmental stages: 1 nauplius, 5 copepodits and the adult. The first copepodit is infective in both sexes. Females attach to the host, while dwarft males attach near the genital

aperture of the females, where they pass though the additional copepodit stages before reaching maturity. Fertilization does not take place before the female has transformed into a sexually mature adult. Heegaard (1947) suggested, that only a small number of females survive the winter on fish from western Swedish waters, as young females were mainly found from May to June.

The distribution of larval stages of this parasite on the host differs from the adults. Larvae show a preference for the gill arches, while the adult are mainly found on the walls of the branchial and the buccal cavity (Van den Broek 1979).

In the present study, *A. cornuta* was one of the most regularly occurring and most abundant parasite species in flounder from the German Bight. 63.7% of all flounder examined were infected with *A. cornuta*, representing a total of 5 495 individuals (table 3). The parasite occurred at all sampling sites during all sampling points. Corresponding to the descriptions of Van den Broek (1979), larvae were found on the gill arches, while the adults were found in the branchial and buccal cavity of the flounder. Mean prevalence was highest in flounder from Helgoland, Outer Eider estuary and Spiekeroog, while lowest were found at the estuarine sites. Mean intensity was lower in flounder from the Elbe estuary compared to flounder from all other sites (table 24). During single sampling periods, prevalence ranged between 13-100%, intensity between 1-44 individuals per fish. Prevalence and intensity of *A. cornuta* showed seasonal fluctuations in flounder from the Outer Eider estuary. At this site, infection levels were significantly higher in autumn ($p < 0.001$) than in spring. Detailed information about the infection characteristics of *A. cornuta* in flounder from the German Bight is given in chapter 3.

Table 24: Prevalence [%] and range of intensity (in brackets) of ***Acanthochondria cornuta*** at five locations in the German Bight, North Sea, during sampling points from 1995-1997 and 1999-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95	13 (1-5)	34 (1-9)	73 (1-23)	nd	nd
Sep 95	33 (8)	87 (1-44)	100 (2-32)	nd	nd
Jan 96	31 (3-14)	77 (1-38)	97 (1-16)	nd	nd
Apr 96	10 (4-12)	70 (1-23)	86 (1-21)	nd	nd
Jul 96	13 (1-7)	44 (1-28)	77 (1-13)	nd	nd
Oct 96	13 (1)	93 (1-27)	97 (2-33)	nd	nd
Jan 97	-	89 (1-13)	100 (1-9)	nd	nd
Apr 97	27 (1-11)	86 (1-35)	97 (2-25)	77 (1-24)	nd
Sep 97	20 (1-9)	97 (2-40)	100 (1-34)	93 (1-29)	nd
Apr 99	20 (1-2)	53 (1-12)	85 (1-23)	67 (2-15)	40 (1-23)
Sep 99	25 (1-11)	86 (2-22)	100 (2-25)	95 (1-31)	-
Apr 00	25 (1-3)	75 (1-30)	90 (1-23)	78 (1-17)	39 (2-16)
Sep 00	55 (1-10)	100 (1-16)	95 (1-28)	95 (1-11)	-
Mean	23.8 (3.7)	76.2 (8.2)	92.1 (8.9)	84.2 (8.5)	39.5 (7.3)
S.D.	12.4 (3.6)	20.7 (7.6)	9.2 (7.0)	11.8 (6.6)	0.7 (6.6)

A study of Van den Broek (1979) on flounder from the Dutch coast, revealed corresponding results to the present study, referring to seasonality in the infection levels of the parasite and its relation to host size. *A. cornuta* was present throughout the year at the Dutch coast. Prevalences of *A. cornuta* showed a maximum of 100% from October to December and a minimum of only 21.4% between February and June.

Suborder: Siphonostomatoida
Family: Caligidae Dana, 1852
Subfamily: Caliginae Dana, 1852
Genus: *Caligus* Müller, 1785

Caligus elongatus Nordmann, 1832

This copepod species has been recorded from more than 80 fish species, both teleosts and elasmobranches, belonging to 17 orders and 43 families, from most oceanic regions of the world. It is probably the most common parasitic copepod of the North Sea and adjacent waters (Kabata 1979). In flounder, *C. elongatus* was reported by Ronald (1959) as *C. rapax*, by Boxshall (1974 a) from North England and by Lile (1989) and Levsen (1990) from North and West Norway. Parker (1969) re-described the species and reviewed its classification. The life cycle of *C. elongatus* was experimentally shown by Piasecki and MacKinnon (1995). It consists of 8 developmental stages, separated by moults: 2 nauplii, 1 copepodid, 4 chalimi, and the adult. A separate preadult stage does not occur, in contrast to other known life cycles of caligid copepods. The copepodid is infective and all subsequent stages live on fish. The morphology of all developmental stages was described in detail by Piasecki (1996).

The generation time under laboratory conditions was 43.3 days. Males probably die shortly after copulation. Under natural conditions, their relative proportion dropped during winter and they were completely absent from February to April. Overwintering females are supposed to go through a diapause, due to their immobility, torpidity and the possession of nonviable eggs (Piasecki and MacKinnon 1995). The females can lay eggs at least twice during their life- time. Fertilized eggs are susceptible to low oxygen contents of the water (Piasecki and MacKinnon 1995).

In the present study, *C. elongatus* occurred sporadically on the surface of the skin of flounder from all sites, except of Inner Eider estuary. 3.3% of all flounder investigated were infected with a total of 69 individuals of *C. elongatus* (table 3). Highest prevalences were found in flounder

from Helgoland, lowest one at Elbe estuary (table 25). During single sampling points, prevalences ranged between 3-40% at single locations, intensities between 1-14 individuals per fish. In April of 2000, *C. elongatus* was found in flounder from all four stations (table 25).

Table 25: Prevalence [%] and range of intensity (in brackets) of ***Caligus elongatus*** at five locations in the German Bight, North Sea, during sampling points from 1995-1997 and 1999-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog
Oct 96		3 (1)		nd
Apr 97		7 (1)	7 (1)	20 (1-5)
Sep 97		3 (3)	23 (1)	
Apr 99			10 (1)	
Apr 00	5 (1)	15 (1-3)	40 (1-14)	11 (1)
Sep 00			5 (1)	
Mean [%]	5	2.2	6.5	5.2
S.D.		4.4	12.1	8.5

Although *C. elongatus* has no pathological effect on infected individuals under natural conditions, it is an important pathogen to sea- farmed Atlantic salmon. Losses attributed to crustacean parasites count in millions of dollars, due to direct mortalities as well as to slower growth rates of infected fish and the costs of prevention and treatment.

Suborder: Siphonostomatoida

Family: Caligidae Dana, 1852

Subfamily: Lepeophtherinae Yamaguti, 1963

Genus: *Lepeophtheirus* Nordmann, 1832

Lepeophtheirus pectoralis Müller, 1777

This copepod species is parasitic predominantly on pleuronectid flatfishes (Boxshall 1974c, 1976; Kabata 1979). It is distributed along the Atlantic coast of Europe, as well as in the Faroes, Iceland and the White Sea (Boxshall 1974c; Kabata 1979; Zeddam et al. 1988). *L. pectoralis* was reported on flounder from offshore and estuarine sits in the North Sea (MacKenzie and Gibson 1970; Gibson 1972; Boxshall 1974; Van den Broek 1979; Zeddam et al. 1988; Lile 1989; Levsen 1990; Wichowski 1990; Pattipeiluhu 1995; Broeg et al. 1999; El-Darsh and Whitfield 1999) as well as from the western Baltic Sea (Möller 1974, 1978; review Fagerholm and Køie 1994).

Detailed information on morphology, life cycle and population dynamics are given by Boxshall (1974 b, 1974c, 1974d). According to these descriptions, the development of *L. pectoralis* follows the typical caligid pattern: two nauplii, one copepodid, four chalimus, two preadult stages and the adult.

In the present study, *L. pectoralis* was one of the most abundant species in flounder from the German Bight. It was present at all sampling sites during all sampling points (table 26).

Table 26: Prevalence [%] and range of intensity (in brackets) of ***Lepeophtheirus pectoralis*** at five locations in the German Bight, North Sea, during sampling points from 1995-1997 and 1999-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95	41 (1-5)	62 (1-27)	53 (2-31)	nd	nd
Sep 95	97 (1-16)	97 (1-29)	78 (2-10)	nd	nd
Jan 96	52 (1-9)	90 (1-28)	93 (1-29)	nd	nd
Apr 96	23 (1-8)	77 (1-19)	93 (1-28)	nd	nd
Jul 96	67 (1-15)	100 (1-30)	90 (1-20)	nd	nd
Oct 96	93 (1-12)	93 (4-50)	100 (1-21)	nd	nd
Jan 97	-	89 (2-31)	100 (5-9)	nd	nd
Apr 97	28 (1-21)	79 (1-26)	90 (1-29)	83 (1-27)	nd
Sep 97	93 (2-27)	97 (3-25)	97 (1-31)	97 (4-46)	nd
Apr 99	15 (1-3)	58 (1-10)	80 (2-35)	78 (1-14)	33 (2-9)
Sep 99	100 (3-30)	100 (1-29)	100 (1-29)	100 (2-38)	-
Apr 00	40 (1-8)	85 (1-20)	95 (1-20)	67 (2-12)	39 (3-24)
Sep 00	90 (1-30)	100 (7-20)	100 (1-35)	95 (3-29)	-
Mean	61.9 (6.7)	86.7 (10.6)	89.9 (9.4)	86.7 (13.1)	36 (7.0)
S.D.	32.1 (6.3)	14.1 (7.5)	13.2 (6.8)	12.9 (9.1)	4.2 (6.5)

Adult female attached under the pectoral and pelvic fins, while preadults and adult males were found all over both sides of the body surface. This corresponded to the findings of Boxshall (1974b). 78.4% of all flounder examined were infected with *L. pectoralis*, representing a total of 8 045 individuals (table 3). Mean prevalence and intensity was highest in flounder from the coastal and offshore sites, and lowest in flounder from the estuarine sites. During single sampling periods, prevalences ranged between 28-100%, intensity between 1-50 individuals per host (table 26).

Infection characteristics of *L. pectoralis* in flounder from different sites in the German Bight as well as the impact of natural parameters are presented and discussed in detail in chapter 3.

Suborder: Siphonostomatoida

Family: Pennellidae Dana, 1853

Genus: *Lernaeocera* de Blainville, 1822

Lernaeocera branchialis L.

The distribution of *L. branchialis* appears to be restricted to both the European and the American side of the North Atlantic and adjacent seas (Kabata 1961, 1979).

L. branchialis is a parasite that has a two-host life cycle. The early larval stages are found on flatfish, while definite hosts are gadoids, mainly cod or whiting. A list of intermediate and definite host species of this copepod species is given by Kabata (1960, 1979). On flounder *L. branchialis* was reported by various authors from offshore and estuarine sites in the North Sea (MacKenzie and Gibson 1970; Boxshall 1974; Van den Broek 1979; Lile 1989; Levsen 1990; Wichowski 1990; Pattipeiluhu 1995; Van Damme and Ollevier F 1996; Van Damme et al. 1997; Broeg et al. 1999; El-Darsh and Whitfield 1999) as well as from the western Baltic Sea (Lüthen 1989; review Fagerholm and Køie 1994; Køie 1999). As *L. branchialis* is a marine species, its occurrence in the Baltic most likely depends on immigration of fish from the North Sea (Lüthen 1989).

Detailed descriptions on the morphology of larval stages and the adults are given by Sproston (1941), Schuurmans-Stekhoven (1936) and Kabata (1979). Larval development comprises two nauplius stages, a free-living infective copepodite stage, followed by a premetamorphic part of the life cycle, which occurs on the gill tips of the intermediate host. This part includes four chalimus stages. This development culminates in mating. After copulation the male dies, while upon fecundation the female leaves the intermediate host, lives free-swimming for a short period until it attaches to the gills of the final host. After metamorphosis to the adult stage, which occurs within a few days, the parasite is deeply fixed in the host tissue. Excesses of the parasite's head extend to the heart of the host, where it feeds on the host's blood (Sproston 1941; Kabata 1979; Möller and Anders 1986).

A classification of the adult female into substages was established by Sproston and Hartley (1941) and redescribed by Whitfield et al. (1988), who introduced new subdivisions for the female. Van Damme and Hamerlynck (1992) incorporated these changes and gave an overview over all the stages and their characteristics. Following these descriptions, the adult female passes ten different stages including two pennella, an immature, a mature pregravid, four mature gravid stages and three stages of the dead parasite.

The presence of more than one adult female of *L. branchialis* can have serious if not lethal effects on the final host, while the presence of more than 700 juveniles on the intermediate host appears to produce no pathological effects (Kabata 1979).

Chalimus stages attach to the intermediate host by a double chitinous filament produced by the frontal gland, which provoke swellings on the gill tips. Swellings continue to exist several days after detachment of the larvae and feign pollution-induced hyperplasia of the gills (Möller and Anders 1986; Wichowski 1990).

Information on the population dynamics of *L. branchialis* in Dutch waters was presented by Schuurmans-Stekhoven (1936), Van Damme and Hamerlynck (1992), Van Damme and Ollevier F (1996) and Van Damme et al. (1997). Following these descriptions, *L. branchialis* shows a one- year life cycle. While in intermediate hosts prevalences were more or less constant throughout the year, the infection intensity reached a maximum in spring, which was followed by a progressively increasing infection of the final hosts cod (*Gadus morhua*) and whiting *(Merlangius merlangus*).

Copepodits of *L. branchialis* do not regulate internal osmotic pressure. Therefore, the survival of the parasite is impaired in water salinities below 10-15‰. This notion is supported by the absence of the parasite from the central part of the Baltic Sea with water salinities below 10‰ (Lüthen 1989; Knudsen 1998). Although copepodits of *L. branchialis* in general occur on marine flounder (Kabata 1979) and do not appear to reproduce in the Kieler Bight (Arntz 1972), they are probably introduced into the more saline areas of the western Baltic Sea by migration activity of single host individuals (Lüthen 1989). Anyway copepodits are quickly lost, when flounder migrate into upper areas of estuaries, or when salinity dropped to 5-10 ‰ (Möller 1978; Wichowski 1990).

In the present study, *L. branchialis* was the most abundant species in flounder from the German Bight. In the present study, 92.6% of all flounder examined were infected with *L. branchialis*, representing a total of 74 708 individuals (table 3). During single sampling points, prevalences ranged between 35-100%, intensity between 1-1186 individuals per host (table 27). Both prevalences and intensities were lower in flounder from the Elbe estuary than at all other sites.

Table 27: Prevalence [%] and range of intensity (in brackets) of ***Lernaeocera branchialis*** at five locations in the German Bight, North Sea, during sampling points from 1995-1997 and 1999-2000. For key, see table 4.

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Jun 95	100 (1-1046)	100 (19-1186)	100 (25-495)	nd	nd
Sep 95	97 (2-38)	100 (1-74)	56 (1-3)	nd	nd
Jan 96	86 (1-419)	97 (15-304)	100 (11-280)	nd	nd
Apr 96	87 (1-246)	100 (10-200)	100 (6-725)	nd	nd
Jul 96	63 (1-79)	100 (19-97)	100 (27-336)	nd	nd
Oct 96	100 (1-66)	97 (1-127)	90 (2-46)	nd	nd
Jan 97	-	100 (15-677)	100 (3-28)	nd	nd
Apr 97	83 (1-234)	100 (4-420)	100 (14-228)	100 (33-398)	nd
Sep 97	40 (1-2)	97 (1-38)	100 (2-34)	73 (1-9)	nd
Apr 99	85 (1-56)	100 (10-198)	100 (8-115)	100 (21-154)	100 (2-102)
Sep 99	95 (1-145)	100 (11-295)	100 (6-47)	100 (7-109)	-
Apr 00	35 (1-139)	95 (2-199)	100 (18-278)	100 (18-142)	100 (3-284)
Sep 00	100 (1-141)	100 (23-155)	100 (3-69)	100 (8-250)	-
Mean	80.9 (45.7)	98.9 (94.4)	95.8 (78.9)	95.5 (76.5)	100 (90.7)
S.D.	22.8 (97.3)	1.8 (144.7)	12.3 (91.2)	11.0 (77.3)	0 (63.09

Detailed information about the infection characteristics of *L. branchialis* in flounder from the German Bight are given in chapter 3. Natural influences on the distribution of the parasite and its infection levels in flounder are discussed.

Chapter 3
Infection characteristics of potential indicator species

Abstract

As part of an integrated biological effect monitoring, the parasite fauna of flounder (*Platichthys flesus* L.) was investigated from 5 locations in the German Bight, in order to use parasite species as bioindicators. Over a period of six years, parasites from 30 different taxa were identified, but only 7 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 Ciliophora *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, results of individual sampling points and the long-term data set compared. 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 Elbe, as the most polluted site, and the less polluted coastal and marine locations, Helgoland, Outer Eider and Spiekeroog, especially in the long-term data set. Gradual differences between Elbe, Outer Eider and Helgoland, which were not detected in individual sampling points, 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, established between the sites. Seasonal variation in the infection parameters affected the spatial distribution of the copepod species, *L. pectoralis* and *L. branchialis*. Annual variations are considered to occur in the range of natural variability, thus no trend of increasing or decreasing infection levels of the parasites was found with the course of the study.

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 mediated from natural effects. As 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 bioindicators.

This approach was followed in a study by Broeg et al. (1999) who in the framework of a biological effects monitoring investigated the parasites of flounder (*Platichthys flesus* L.) at different locations in the German Bight for their potential use as bioindicators for pollution effects and for the first time, supplemented the parasitological data by well-known biochemical and histochemical biomarkers, which were recommended by the ICES Advisory Committee on the Marine Environment (ACME) for application in biological effects monitoring programs (ICES 1996).

The results of this study were promising, because infection levels of several parasite species were reduced in the Elbe estuary, the site with highest contamination load, when compared to less contaminated sites. These parasitological findings were supported by the responses of other biomarkers which largely correlated to the parasitological data (Broeg et al. 1999), but especially the responses of very abundant Crustacea, Nematoda and Digenea to pollutants remained preliminary, because little was known on the effects of natural influences on distribution and infection levels of these parasites.

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

Special emphasis was given to natural variations of 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 discussed, in order to evaluate their influence on the distribution pattern 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 in chapter 5.

Materials and methods

Sampling

During spring and autumn of the years 1995 to 2000, nine sampling points were carried out in the North Sea, where a total of 802 flounder, *Platichthys flesus* (L.), were collected from selected sampling locations in the German Bight. At each location 9 to 30 flounder of the size class 18-25 cm were investigated. Numbers of evaluated fish specimen for each sampling point at each site are given in table 1.

Details of sampling procedure, study area, examination of flounder and ecological terminology for the description of parasite populations are given in chapter 2.

Table 1: Sampling program on flounder parasites. Flounder were collected at five different locations in the German Bight, during sampling points from 1995 to 1997 and 1999 to 2000. Given are numbers of flounder specimen evaluated for parasitological investigation at the sampling sites during individual collections. The sampling periods are identified by month and year (Apr= April, Sept= September, Oct= October).

Sampling period	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Sept 95	30	30	9	-	-
Apr 96	30	30	14	-	-
Oct 96	30	30	30	-	-
Apr 97	30	28	30	30	-
Sept 97	30	30	30	30	-
Apr 99	20	19	20	9	15
Sept 99	20	21	20	20	0
Apr 00	20	20	20	9	18
Sept 00	20	20	20	20	0
Total	230	228	193	118	33

Evaluation of data

Data analysis was carried out for single sampling points 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, because the number of infected hosts sometimes was too low to compare intensities. For the long-term data set, mean values of prevalences, abundances as well as intensities are given, separated by season.

Evaluation of seasonal fluctuations of 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 points during the course of the study, was based on the abundance data of different sampling points, 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 an identification of 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 of intensity were normalized by logarithmic transformation ($\log_{10}(N+1)$). The prevalence of a particular parasite at different locations or sampling points was compared by a chi-square test. Comparison of two groups were performed with Student's t-test or Mann-Whitney's U-test: Comparison of more groups were made using ANOVA and Tukey's post hoc comparison of means or Kruskal-Wallis ANOVA and Dunn's post hoc test. Differences were considered to be statistically significant a probability of error $p< 0.05$. Correlation coefficients were calculated with the parametric Pearson's Product Moment Correlation or Spearman's correlation on ranks. Correlations were considered to be significant at a probability of error $p< 0.05$.
The analyses were carried out using the computer programs SigmaStat® 2.0 and STATISTICA 6 (StatSoft).

Results

During sampling points in spring and autumn of 1995-1997 and 1999-2000, a total of 802 flounder were dissected. In total, 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 chapter 2, table 2.
Prevalence, mean intensity and mean abundance of all parasite taxa are summarized in table 2 and 3, separated by sampling site and season.
In general, all of the fish examined were infected with 1 or more parasite species. The majority of parasite taxa showed an aggregated distribution pattern between hosts.
Parasites of 17 taxa were present at all sampling sites, but not all of them were found during both seasons or during each sampling point. Only 7 species/taxa were regularly present and sufficiently abundant over the whole sampling period to be considered as potential indicator species:

the Ciliophora *Trichodina* spp., the 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 points. Ecological measurements are compared referred to their suitability of indicating differences between sites. The results of single sampling periods are compared with those obtained for 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 there can be observed a trend of increasing or decreasing infection levels of the parasites during the course of the study. Relation of the infection levels of the parasites to 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 on species level.

During all sampling periods, prevalences and abundances of this parasite were significantly higher at the estuarine sites than at Helgoland (figure 1). When the spring sampling periods of the pooled data set (table 4) were considered, gradual differences between sites were found in the prevalence and the abundance of *Trichodina* spp. in an 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 when compared to 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 caused by differences in prevalence and not in infection intensity. Infection levels were not related to the sex of the flounder.

Table 2: Prevalence of parasites from flounder collected at 5 sampling sites in the German Bight in 1996-2000. Given are mean prevalence ± standard deviation calculated from specimen collected during 2-4 sampling points in spring and autumn. The number of specimen evaluated is given in table 1. Upper panel: most abundant species, lower panel: other species of the component community.

Species	**Elbe**		**Outer Eider**		**Helgoland**		**Spiekeroog**		**Inner Eider**
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
No. of sampling points	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
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
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
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
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
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
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
Goezia sp.	0	3.3 ±2.0	-	1.3 ±1.8	0	-	0	6.1 ±7.9	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
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	x
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
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
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

Table 3: Infection parameters of parasites from flounder collected at 5 sampling sites in the German Bight in 1996-2000. Given are mean intensity and confidence interval (-0,95%; 95%) as well as mean abundance, calculated from specimen collected during 2-4 sampling points in spring and autumn. Number of specimen evaluated is given in table 1. Upper panel: dominant species, intermediate panel: other component species, lower panel: rare species (mean intensity and range only)

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

Table 3: continued

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
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
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

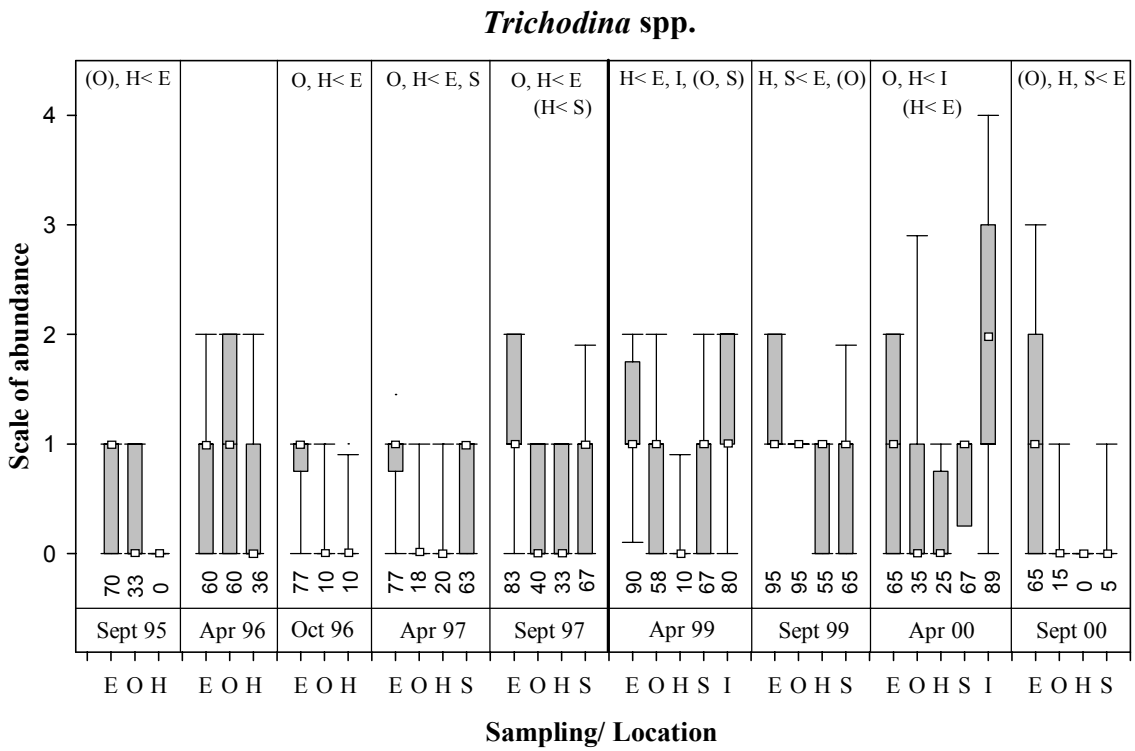


Fig. 1: Abundance of the *Trichodina* spp. on an arbitrary scale (0= absent, 1= 1-3, 2= 4-10, 3=10-20, 4= > 21 individuals per slide) at different sampling locations during the course of the study. Given are median abundance (□) with 25-75% percentiles (boxes in grey) and 10-90% percentiles (whiskers). 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). Differences in the abundance, observed between sites are given on the top of the graph. Information, given in parenthesis, was additionally observed in the prevalences; levels of significance, $p < 0.05$. E= Elbe, O= Outer Eider, H= Helgoland, S= Spiekeroog, I= Inner Eider. For numbers of flounder evaluated during the sampling points see table 1.

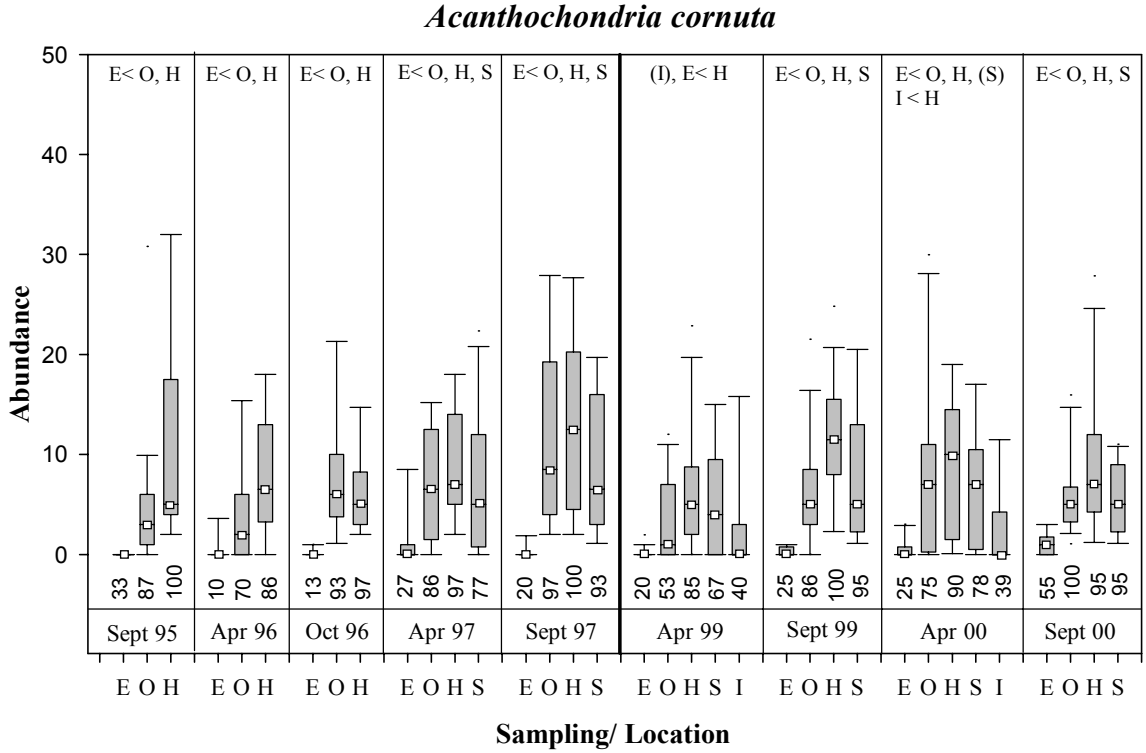


Figure 2: Abundance of *Acanthochondria cornuta* at different sampling locations in the German Bight during the course of the study. For key, see Figure 1.

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 during the course of the study. In all sampling points, prevalences and abundances were lower at the Elbe estuary than at the coastal and offshore sites, whereas prevalences and abundances at the Inner Eider estuary were only lower than at Helgoland. These differences were found to be statistically significant in most of the sampling periods (figure 2). In the spring sampling periods of the pooled data set, prevalences 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 periods, significantly lower prevalences and intensities were found in fish from the Elbe estuary compared to the other locations. Gradual differences, however, were found when abundances were considered (table 4).

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) compared to spring. This variation had no influence on the distribution pattern of *A. cornuta* among the sites. Annual fluctuations were only found in autumn sampling periods (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), caused by prevalence as well as by intensity. Infection levels, however, were not related to sex and condition factor of the flounder.

Lepeophtheirus pectoralis is a monoxenous, marine copepod that parasitizes 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 10-40% in spring and 90-100% in autumn. These changes had a clear effect on the site-specific distribution of the parasite. During the spring sampling periods, prevalences and abundances were significantly lower at the Elbe station than at the costal and offshore sites. Prevalence and abundance at the Inner Eider station were lower only than at Helgoland. In the autumn sampling periods, differences between sites were only found in the abundance (figure 3).

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 compared to 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 4).

Annual fluctuations were only found in autumn sampling periods (Elbe: all years< 99; Helgoland: 95, 96< 97; $p< 0.05$), but did not correspond between 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, however, were not influenced by sex and condition factor of flounder.

Lernaeocera branchialis was the most common and most numerous species in the community. The larval stages of this heteroxenous, marine copepod use flounder as their preferred intermediate host and parasitize the gills. The final hosts are gadoid fish.

In all spring sampling periods, abundances were significantly lower at the Elbe station than at all other sites, including the Inner Eider estuary (figure 4). 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 4). In spring, also the number of parasite individuals was 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 in1999 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 caused by differences in intensity and not in prevalence. The infection levels were not related with the sex of the flounder.

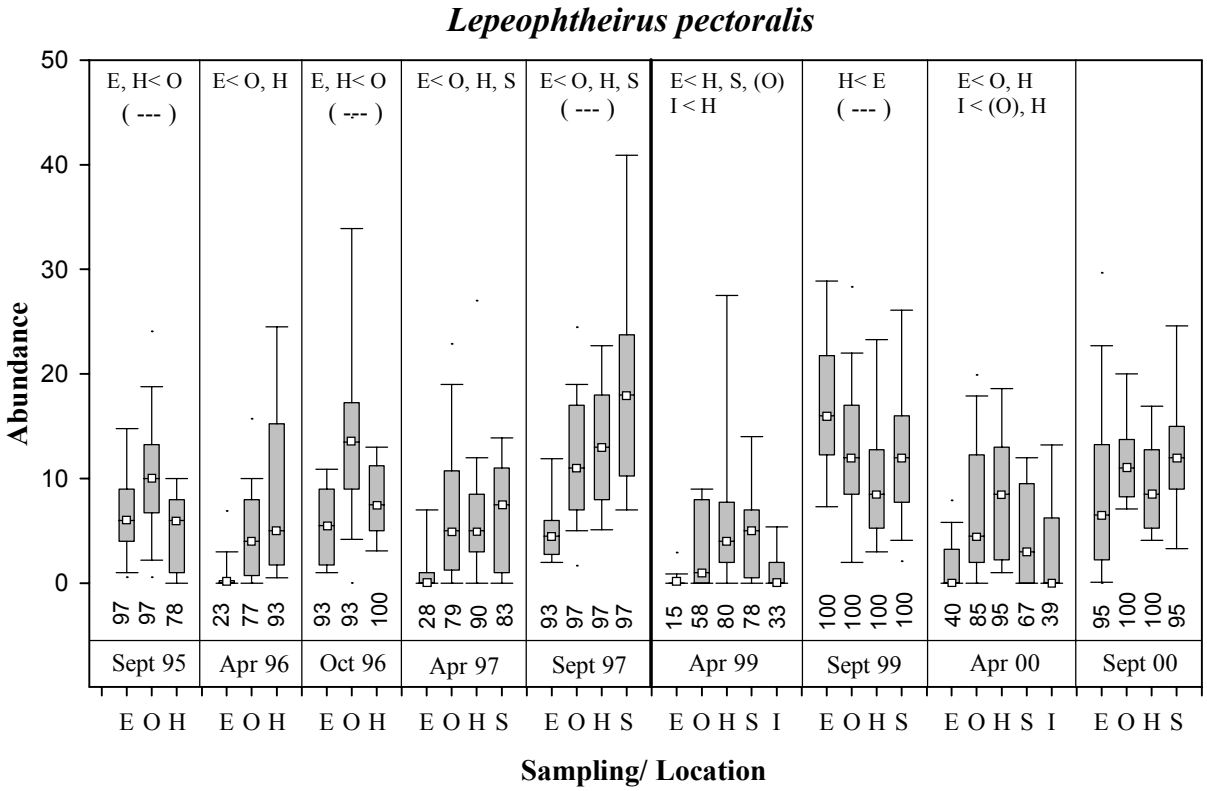


Figure 3: Abundance of *Lepeophtheirus pectoralis* at different sampling locations in the German Bight during the course of the study. For key, see Fig. 1.

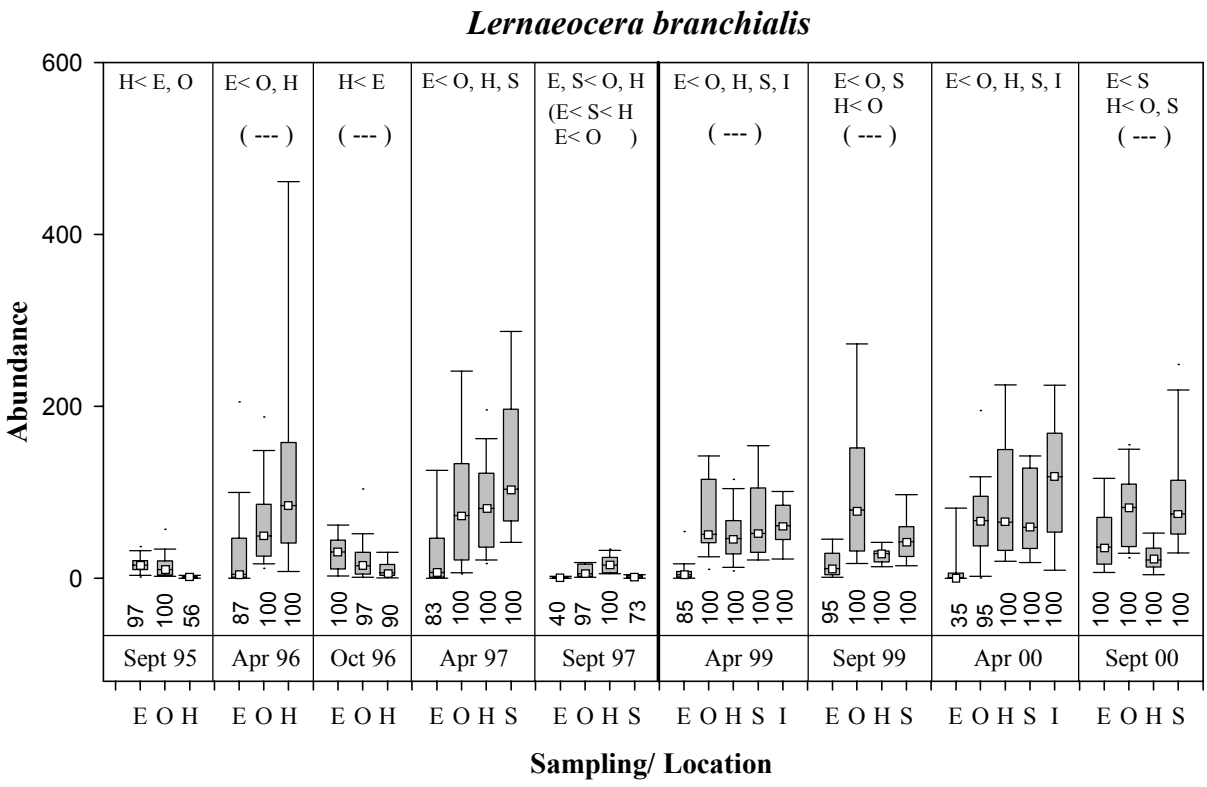


Figure 4: Abundance of *Lernaeocera branchialis* at different sampling locations in the German Bight during the course of the study. For key, see Fig. 1.

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 (figure 5).

Gradual differences were found in increasing order between Elbe < Outer Eider < Helgoland in the first three sampling periods (figure 5) 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. During the following sampling periods, it slowly recovered (figure 5).

Annual fluctuations were more pronounced in autumn compared to 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 caused by 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 (figure 6). Infection levels at the stations Outer Eider and Spiekeroog varied, in some sampling periods they were similar to Elbe, 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 of Elbe < Outer Eider < Helgoland (E< H; $p< 0.001$, E< O and O< H; $p< 0.05$) during both seasons. Significant differences between Elbe < Spiekeroog ($p< 0.001$) were only found in spring. In autumn, the intensity was lower in fish from Elbe estuary than in fish from Helgoland (table 4).

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 ($R= 0.340$; $p< 0.001$), caused by 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.

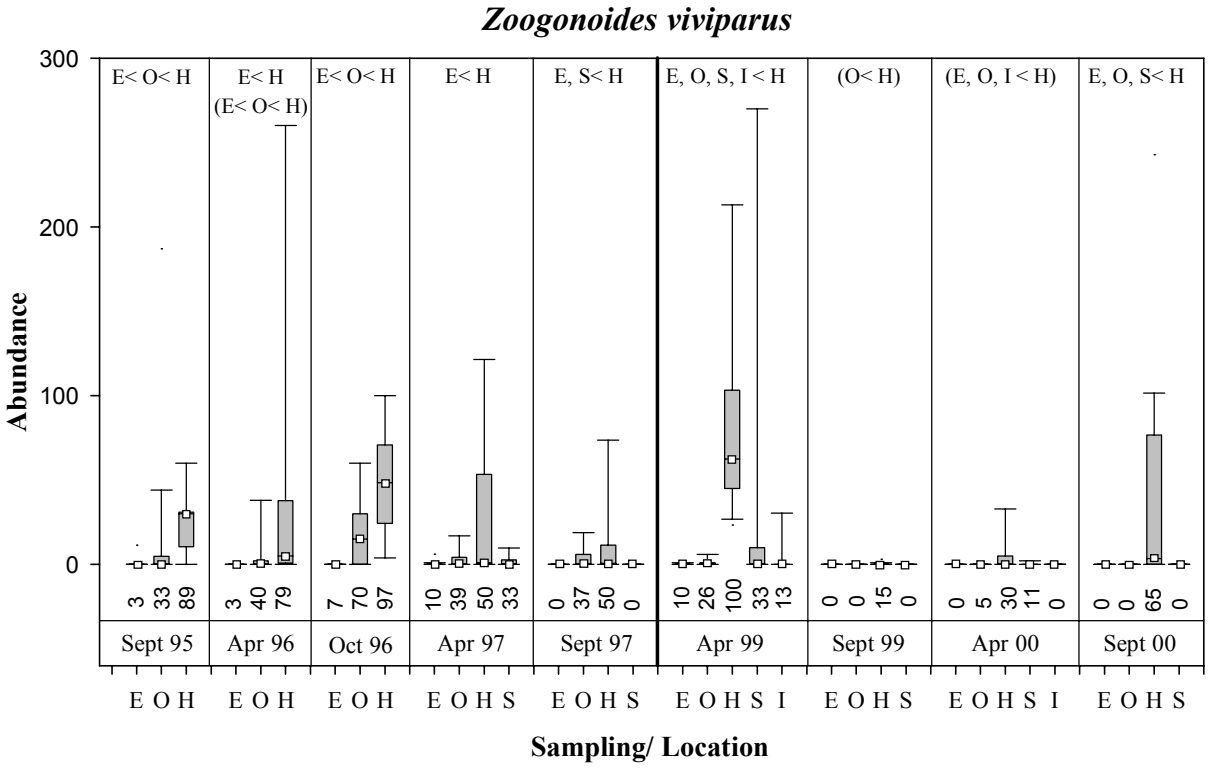


Figure 5: Abundance of *Zoogonoides viviparus* at different sampling locations in the German Bight during the course of the study. For key, see Fig. 1.

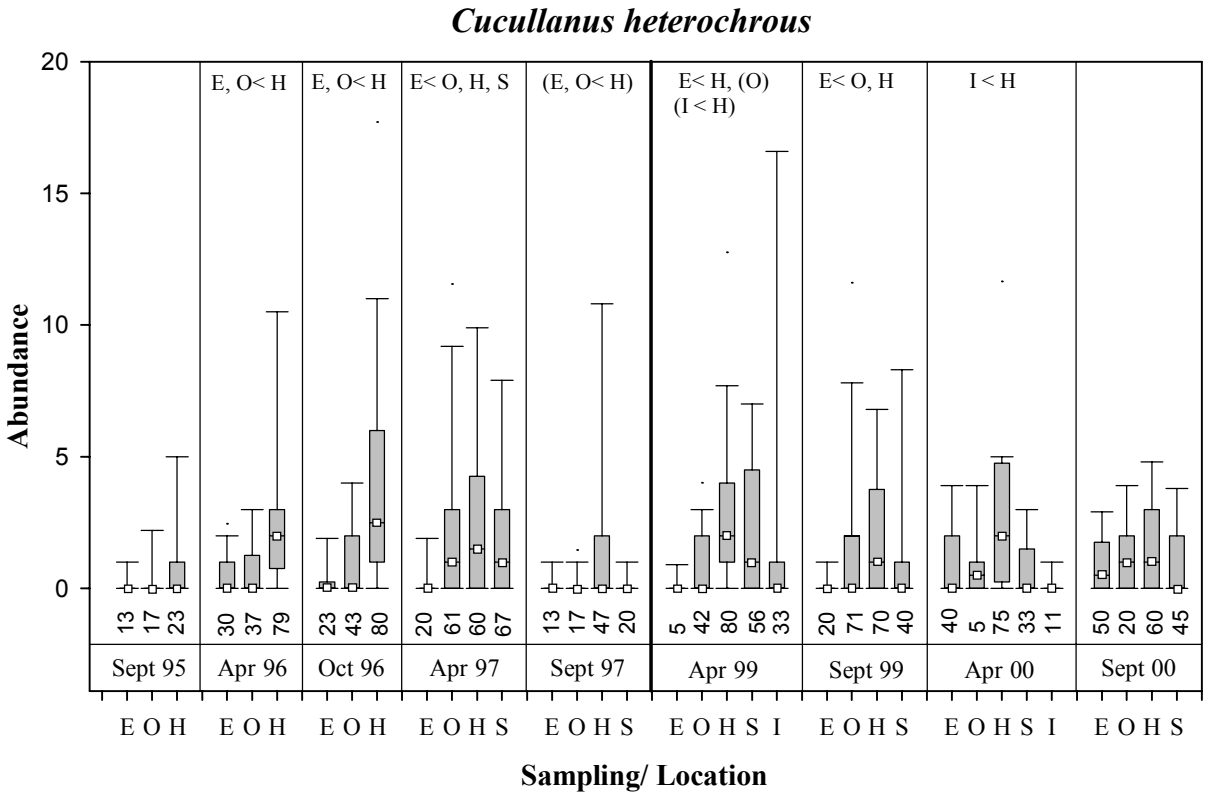


Figure 6: Abundance of *Cucullanus heterochrous* at different sampling locations in the German Bight during the course of the study. For key, see Fig. 1.

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 single flounder specimen. The maximum number of metacercaria found on an individual flounder was 1237.

In half of the sampling points, significant differences were found between sites, but the results varied between the sampling periods (figure 7). In the spring sampling periods of 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 the Outer Eider estuary. Intensity was lower in fish from Outer Eider estuary than in fish from Spiekeroog, but only in autumn (table 4).

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 with the infection levels of the metacercaria.

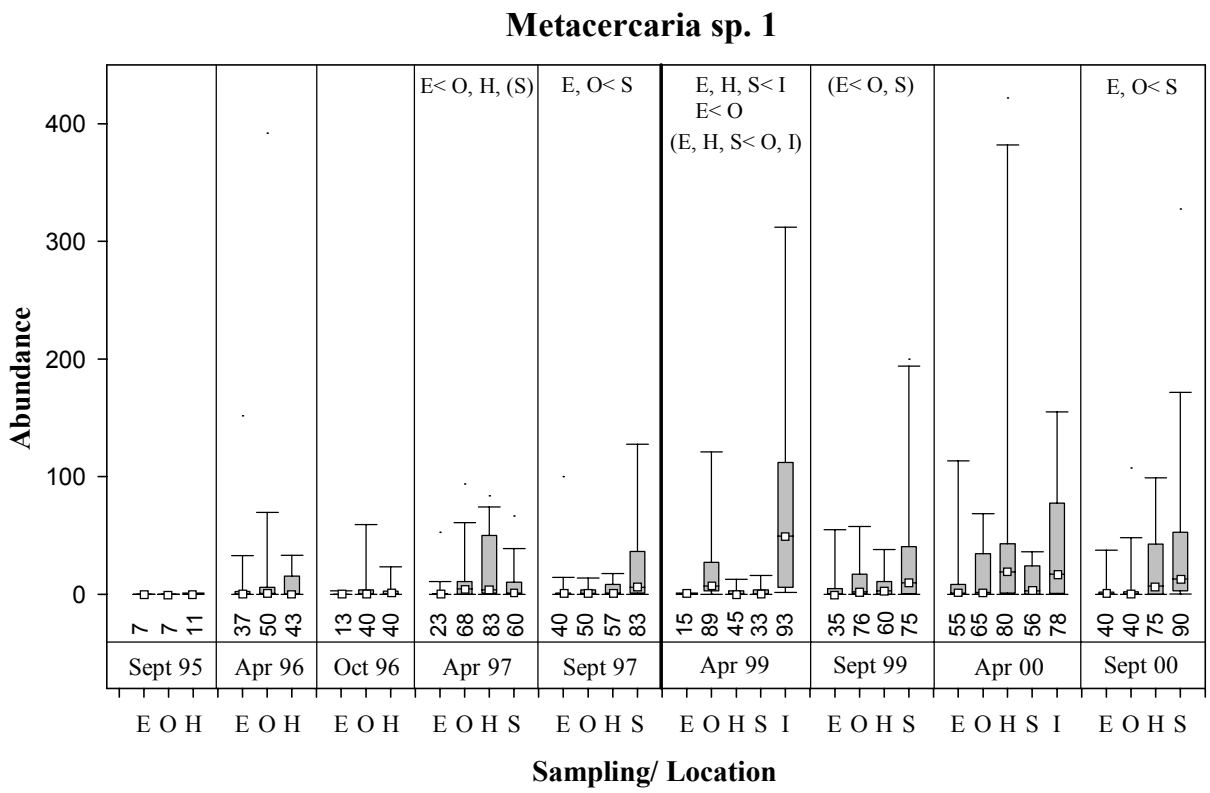


Figure 7: Abundance of the unidentified metacercaria at different sampling locations in the German Bight during the course of the study. For key, see Fig. 1.

Table 4: Spatial differences in the parasite fauna of flounder in the German Bight. Given are statistically significant differences between sites found in the infection levels of the dominant 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

Parasite species	**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

Summary of the results

From a total of 30 parasite taxa, which were found during the course of the present study, only 7 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 lower in fish from the Elbe estuary or from both of the estuarine sites compared to fish from the marine and coastal sites. *Trichodina* spp. was the only species, which had an opposite distribution pattern 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 *L. branchialis* and *L. pectoralis*, differences between sampling sites were found in almost all sampling periods in their abundance, but less frequent in their prevalence. In the pooled data set, differences were found in prevalence as well as in the abundance.

Gradual differences of infection levels between sites, coinciding with a described pollution gradient (Elbe < Outer Eider < Helgoland) (Broeg et al. 1999; Schmolke et al. 1999), were rarely detected in single sampling points, but in the pooled long- term data set they were found in prevalence and abundance for 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 for the prevalence of *Trichodina* spp. Gradual differences were also observed in the abundance of *Trichodina* spp. and *A. cornuta*.

Only two species, *L. pectoralis* and *L. 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 between autumn sampling periods, and they were less prominent between 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 for *Trichodina* spp., which was negatively correlated with fish length. As 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 there was also found a relation with the condition factor of flounder, 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 fish parasites to assess environmental changes (Kennedy 1997; Overstreet 1997; Gelnar et al. 1997; Yeomans et al. 1997). The primary agents that impact on natural communities, apart from natural environmental fluctuations, are anthropogenic disturbances. Because perturbances caused by humans 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 there are numerous ways how they reflect the host-environment situation. Parasites can respond directly to immunosuppression of their host by a rapid multiplication. Within days an infection with a monoxenic parasite such as *Ichthyobodo necator* can rise from a moderate infection to severe levels (Woo and Poynton 1995). A reduction in biodiversity can result in an extinction of invertebrate species, which serve as intermediate hosts for a heteroxenous parasite that 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 causal mechanism and to identify anthropogenic impact from natural environmental fluctuations. In order to use parasite species or community data as bioindicators 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 bioindicators, 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 5 different 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, here it was possible to identify some of the taxa, which remained undetermined in the previous work.

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

In order to use these parasite species as bioindicators, only 7 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 *Ciliophora Trichodina spp*., *the copepods A. cornuta*, *L. pectoralis* and *L. 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 programs, is given in table 6. The response of these parasites to selected pollutants however, is completely unknown, except for *Trichodina* spp.

Each of these parasite taxa displayed a specific infection pattern. Infection levels of some of the species varied within wide ranges during single sampling points, but in the combined, long-term data set, all of the species showed significant differences in their infection levels between 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 between the sites by Broeg et al. (1999) with respect to the residues of chlorinated hydrocarbons in the muscle and the liver tissue of flounder, and by Schmolke et al. (1999) with respect to the residues of heavy metals in sediments and blue mussel tissue, could be confirmed. These differences were observed when the prevalence of the four species *Trichodina* spp., *A. cornuta*, *Z. viviparus* and *C. heterochrous* over the 5 years sampling period were 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 between the sampling sites, which clearly underlines the necessity of prolonged observations when parasites are used in pollution monitoring.

The sites specific distribution of the species, *L. pectoralis* and *L. 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, also with highest infection intensities from spring to summer and lowest intensities in autumn. He described that in spring, males and females accumulate 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 (1974b), Pattipeiluhu (1996) and Van den Broek (1979) described an annual cycle for *L. 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 which influences 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 to temperature effects. In spring, when salinity is lowest in the estuary, due to a high freshwater inflow (Möller 1990), prevalences also were significantly lower than in autumn, when salinity is highest (Möller 1990). Möller (1974) observed a similar distribution pattern 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 pattern of parasites coastal waters. Möller (1978) considered stenohalinity of parasites and their hosts as the main reason for a natural reduction of the parasitic fauna in brackish water. Water salinities of about 16‰ provide unfavourable conditions for marine as well as for freshwater species. Especially ectoparasites and digeneans are affected by low salinity. Ectoparasites are directly affected by low salinities, for digeneans it is due to the lack of molluscs that serve as intermediate hosts (Möller 1978; MacKenzie et al.1995).

In the present study, salinity characteristics differed remarkably between 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) as well as Anders and Möller (1991) described considerable salinity fluctuations in the Elbe (2.4-20‰) and Inner Eider estuary (6-29‰). In 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‰ were found downstream from km 720, close to the sampling site of these present investigation. Highest salinities were measured between October and December and lowest salinities from April to June.

Five of the 7 species, considered as bioindicators (all copepods as well as *Z. viviparus* and *C. heterochrous)*, are marine species that are not able to complete their life cycles in waters with low or changing salinities.

Copepods were found to be stenohaline (Kabata 1979; Knudsen and Sundnes 1998) and are quickly lost, when a host individual entered the estuaries (Gibson 1972). Wichowski (1990) found that the distribution of the copepods *L. pectoralis* and *L. 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 ranged between 10-16‰ (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 between copepod species. Möller (1978) observed differences in the susceptibility of copepods and described a lower capacity to survive reduce salinities for *Acanthochondria depressa* (= *A. cornuta*, Kabata 1979) than for *L. pectoralis*. Kabata (1959) also suspected that abiotic factors might influence the distribution of *A. cornuta*, but exact physiological requirements of this species still remain unknown. A higher susceptibility to

reduced or fluctuating salinities 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 range of its first intermediate host, *Buccinum undatum* (Gibson 1972; Möller 1978; Køie 1976, 1983). As *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*, polychaets of different species and ophiurids as *Ophiura albida*, which act as first and second intermediate hosts, occur in high numbers and provide excellent conditions for the parasite to infect flatfish 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 probably is the most important intermediate host for this parasite (Køie 2000). Thus, reduced prevalences of *C. heterochrous* at estuarine sites in the German Bight, as observed in the present study, might be due to the susceptibility of its eggs, which embryonate in seawater, to fluctuating salinities, rather than to the absence of the intermediate host in the estuaries. They might also sustain lower, but more constant salinities as 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 conditions were similar at the estuarine sites, infection levels of the parasites differed. Similar prevalences at Elbe and Inner Eider estuary were only found for *Trichodina* spp., *L. 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 pattern also was observed in the prevalences of *L. 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* also were 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 between fish from the Inner Eider estuary and fish from the coastal and marine sites (table 5). Significantly elevated infection levels of some parasite species in fish

from the Inner Eider estuary, when compared to flounder from the Elbe estuary. Between these sites, a contamination gradient (Elbe > Inner Eider > Helgoland; Schmolke et al. 1999) was found which with respect to residues of heavy metals in sediments and blue mussel tissue. 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, Outer Eider estuary and Helgoland, which are characterized by relatively constant salinity levels. Prevalences of four parasite species, *Trichodina* spp., *A. cornuta*, *Z. viviparus* and *C. heterochrous*, were significantly lower in fish from Outer Eider estuary than in flounder from Helgoland (table 5). Between these sites, Broeg et al. (1999) found a contamination gradient (E > O > H) when residues of chlorinated hydrocarbons in muscle and liver tissue of flounder were considered. A similar gradient could be seen with respect to 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 most likely is not the only factor, which 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 chapter 5.

Table 5: Summary of biological information on dominant 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= Metacercaria sp. 1, Cuc= *C. heterochrous*, *= yes or positiv; * (a) = organic pollution; 1= Kabata (1959), 2= Boxshall (1974, 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 (1972); 9= Køie (2000); 10= Køie (1976).

Biological information	Trich	Acan	Lep	Lern	Zoog	Meta	Cuc
1) regularly present at all sites investigated	*	*	*	*	*	*	*
2) infection levels different between sites	*	*	*	*	*	*	*
3) common parasite species of flounder	?	*	*	*	*	?	*
3) sampling and identification easy	no	*	*	*	*	?	*
4) distribution and habitat requirements known	no	*	*	*	*	no	*
5) biology and life-cycle known	no	1	2, 3	4-7	8, 9	no	10
6) low seasonal fluctuations	*	*	no	no	*	*	*
7) low annual fluctuations: spring	*	*	*	*	no	*	*
autumn	no	no	no	no	no	*	no
8) relation with fish length	*	*	*	*	*	no	*
9) relation with sex of fish	no	no	no	no	no	no	no
10) dependence on salinity	*	*	*	*	*	no	*
11) response to selected contaminants known	* (a)	no	no	no	no	no	no

The trichodinids and the metacercaria could not be identified at the species level, which did not allow to decide, whether they were of marine or estuarine origin. In general, trichodinids live on the surface of skin and gills of fish and feed on bacteria and organic matter from the water column and 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 works effluents. In cultured eel (*Anguilla anguilla*), the infection level of *Trichodina jadranica* was positively correlated to 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 predominantly were found in estuarine sites with increased organic load (Möller-Buchner 1987). A possible link between anthropogenic eutrophication and infection levels of trichodinids is discussed in chapter 5.

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

Conclusions

In the present study it was shown that all parasite species under study were useful indicators for differences between the sampling sites in the German Bight. The necessity of long-term studies, when parasites are used in environmental monitoring programs, was also underlined. While a high variability in the infection characteristics of the parasites was observed between single sampling periods, specific infection patterns were detected for all of the parasite species when data were pooled over all sampling points over a period of 5 years.

In the long-term data set it became evident that seasonal variations had an influence on the spatial distribution of the parasites. Differences between the sampling sites were more pronounced in spring than in autumn, especially in the infection levels of *L. pectoralis* and *L. branchialis,* where spatial differences were exclusively observed in spring. As a consequence, a direct comparison of parasitological data from different seasons is not recommended, because these variations might cause misleading results. All of the species under study showed significant differences in their infection levels between Elbe estuary, as the most polluted site, and the less polluted coastal and marine locations, Helgoland, Outer Eider estuary and Spiekeroog. Gradual differences between Elbe, Outer Eider and Helgoland, which were not observed during single sam-

pling points, also became evident in the prevalence of *Trichodina* spp. (E > O > H), as well as in that of *A. cornuta*, *Z. viviparus*, and *C. heterochrous* (E < O < H). In addition, significant differences were found in the prevalence of Metacercaria sp. 1 and the intensity of *A. cornuta* and *L. branchialis* between the estuarine sites Elbe and Inner Eider estuary. These differences, which were observed between sites with similar salinity conditions, corresponded to a contamination gradient (Elbe > Outer Eider, Inner Eider > Helgoland), established between these sites by residue analyses in sediments and biota. Thus in addition to salinity, which is considered as the most important natural factor influencing the distribution of parasites and its intermediate hosts in coastal environments, man- made effects such as pollution also most likely had an impact on the distribution pattern of the parasites at the locations under study. *Trichodina* spp. showed increasing infection levels at sampling sites with elevated concentration of organic substances, which supported the hypothesis, that trichodinids may act as indicators for organic contamination.

As little information on specific effects of known pollutants on the physiology of the parasites is available, the impact of pollutants has to be backed up by an integrated use of parasitological data in combination with the responses of established biomarkers to pollution exposure, as shown in chapter 5.

Chapter 4
Community structure and fish parasite biodiversity

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 from different locations in the German Bight during spring and autumn of 1995-2000, using established ecological concepts. Species richness (S), Shannon- Wiener's Index of Diversity (H'), Evenness (E), inverse Simpson's Index of Diversity (1/D) were calculated on the infra-community level. In addition, the number of monoxenous (M_{sp}) and heteroxenous species (H_{sp}) and the ratio between heteroxenous and monoxenous parasite species (H/M_{sp}), were calculated according to D'Amelio and Gerasi (1997). On the component community level, an estimation of true species richness by the analysis of exponential accumulation curves was applied, as suggested by Walther et al. (1995), the number of component species, according to Bush et al. (1990) and Sorenson's indices of similarity calculated.

Although the parasite community composition was very similar at the component community level, number of component species as well as the species accumulation curves showed clear differences between 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 when compared to 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 in individual sampling pointss, became evident in the measurements of S, N, H_{sp}, H' and 1/D and corresponded to a contamination gradient (Elbe > Inner Eider, Outer Eider > Helgoland), established by Broeg et al. (1999) and Schmolke et al. (1999) during both seasons. Despite of seasonal variations, which were observed in almost all measurements, these gradual differences were found in both seasons.

Introduction

The analysis of fish parasite community structure and the use of ecological richness and diversity measurements are widely used 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 to a reduction of diversity (Kennedy 1997).

The simplest and oldest diversity measurement is the number of species, or species richness, which registers only the presence of a parasite species in the 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 of the location under study by the analysis of exponential accumulation curves, in order to determine the optimal size of sampling effort. Species diversity indices combine the information of species richness and the relative abundance of each species. Commonly used indices are Shannon-Wiener index and inverse Simpson index of diversity, as well as Shannon's Evenness (Magurran 1988). Frequently discussed are the problems that these indices are sensitive to the presence or absence of one or few dominating species in the community and that protistans and procaryotes must be excluded from these calculations, due to the difficulties in counting individuals (D'Amelio and Gerasi 1997). Anyway, the use of biotic indices has many advantages. Without the 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 heteroxenous parasites with complex, multi-host life cycles depend for transmission on the presence of a variety of invertebrate and vertebrate intermediate hosts. These parasites only persist in environments, where all species required as intermediate and definite hosts can co-exist. In disturbed habitats, where the overall diversity is reduced, the number of heteroxenous parasite species may also decline, due to the lack of required hosts. In such environments, monoxenous species, which only need a single host species to reproduce, may dominate (D'Amelio and Gerasi 1997).

In the framework of a biological effects monitoring, Broeg et al. (1999) investigated the parasite community of flounder *Platichthys flesus* (L.) at different locations in the German Bight, by means of the two concepts mentioned above and assessed their potential use as bioindicators for

pollution effects. Applied were species richness and the ratio of heteroxenous to monoxenous species. 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 was to separate natural influences on the parasite community from pollution mediated effects (Broeg et al. 1999).

The present study was done in continuation of the previous report by Broeg et al. (1999), the flounder were collected at the same locations, the two data sets were combined in order to obtain a more extensive data base collected over a period of 6 years. This allowed a detailed investigation of the parasite community of flounder in the German Bight using common and established ecological concepts 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 in chapter 5.

Materials and 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.

A characterisation of the sampling sites as well as a detailed description of the sampling procedure and the examination of flounder and parasite species are given in chapter 2. Numbers of evaluated fish specimen for each sampling points 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 in chapter 3.

The parasite community structure of the flounder was examined a) at the infra-community level, which is the community of parasite infrapopulations in a single host and b) 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 as well as 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 (1995) was calculated according to the formula: $Y = a(1-e^{-bx})/b$, with a = increase in species richness at the beginning of sampling, b = parameter that sets the species richness asymptote $R = a/b$, x = unit of sampling effort.

Similarity in the parasite community between investigated sites were measured using Sorenson's Index, according to Magurran (1988), which was calculated qualitatively according to: $C_S = 2j / (a+b)$ with j = number of species found jointly in two samples, a = number of species in the first sample, b = number in the second sample. It also was calculated quantitatively according to the formula: $C_N = 2j_N / (a_N+b_N)$ with a_N = number of individuals in sample a, b_N = number of individuals in sample b, jN = sum of the lower of the two abundances of species which occur in the two samples. Sorenson's indices, which surpass values of 0.6, are considered to indicate similarities, and values of more than 0.8 great similarities.

Measures of the **infra community** were the mean number and range of parasite species found on flounder individuals, 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:

Shannon-Wiener's Index of Diversity ($H' = -\sum (p_i \ln p_i)$), where p_i = relative intensity of parasite species i; completed by Evenness ($E = H' / \ln S$, where S = total number of parasite species) and Inverse Simpson's Index ($D = 1/ \sum p_i^2$). Shannon-Wiener's Index is weighted towards the richness of a community, and Simpson's 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 between hosts, values of 1 a totally even distribution. All indices were calculated according to Magurran (1988).

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

Statistical analysis

Most of the data were not normally distributed (Kolmogorov- Smirnow test). Data of Simpson's Index were normalized by logarithmic transformation ($\log_{10}(N+1)$), those of Evenness by potential transformation (x^2). Normalized data were compared by Student's t-test or ANOVA and Tukey's post hoc test, otherwise nonparametric tests as Mann-Whitney's U-test or Kruskal-Wallis ANOVA and Dunn's post hoc test were used. Differences between groups were considered as significant at a probability of error $p< 0.05$. Correlation coefficients were calculated with the parametric Pearson's Product Moment Correlation or Spearman's correlation on ranks. Correlations were considered as significant at a probability of error $p< 0.05$. The analyses were carried out using the computer programs SigmaStat® 2.0 and STATISTICA 6 (StatSoft).

Results

Component community

During 9 sampling pointss in spring and autumn of 1995-1997 and 1999-2000, 802 flounder individuals 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), which displayed a total of 77 611 individuals, and 6 species of non-countable microparasites (1 Apicomplexa, 2 Microsporea, 1 Ciliophora and 2 Myxozoa) were recorded. 9 taxa had a monoxenous development and 21 taxa a heteroxenous life cycle.

A list of all parasite taxa and information about the relative abundance of the macroparasites and the presence of microparasites are given in table 1 for all locations investigated.

17 parasite 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). Out of these, 7 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*, not determined metacercaria and the ciliate protozoan *Trichodina* spp. These taxa dominated the parasite community both by prevalence and by intensity and, except of 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 predominating species in prevalence and intensity, followed by the metacercaria. The infection characteristics of these 7 parasite taxa, some of the natural factors influencing their infection levels as well as their potential use as indicator species are presented in detail in chapter 3.

Table 1: List of parasites species recovered from flounder in the German Bight during sampling points in spring and autumn from 1995-2000. For macroparasite species, the relative abundance is given as the proportion (p_i) of the total number of all macroparasites of all species at the 5 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.

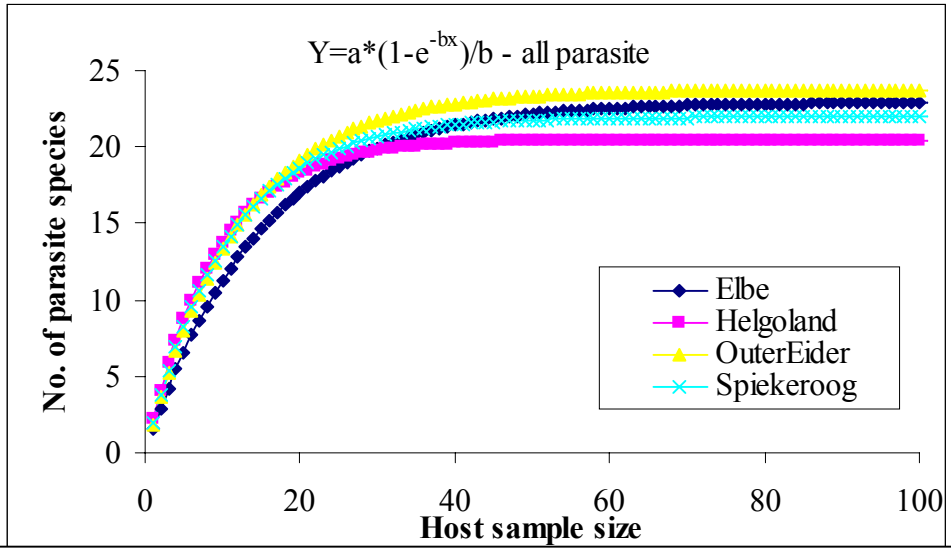
				Localities				
Taxonomic group	Parasite taxa	Target organ	Life cycle [1]	Elbe	Outer Eider	Helgoland	Spiekeroog	Inner Eider
Apicomplexa	*Epieimeria* sp.	gut	m	* /c	* /c	* /c	* /c	* /c
Ciliophora	***Trichodina* spp.**	gills	m	* /c	* /c	* /c	* /c	* /c
Microsporea	Microsporea sp.1	kidney	m	* /c	* /c	* /c	* /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

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

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

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

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.



Elbe: $r^2 = 0.88$; R (a/b) = 22.9; C = 124, **Helgoland:** $r^2 = 0.78$; R (a/b) = 20.48; C = 78, **Outer Eider:** $r^2 = 0.83$; R (a/b) = 23.71; C = 95, **Spiekeroog:** $r^2 = 0.88$; R (a/b) = 21.95; C = 87

Figure 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

Table 3: Seasonal changes in the total species number of the parasite component community of flounder at different sampling locations in the North Sea.

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

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

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 ranged between 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, varied in a greater range from 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 Elbe estuary and the offshore sites Helgoland and Outer Eider. The highest similarity was found between Inner Eider estuary and Spiekeroog as well as between Helgoland and 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).

Table 4: Similarity of the parasite component community of flounder at different sampling locations in the German Bight of the North Sea. a) in spring and b) in autumn, upper half of the panel: qualitative Sorenson's indices, lower half: quantitative Sorenson's indices, c) Comparison between seasons at each site. E = Elbe, O = Outer Eider, H = Helgoland, S = Spiekeroog, I = Inner Eider

a)

Spring	E	O	H	S	I
E	*	0.84	0.79	0.83	0.76
O	0.53	*	0.89	0.86	0.84
H	0.45	0.87	*	0.86	0.84
S	0.74	0.76	0.66	*	0.88
I	0.8	0.71	0.61	0.94	*

b)

Autumn	E	O	H	S
E	*	0.84	0.89	0.86
O	0.64	*	0.99	0.83
H	0.63	0.82	*	0.78
S	0.81	0.82	0.81	*

c)

Sites	qualitative	quantitative
E	0.82	0.91
O	0.87	0.96
H	0.86	0.86
S	0.77	0.98

Infracommunity

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 Outer Eider estuary. During single campaigns, the mean number of parasite taxa (species richness = S) ranged between 3 and 7. In almost all campaigns, fish from the Elbe estuary harboured significantly less parasite species than fish from Helgoland and the Outer Eider estuary (figure 2). The mean number of macroparasite individuals per fish (N) ranged between 5 and 180. In most of the campaigns, flounder from the Elbe estuary were significantly less infected with macroparasite individuals than fish from Helgoland or the Outer Eider estuary (figure 3).

Mean values of Shannon- Wiener's Index of diversity (H') per fish ranged between 0.12 and 1.25, mean values of the inverse Simpson Index (1/D) between 1.1 and 3.1. During the sampling points of 1995-1997, fish from the Elbe location exhibited significantly lower diversity values for both measurements (H' and 1/D) than fish from Helgoland or Outer Eider. Between the latter two sites, differences in diversity were not found in 1995-1997, but from 1999 to 2000, diversity values were highest in fish from Helgoland compared to all other sites under study (figure 4 and 6). Mean values of evenness (E) ranged between 0.2 and 0.8. In only half of the campaigns, significant differences were found between the sampling sites. The results were highly variable, and only in two campaigns, evenness was significantly lower in fish from the Elbe estuary than in fish from Helgoland or Outer Eider estuary (figure 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 compared to fish from the Outer Eider estuary and Helgoland in almost all campaigns (figure 7), M_{sp} exhibited the same differences in only half of the campaigns (figure 8). Mean values of the H/M_{sp}-ratio were found in the range of 0.45 and 1.1. Significant differences between sites were only found during three campaigns. Then fish from Elbe had lower H/M_{sp} values than fish from the coastal and offshore locations (figure 9).

All parasitological measurements, except for species richness (S), 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/M_{sp}-ratio were significantly higher in spring than in autumn ($p< 0.001$).

Thus, data were summed across the sampling pointss and separated by seasons in order to evaluate observations over the complete sampling period. The results are given in table 5, separated by location and season.

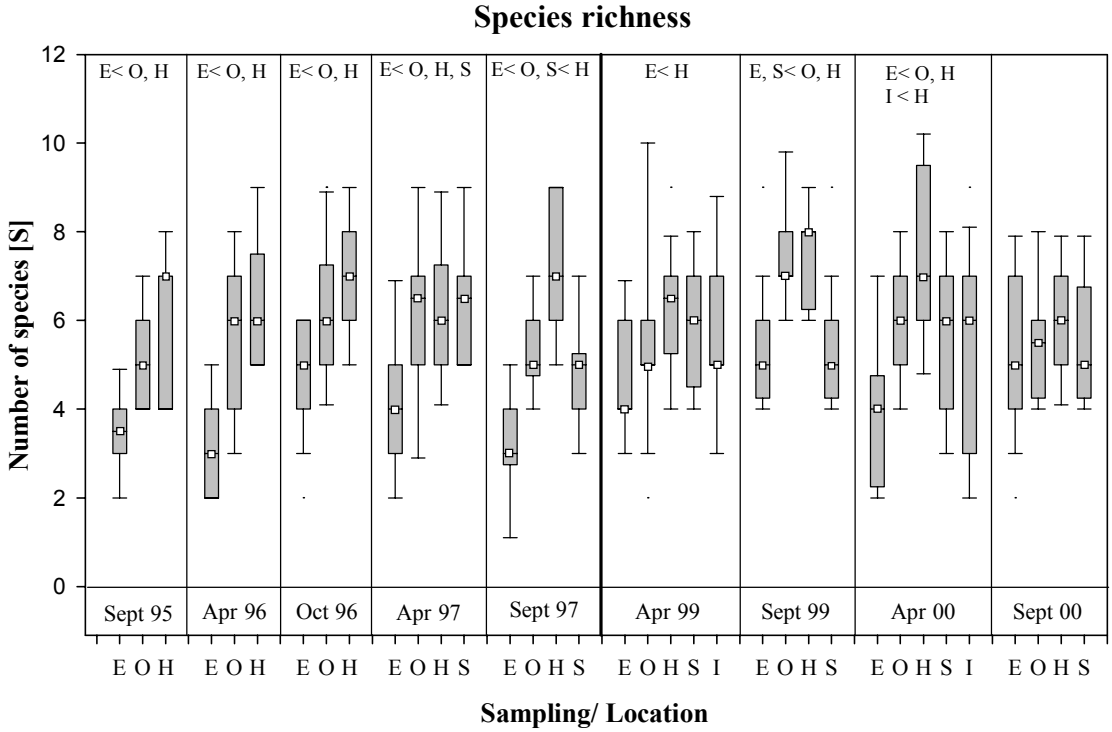


Figure 2: Species richness [S] of the parasite community of flounder at different sampling locations from 1995-1997 and 1999-2000. Given are median values (□), with 25-75% percentiles (boxes in grey) and 10 –90% percentiles (whiskers). The campaigns, indicated below the box-plots, are identified by month and year (Apr= April, Sept= September, Oct= October). Differences in the species richness, observed between sites are given on the top of the graph. Information, given in parenthesis, was additionally observed in the prevalences; levels of significance, $p < 0.05$. E= Elbe, E= Outer Eider, H= Helgoland, S= Spiekeroog, I= Inner Eider. For numbers of flounder evaluated during the sampling point see chapter 4, table 1.

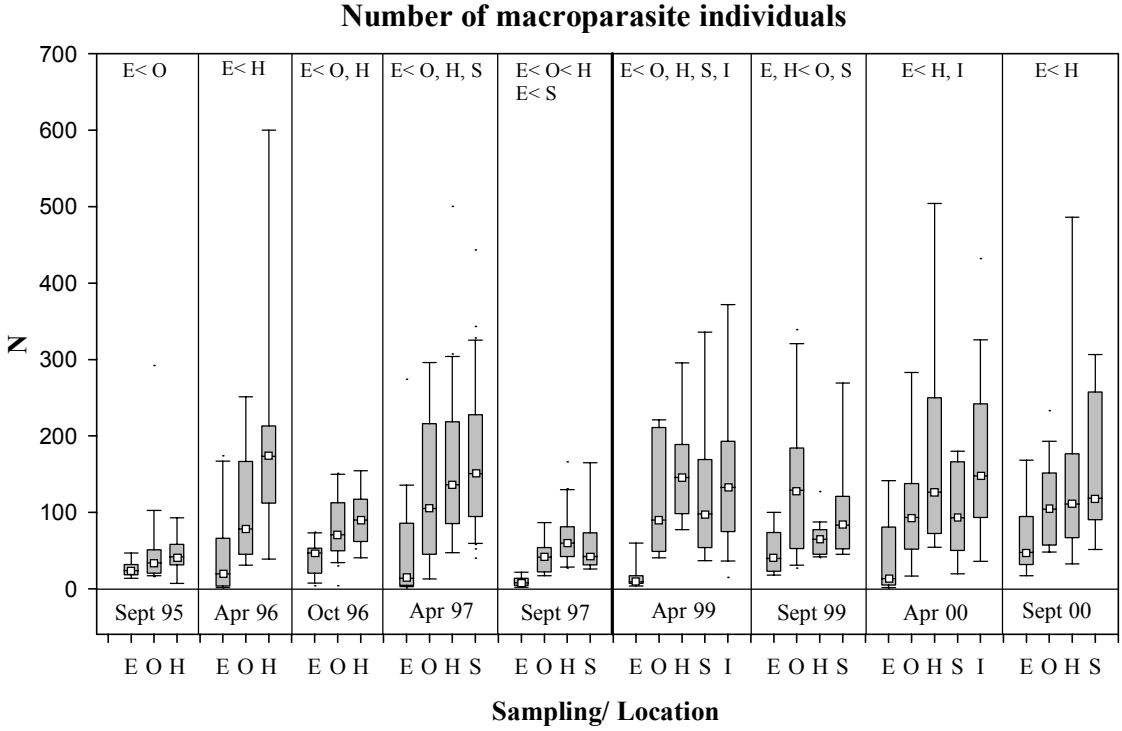


Figure 3: Number of macroparasite individuals [N] in the parasite community of flounder at different sampling locations from 1995-1997 and 1999-2000. For key, see fig. 2.

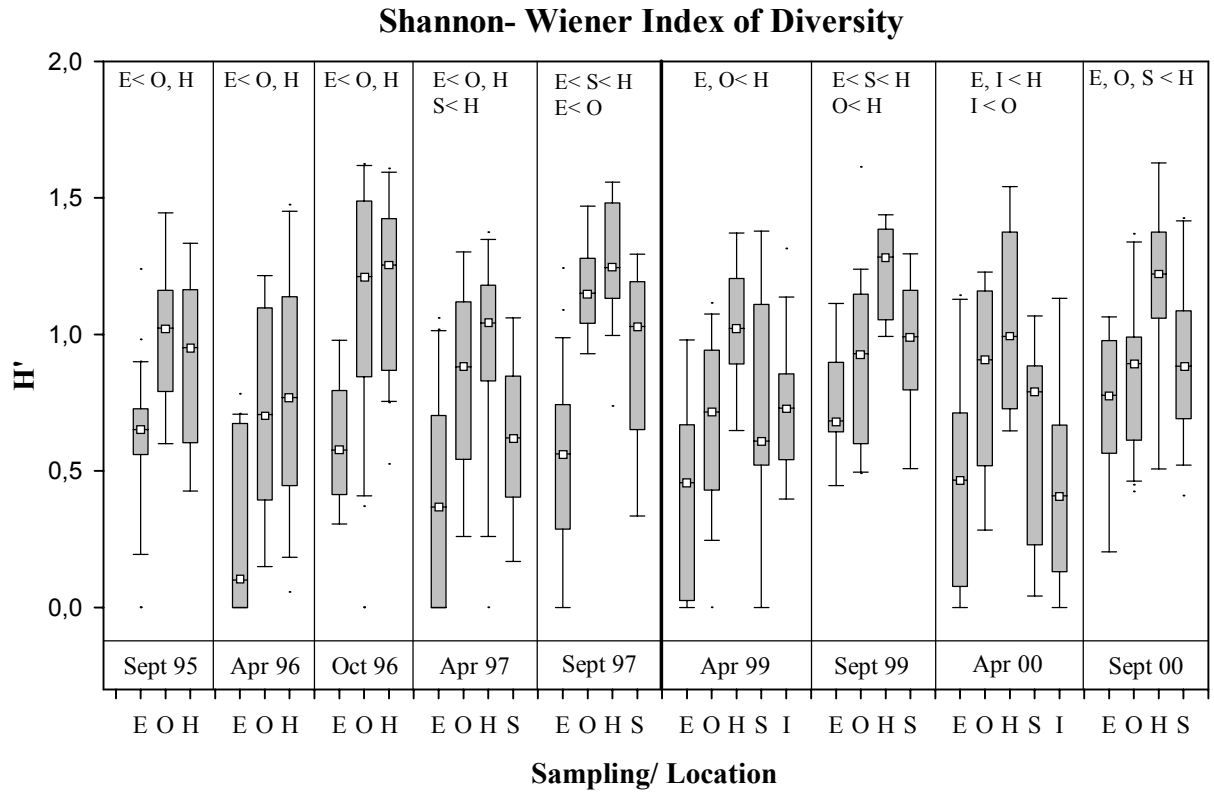


Figure 4: Shannon-Wiener's Index of Diversity [H'] of the parasite community of flounder at different sampling locations from 1995-1997 and 1999-2000. For key, see fig. 2.

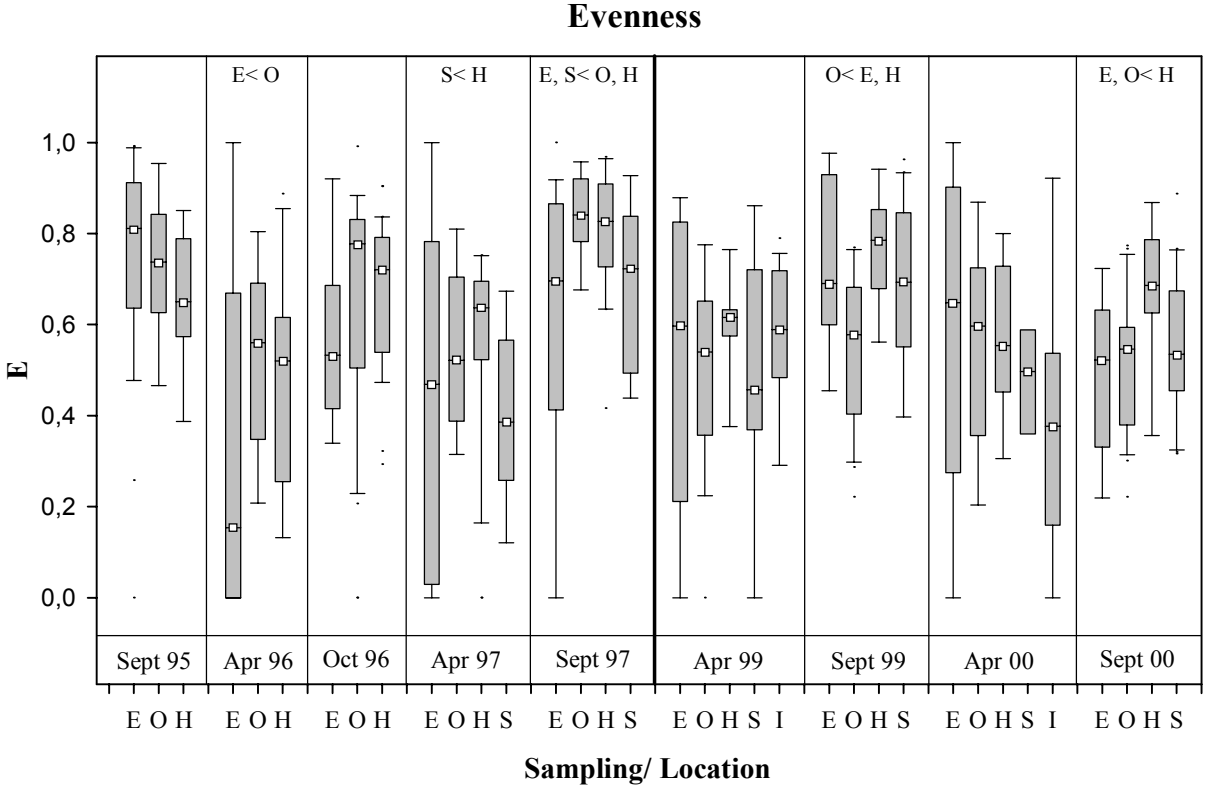


Figure 5: Evenness [E] of the parasite community of flounder at different sampling locations from 1995-1997 and 1999-2000. For key, see fig. 2.

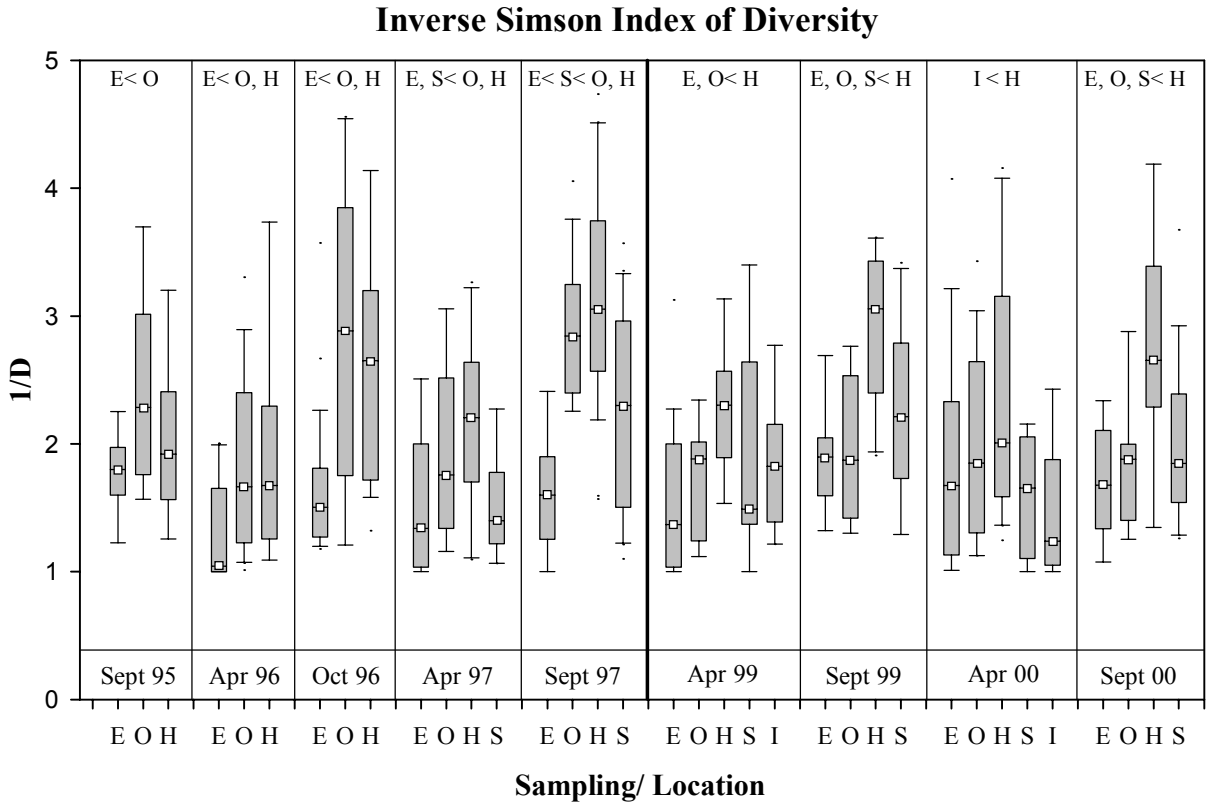


Figure 6: Inverse Simpson's Index of Diversity [1/D] of the parasite community of flounder at different sampling locations from 1995-1997 and 1999-2000. For key, see fig.2.

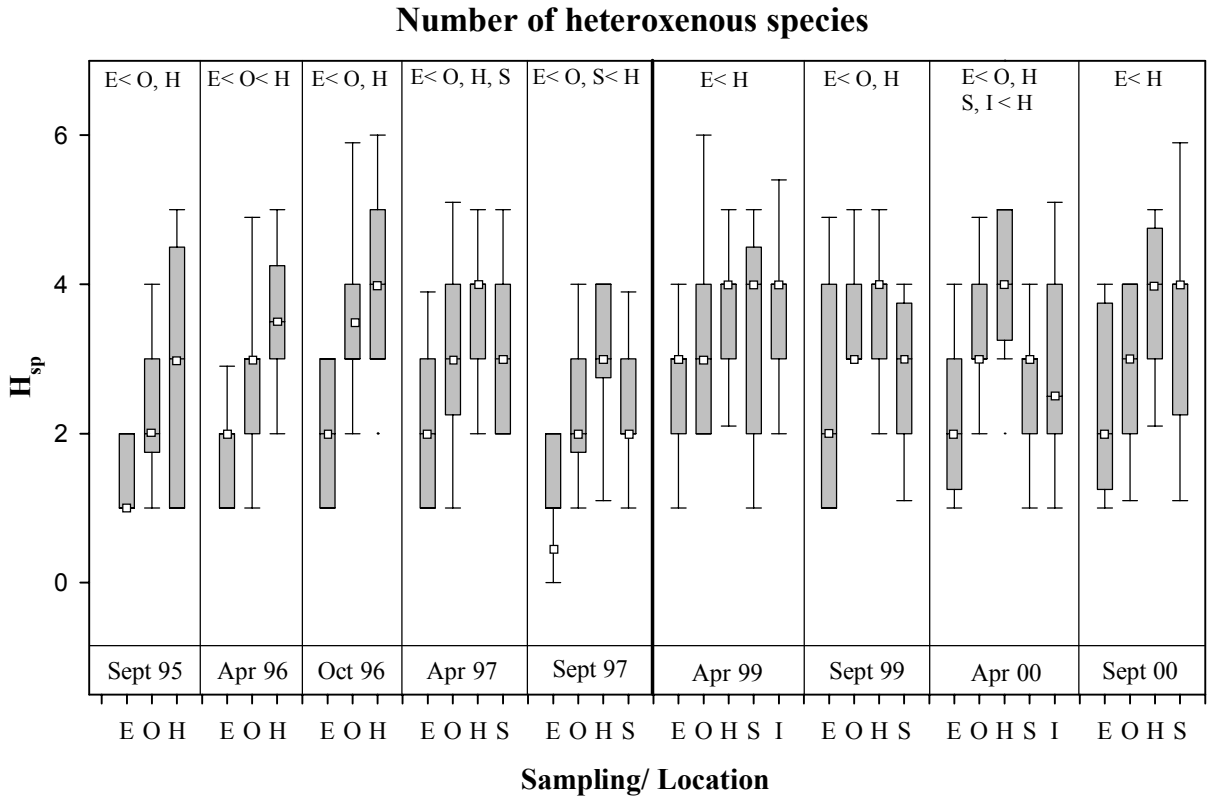


Figure 7: Number of heteroxenous parasite species [H_{sp}] in the parasite community of flounder at different sampling locations from 1995-1997 and 1999-2000. For key, see fig. 2.

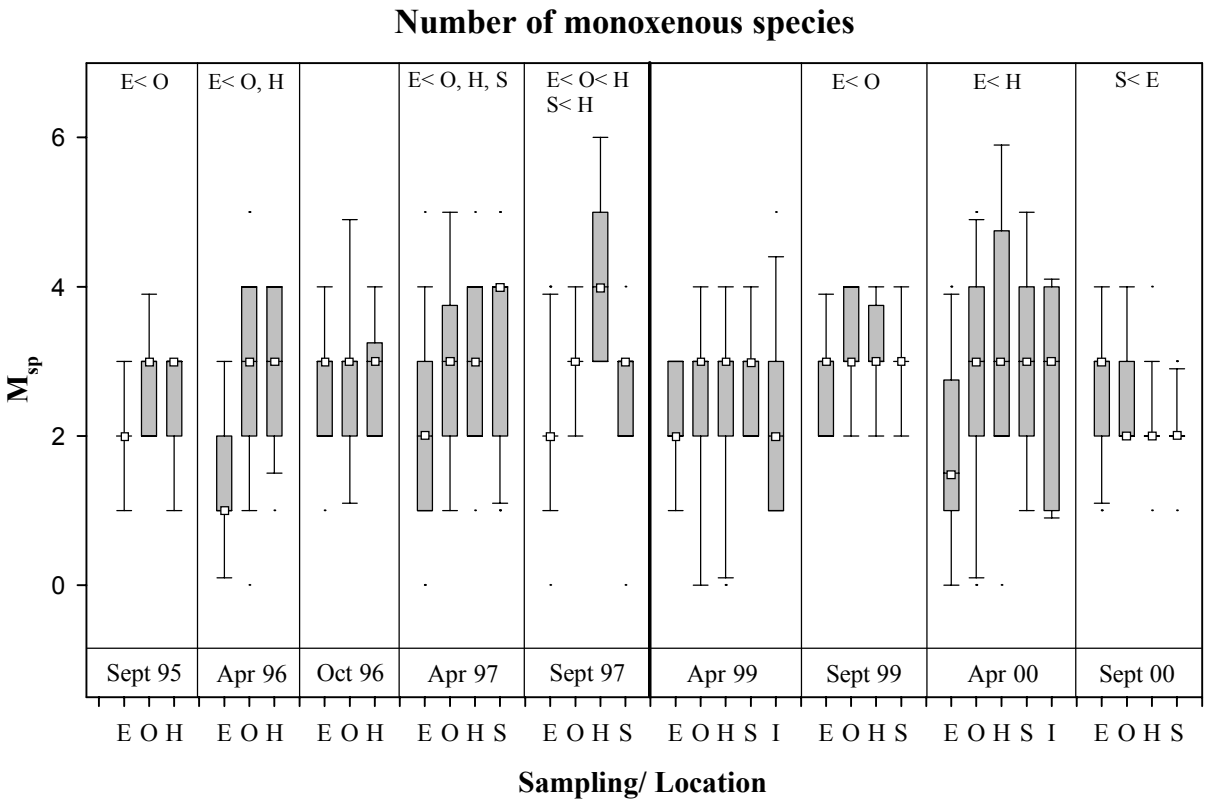


Figure 8: Number of monoxenous parasite species [M_{sp}] in the parasite community of flounder at different sampling locations from 1995-1997 and 1999-2000. For key, see fig. 2.

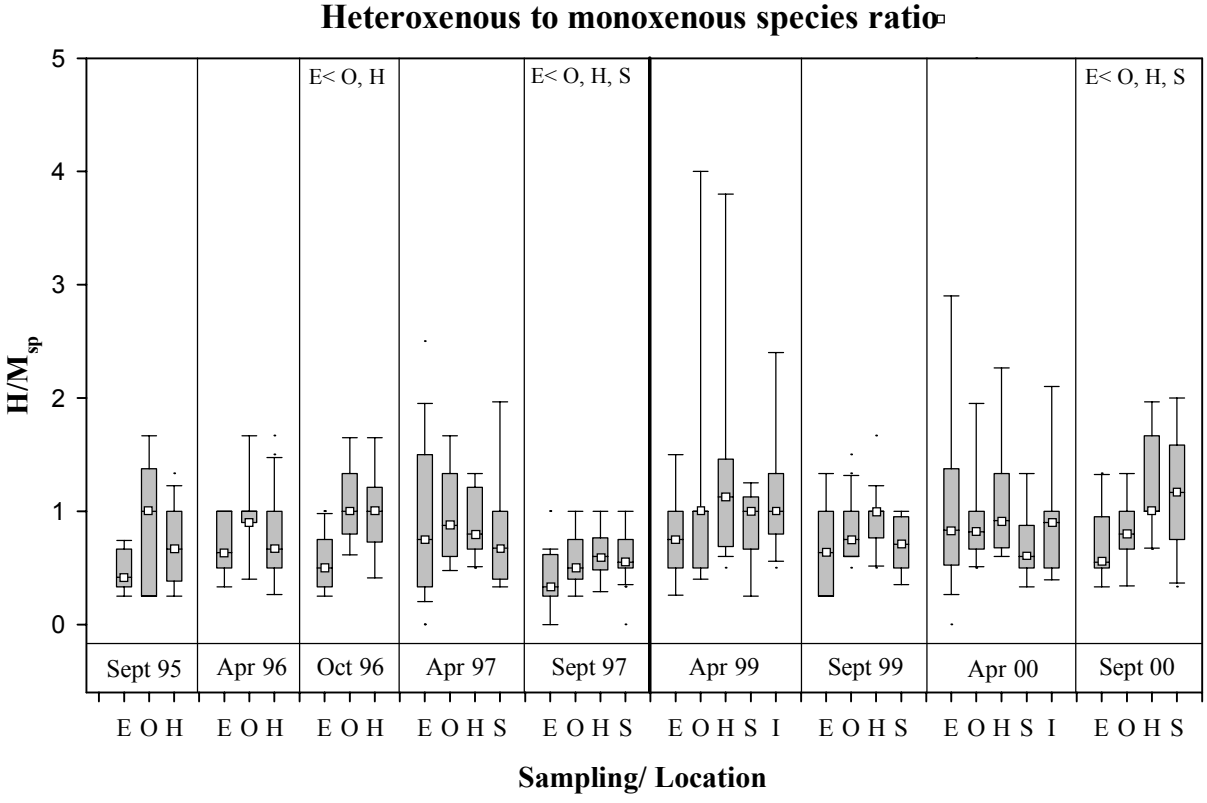


Figure 9: Ratio of heteroxenous and monoxenous parasite species [H/M_{sp}] in the parasite community of flounder at different sampling locations from 1995-1997 and 1999-2000. For key, see fig. 2.

Table 5: Seasonal changes in the diversity characteristics of the parasite infra community of flounder at different sampling locations in the North Sea. For all ecological measurements mean values ± standard deviation are given, which were calculated from specimen collected during 2-4 sampling points in spring or autumn. S= species richness, N= no. of macroparasite individuals, H'= Shannon- Wiener's 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/M_{sp} = heteroxenous to monoxenous species ratio.

Diversity characteristics	Elbe		Outer Eider		Helgoland		Spiekeroog		Inner Eider
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
Number of fish evaluated	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/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

Statistical evaluation of these data revealed that in general, species richness (S), the number of macroparasite individuals (N) and of heteroxenous species (H_{sp}) were significantly lower in fish from the Elbe estuary than in fish from all other sites, whereas the Shannon- Wiener Index (H') and the inverse Simpson Index (1/D) were highest in fish from Helgoland (table 6).

For S, H', 1/D and $H_{sp,}$ gradual differences were found during both seasons in an increasing order with Elbe< Outer Eider< Helgoland. For N, these differences were only found in spring (for H' and 1/D = $p< 0.001$ for all comparison; for S, N, H_{sp} = E< O, H, $p< 0.001$; O< H, $p< 0.05$). Gradual differences were also found between 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} (all comparison $p< 0.05$) and between Elbe< Outer Eider< Inner Eider for N (O< I, $p< 0.05$) (table 6).

Table 6: Spatial differences in the parasite fauna of flounder in the German Bight. Shown are statistically significant differences in species richness and diversity measurements of the parasite infra-community between the sampling sites in both seasons. 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/M_{sp}	---	E< O, H

For M_{sp}, H/M_{sp}-ratio and evenness (E), differences between the sampling sites were not that marked. In spring, M_{sp} was significantly lower in fish from Elbe estuary compared to fish from Outer Eider estuary, Helgoland or Spiekeroog. In autumn, differences were only found between fish from Elbe estuary and Helgoland. No differences were found between sites when H/M_{sp}-ratio and E were considered during the spring campaigns, but in autumn H/M_{sp}-ratio was significantly lower in fish from the Elbe estuary compared to individuals from Helgoland, and values of E were significantly lower in fish from Elbe estuary and Spiekeroog compared to fish from Helgoland (table 7).

Table 7: Correlation coefficients (r) between fish length and ecological indices. Level of significance: *** = p< 0.001. For key, 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/M_{sp}	0,200	***

At individual sampling locations, the ecological measurements considered here exhibited annual variations. These variations were more evident in autumn campaigns than in spring campaigns. During the course of 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 were correlated to fish length, except of evenness (tab. 8). As 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 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 on the component community and the infra-community level 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 characterize five locations in the German Bight that were considered to differ in their contamination load.

The basic hypotheses were a) that species richness and species diversity are reduced in contaminated habitats and b) that the ratio of heteroxenous to monoxenous species change in favour of monoxenous species under such conditions.

Component community

In the present study, a total of 30 parasite taxa were recorded from flounder in the German Bight, including 24 macro- and 6 microparasite species. Flounder from individual sampling sites har-

boured 24 - 26 taxa. In comparison to 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 8).

Table 8: Number of parasite taxa, reported in studies on the parasite community of flounder from different areas in the North Sea and the Baltic Sea, *= number of taxa which also were found in the present study

					Parasites		Parasites *	
Study	**Year**	**Area**	**Habitat**	**N (fish)**	**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	South England	Thames estuary	390	1	23	1	16
Koie (1999)	1999	Transect	Baltic Sea	200	-	27	-	11
Present study	2003	Germany	German Bight	802	6	24		

The total number of species in the component community was almost equal at all investigated sites. It is known that the invertebrate and parasitic fauna, in general, is reduced in the central Elbe estuary due to natural variations in habitat conditions (Möller 1990), but parasite species are occasionally introduced from marine as well as from limonitic environments into the estuary by invading hosts. This explains that 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 was 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 chapter 3). 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 numbers 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 caused by the high number of species that occurred at three, four or all sampling locations. The high similarity in parasite numbers, espe-

cially between the offshore sites, Outer Eider estuary and Helgoland, and between Spiekeroog and Inner Eider estuary was mainly due to similar numbers of parasite individuals of the dominating 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 specimen 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 host from Helgoland and 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 in mean with a maximum of 8 species, the lowest parasite number of flounder from the study area.

Studies, which investigate the parasite community of flounder on the infra-community level, are lacking from 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 numbers 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 from all flatfish species investigated flounder exhibited the richest parasite community. Thus it is not surprising that the species richness of dab specimen was lower than of flounder individuals 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 with respect to the residues of chlorinated hydrocarbons in the muscle and the liver tissue of flounder (Broeg et al. 1999) and with respect to the residues of heavy metals in sediments and blue mussel tissue (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 (chapter 3) and in the present study, the measurements of species richness (S) and diversity (H' and 1/D) allowed to establish these gradual differences even for shorter periods of three years in 1995-1997 and in 1997-2000. The Shannon- Wiener index of diversity (H') displayed these differences already, when data from the April campaigns of 1999 and 2000 were considered. These were the campaigns, which included samplings at the Inner Eider estuary location.

The index of evenness (E) was less suitable for a separation of the sampling sites. Especially in spring it was low at all stations. Then the values ranged between 0.41- 0.57. In autumn the values were significantly higher and then, flounder from Helgoland had significantly higher values of evenness compared to individuals from the Elbe estuary or from Spiekeroog. In helminth communities of fish, evenness was strongly influenced by presence or absence of a few, very abundant species (Poulin 1996). When these species were present in the community, they dominated over all other species by number and led to uneven communities. When the dominating 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 *Lernaeocera branchialis* was the predominating species of 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 and not in autumn. In spring, however, the numbers of *L. branchialis* individuals were increased, which most likely caused the reduced diversity and evenness indices compared to autumn, when lower numbers of *L. branchialis* individuals were found on the flounder (chapter 3). 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 in the range of natural variability.

Despite of seasonal and annual variations, a separation of the sampling locations was possible by Shannon- Wiener's Index (H') and inverse Simpson index of diversity (1/D) during both seasons. A separation of sampling sites by the calculation of H/M_{sp}- ratio, however, was not as possible. In autumn, flounder from the Elbe estuary exhibited lower values of H/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 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 (S), 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 (see chapter 3). Thus the values of the H/M_{sp}-ratio did not decrease at the Elbe estuary, the location to be considered challenge.

When the two estuarine locations, Elbe and Inner Eider estuary, were compared, differences were detected by species richness (S), the number of macroparasite individuals (N) and the number of heteroxenous species (H_{sp}). While both species related measurements, S and H_{sp}, showed gradual differences between Elbe < Inner Eider < Helgoland, individuals from the Inner Eider estuary harboured as many macroparasite individuals (N) as flounder from Helgoland. When the biology of the parasite taxa is considered (reviewed in chapter 3), which formed the community, 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 such as reduced or changing water salinities as 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 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 effect, a comparison with the residue analysis and with the responses of several established biomarker, which were additionally applied in the study, is presented in chapter 5.

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 between the sites under study. This was observed at the infra-community as well as at the component community level. Although the parasite community composition on the component community level was very similar at the sites, the analysis of exponential 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 Outer Eider. The number of component species was also lower at 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 summed, even gradual differences were observed between 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 both heteroxenous as well as monoxenous species were reduced in fish from the Elbe estuary, thus the ratio remained constant in most of the campaigns.

Chapter 5
Pollution effects on the parasite community and a comparison to biomarker responses

Abstract

In the frame of an integrated biological effect monitoring, the parasite community of flounder, *Platichthys flesus* (L.), was investigated at different locations in the German Bight from 1995-2000. In order to assess the impact of environmental contamination on the parasite community caused by anthropogenic activities, selected parasitological parameters that displayed significant differences between the sampling sites were subjected to correlation analyses with site-specific contamination and individual pollution load of their fish hosts. In addition, correlation analyses were conducted with the responses of selected genetic, biochemical, histopathological, physiological and immunological parameters of fish, used as potential biomarkers. In total, 802 flounder were analysed for chemical, biological and parasitological parameters. Information on the chemical background at the sampling sites was derived from sediment samples and from 120 blue mussel tissue samples (*Mytilus edulis*), collected at each of the sampling sites. Based on chemical data available from sediments and blue mussel, a pollution gradient was established between the sampling sites. This gradient was not reflected equally in the chemical burden of flounder muscle from the same sites. In contrast, the biological information obtained from some of the measurements in fish samples displayed a regional as well as a temporal pattern. Most of the parasitological parameters significantly reflected the established site-specific contamination gradient, when data were pooled over all sampling campaigns. Significant correlations were also found with the contamination level of individual flounder, including those parasite species that were not correlated to site-specific contamination. Several biomarkers were significantly correlated with the same contaminants in fish as the parasitological data and they were additionally correlated directly to these parasitological parameters. Results showed that the abundance of several metazoan parasite species, species richness and parasite diversity was reduced in contaminated habitats, and that differences between sites were not only due to natural factors, such as salinity, but also to pollution induced stress. The abundance of the protozoan taxa *Trichodina* spp., which is discussed as a potential indicator of organic contamination, were highest at the estuarine sites, where also pollution impact and contents of nutrients were highest. Short-term responses to new contamination events, found for some of the biomarkers, were not

reflected by the parasitological data, but long-term data of the parasitological parameters were most appropriate to give a general characterisation of the sampling sites. A general impoverishment in the parasite community of flounder in the German Bight could not be observed over a study period of six years.

Introduction

Field bioassessment studies should include a combination of rapidly responding sensitive biomarkers and the more ecologically relevant bioindicators (Adams 2002).

In general, biomarkers are used to indicate exposure of an organism to a stressor and help to identify the mechanistic basis of causal relationships between stressors and its effect. They are measured on the suborganism level, are sensitive to stressors and show a direct, often highly variable response to these stressors on a short time scale. Bioindicators respond on higher levels of biological organisation, like the population or community level. They are of high ecological significance, but due to the complexity of processes in population and community dynamics, provide little information for helping to understand the underlying causal mechanisms between stressors and effects. Their sensitivity to specific stressors is low and they tend to respond to effects of multiple stressors over a large spatial and temporal scale (Adams 2002). A combined use of biomarkers and bioindicators with a focus on the organism level could provide a link between mechanistic understanding and the ecological consequences, and increase the ability to interpret biological data (Adams 2002).

The use of fish parasites as indicators for marine pollution was widely and controversially discussed in the last years, because distribution and infection levels of parasites are not only influenced by environmental contaminants but also by a variety of natural factors (Möller 1986; Khan and Thulin 1991; MacKenzie et al. 1995; Kennedy 1997; Overstreet 1997). Therefore 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 biomarkers.

In the present study, environmental deterioration was assessed in the frame of an integrated biological effect-monitoring. Parasite community of flounder (*Platichthys flesus* L.) was investigated from different sites in the German Bight over a period of six years and proved as potential indicator of anthropogenic impact at the population and community level.

The basic assumptions are that biotic diversity is highest in undisturbed environments, whereas man-made stress, as pollution, leads to a loss of species, and to a reduction of diversity (Kennedy 1997).

In the present study, additionally, standard chemical parameters as well as a set of genetic, biochemical, physiological and immunological biomarkers, which are partly recommended as standard methods for general monitoring of the biological effects of contaminants by the ICES Working Group on Biological Effects of Contaminants (WGBEC) (1996, 2002), were sampled and evaluated at the suborganism level from the same individual fish, used for parasitological investigation. Parasitological data were compared to site- and host-specific residues analyses and to responses of biomarkers under study.

Material and Methods

Sampling

During spring and autumn of 1995-1997 and 1999-2000, 802 individuals of European flounder, *Platichthys flesus* (L.), were sampled by research vessel trawl catches at five different locations (Elbe estuary, Outer Eider estuary, Helgoland, Spiekeroog, Inner Eider estuary) in the German Bight, North Sea.

To avoid size or age effects, only individuals of a narrow size range (18-25 cm total length) were included in the analysis. Details of sampling, and examination of flounder as well as numbers of evaluated fish specimen for each sampling campaign at each location are described in chapter 3 and 4. A list of the parasite taxa recorded during the course of the study is given in chapter 2, information about their infection levels as well as parasite community characteristics at the sampling locations are presented in detail in chapters 3 and 4.

Parameters under study

In the present study, those parasitological parameters, which indicated differences between the sampling locations and thus were used for correlation analyses, are listed in table 1: Abundance of selected parasite species, according to the definitions provided by Bush et al. (1997), and community measurements as species richness (S), the number of heteroxenous species (H_{sp}), the number of macroparasite individuals (N), Shannon-Wiener Index of diversity (H') and inverse Simpson's Index of diversity (1/D). All measurements were calculated for individual fish, the indices were used according to Magurran (1988). For details see chapter 3 and 4.

Table 1: Summary of parasitological parameters used in correlation analysis with residues and biomarker responses. *= Abundance of single parasite taxa, according to Bush et al. (1997). Given are parameter abbreviations (Parameter Abb) and full name (Parameter Full)

Parameter Abb	Parameter Full
Trich*	*Trichodina* spp. (Ciliophora)
Acan*	*Acanthochondria cornuta* (Copepoda)
Lep*	*Lepeophtheirus pectoralis* (Copepoda)
Lern*	*Lernaeocera branchialis* (Copepoda)
Meta*	Metacercaria sp. 1 (Trematoda / Digenea)
Zoog*	*Zoogonoides viviparus* (Trematoda / Digenea)
Cuc*	*Cucullanus heterochrous* (Nematoda)
S	Species richness
H_{sp}	Number of heteroxenous species
N	Number of macroparasite individuals
H	Shannon-Wiener Index of diversity
1/D	Inverse Simpson Index of diversity

Table 2: Summary of biomarkers used for correlation analysis with parasitological data. The parameter abbreviation (Parameter Abb), the full name (Parameter Full), the unit and the responsible institute: AWI (Alfred Wegener Institut), TUB (Technische Universität Berlin), THH (Tierärztliche Hochschule Hannover)

Parameter Abb	Parameter Full	Unit	Origin
EROD	EROD activity (the activity of cytochrome P450 dependent monooxygenase Ethoxyresorufin-O-deethylase in flounder liver)	nmol min-1 mg-1 (protein)	AWI
MAA	Macrophage aggregate area (the mean size of macrophage aggregates in flounder liver)	µm²	AWI
MAM	Macrophage aggregate activity (the activity of acid phosphatase in macrophage aggregates in flounder liver)	mean absorbance	AWI
LY1	Lysosomal stability type 1 (the membrane stability of hepatocyte lysosomes (first group of lysosomes displaying an early membrane break down ≤ 10 min)	min	AWI
LY2	Lysosomal stability type 2 (the membrane stability of hepatocyte lysosomes (second group of lysosomes displaying a late membrane break down between 15-50 min)	min	AWI
ChE-f	Cholinesterase activity in flounder muscle	nmol min-1 mg-1 (protein)	TUB
ChE-Br	Cholinesterase activity in flounder brain	nmol min-1 mg-1 (protein)	TUB
DNA-f	DNA unwinding in flounder liver	-log F	TUB
Vitellogenin	Content in the blood plasma	µg/l	TUB
Pin	Pinocytosis activity (endocytic uptake of liquid droplets by head kidney phagocytes)	optical density	THH
ROS	Production of reactive oxygen species of head kidney leucocytes	optical density	THH
ROS-PMA	ROS, stimulated by phorbolmyristate acetate	optical density	THH
Lys	Lysozyme activity in the blood plasma	optical density	THH

Fish individuals that were used for the parasitological investigation, were also analysed for a set of genetic, biochemical, physiological, histopathological and immunological parameters. A list of these parameters is given in table 2. The results of these analyses, which were carried out by other working groups of the study, are presented in detail by Broeg (2002), Dizer et al. (submitted) and Skouras (2002). The raw data of these parameters were kindly provided by all working groups for the correlation analyses of the present study.
As some of the potential biomarkers were influenced by sex of flounder, as EROD and MAM, only adult immature male flounder, which show the clearest results for these parameters (Broeg et al. 1999), were used for all calculations, including biomarkers.

Residue analyses
For a characterization of the sampling sites by their chemical pollution burden, samples from sediment and blue mussel tissue (*Mytilus edulis*) were taken from Elbe estuary, Eider estuary and Helgoland "Tiefe Rinne", and analysed for its content of standard heavy metals and chlorinated hydrocarbons. Analyses were carried out by a chemical laboratory (Dr. Wietz, Dr. Eggert and Dr. Jörissen GmbH, Berlin). A detailed description of all parameters and the results of these analyses are given by Dizer et al. (submitted).
For residue analyses of individual flounder, muscles samples from 10 flounder per sampling site and campaign were analysed for the content of standard heavy metals and chlorinated hydrocarbons also by a commercial laboratory (Labor für Fischgesundheit, Professor Harz, Bremerhaven). The results of these analyses are given in detail by Broeg (2002).
The raw data of residues in sediments and mussel tissue were kindly provided for the correlation analyses of the present study.

Statistical analyses
All correlation analyses were done with pooled data from all sampling campaigns, when data were present. In order to test the relation between parasites and the site- specific residue burden of the sampling locations, correlation analyses were carried out between parasitological data and residues of sediments and blue mussel tissue. These calculations were based on mean values of the parasitological data of 9-30 flounder individuals, single measurements of sediment residues and mean values of residue concentration in 5-10 blue mussels per location and sampling campaign.
The relation between parasitological parameters and the individual contamination level of fish were conducted by correlation analyses between parasitological data and the residue burden

measured in muscle tissue of the same flounder. All residue measurements that were below detection limit were replaced by the 2/3 value of the detection limit, in order to include them into calculations.

The relation between short- term responses of biomarkers and the long-term effects on parasite community induced by contamination were conducted by correlation analyses of both groups, also based on individual fish.

All correlation analyses were carried out using Spearman Rank Correlation (STATISTICA 6.0, SoftStat). Comparison of groups were done by Kruskal-Wallis ANOVA and Dunn's post hoc test (SigmaStat® 2.0).

Results

Sediments and mussel residues

The results of the correlation analyses between parasitological data and residues in sediments and mussel tissue (*Mytilus edulis*) are presented in table 3. Most of the parasitological parameters were negatively correlated with residues, except of the abundance of *Trichodina* spp., which was positively correlated with residues. High correlation coefficients were found with heavy metals and polychlorinated biphenyls (PCBs) in sediments, and with cadmium, β-HCH, p.p.-DDD and ∑PCBs in mussel tissue. Correlations were significant between these residues and the parasitological parameters *Trichodina* spp., *Acanthochondria cornuta*, *Zoogonoides viviparus*, *Cucullanus heterochrous*, species richness (S), the number of heteroxenous species (H_{sp}), the number of macroparasite individuals (N), Shannon-Wiener Index of diversity (H') and inverse Simpson Index (1/D). The results indicate that the abundance of selected parasite species, the number of parasite species and individuals as well as parasite diversity decreased with increasing residue burden at the sampling sites, while the abundance of *Trichodina* spp. also increased.

All of the contaminants in mussel tissue that were correlated with the parasitological data, showed a clear regional pattern, when data were pooled over all sampling campaigns (figure 1). Concentration of β-HCH, p.p-DDE and p.p.-DDD were significantly higher in mussel from the Elbe estuary than from the other sites, while concentration of cadmium (Cd) and ∑PCBs additionally showed a contamination gradient in a decreasing order Elbe> Eider> Helgoland (figure 1).

Table 3: Correlation analyses between parasitological data of male and female flounder and residues in sediments and blue mussel tissue (*Mytilus edulis*). Given are correlation coefficients and the level of significance * p<0.05, ** p<0.01, *** p<0.001. Significant correlations are additionally marked in bold. Samples were taken [1]= only in the year 1996, [2]= in 1996, 1999 and 2000, [3]= only from 1999-2000, ∑ PCBs (PCB 28, 52, 101, 118, 138, 153, 180), [4]= from 1997-2000. For abbreviations see table 1.

Sediments

	n (samples)	Tricho	Acan	Lep	Lern	Meta	Zoog	Cuc	S	N	Hsp	H'	1/D
Mercury [1]	6	0.54	-0.60	-0.37	0.03	-0.09	**-0.94** **	**-0.94** **	**-0.89** *	-0.66	**-0.89** *	-0.77	-0.77
Cadmium [2]	14	0.38	-0.24	0.14	0.02	-0.06	**-0.70** **	-0.44	-0.40	-0.35	-0.39	-0.14	-0.06
Copper [2]	14	**0.54** *	**-0.63** *	-0.04	-0.26	-0.08	-0.49	**-0.74** **	**-0.74** **	**-0.69** *	**-0.75** **	**-0.56** *	-0.34
Lead [2]	14	0.39	**-0.58** *	-0.01	-0.21	-0.02	-0.34	**-0.64** *	**-0.68** **	**-0.57** *	**-0.67** **	-0.51	-0.36
Iron [1]	6	0.49	-0.54	-0.47	0.09	0.09	**-0.89** *	**-0.89** *	**-0.94** **	-0.60	**-0.94** **	**-0.83** *	**-0.83** *
Manganese [1]	6	0.54	-0.60	-0.37	0.03	-0.09	**-0.94** **	**-0.94** **	**-0.89** *	-0.66	**-0.89** *	-0.77	-0.77
Zinc [1]	6	0.49	-0.54	-0.49	0.09	0.09	**-0.89** *	**-0.89** *	**-0.94** **	-0.60	**-0.94** **	**-0.83** *	**-0.83** *
Nickel [1]	6	0.49	-0.54	-0.49	0.09	0.09	**-0.89** *	**-0.89** *	**-0.94** **	-0.60	**-0.94** **	**-0.83** *	**-0.83** *
Aluminium [1]	6	0.49	-0.54	-0.49	0.09	0.09	**-0.89** *	**-0.89** *	**-0.94** **	-0.60	**-0.94** **	**-0.83** *	**-0.83** *
Chromium [2]	14	0.36	-0.48	0.02	-0.19	0.01	-0.11	**-0.62** *	**-0.54** *	-0.46	**-0.57** *	-0.41	-0.40
PCB 138 [3]	8	0.31	**-0.83** *	-0.05	-0.24	-0.43	**-0.76** *	-0.40	**-0.80** *	-0.36	**-0.74** *	-0.64	-0.33
PCB 153 [3]	8	0.34	**-0.88** **	-0.12	-0.19	-0.36	**-0.79** *	-0.50	**-0.85** **	-0.31	**-0.79** *	**-0.74** *	-0.45
∑ PCBs [3]	8	0.12	**-0.71** *	0.00	-0.21	-0.43	-0.63	-0.38	**-0.78** *	-0.21	-0.62	-0.52	-0.38

Mussel

	n (samples)	Tricho	Acan	Lep	Lern	Meta	Zoog	Cuc	S	N	Hsp	H'	1/D
Cd [4]	18	**0.62** **	**-0.73** ***	-0.38	-0.25	-0.04	**-0.48** *	**-0.58** *	**-0.51** *	-0.40	-0.34	**-0.65** **	**-0.54** *
β-HCH [3]	12	**0.74** **	**-0.60** *	-0.22	-0.44	-0.26	**-0.59** *	-0.56	**-0.58** *	**-0.78** **	**-0.73** **	**-0.62** *	-0.47
p.p-DDE [3]	12	**0.63** *	-0.56	0.00	-0.55	-0.52	-0.51	-0.33	-0.51	**-0.77** **	**-0.64** *	-0.41	-0.19
p,p DDD [3]	12	**0.68** *	**-0.75** **	0.14	-0.34	-0.25	**-0.68** *	**-0.64** *	**-0.72** **	-0.49	**-0.76** **	**-0.59** *	-0.51
∑ PCBs [3]	12	**0.87** ***	**-0.88** ***	-0.33	-0.38	-0.38	-0.49	**-0.82** ***	**-0.74** **	**-0.68** *	**-0.80** ***	**-0.90** ***	**-0.78** **

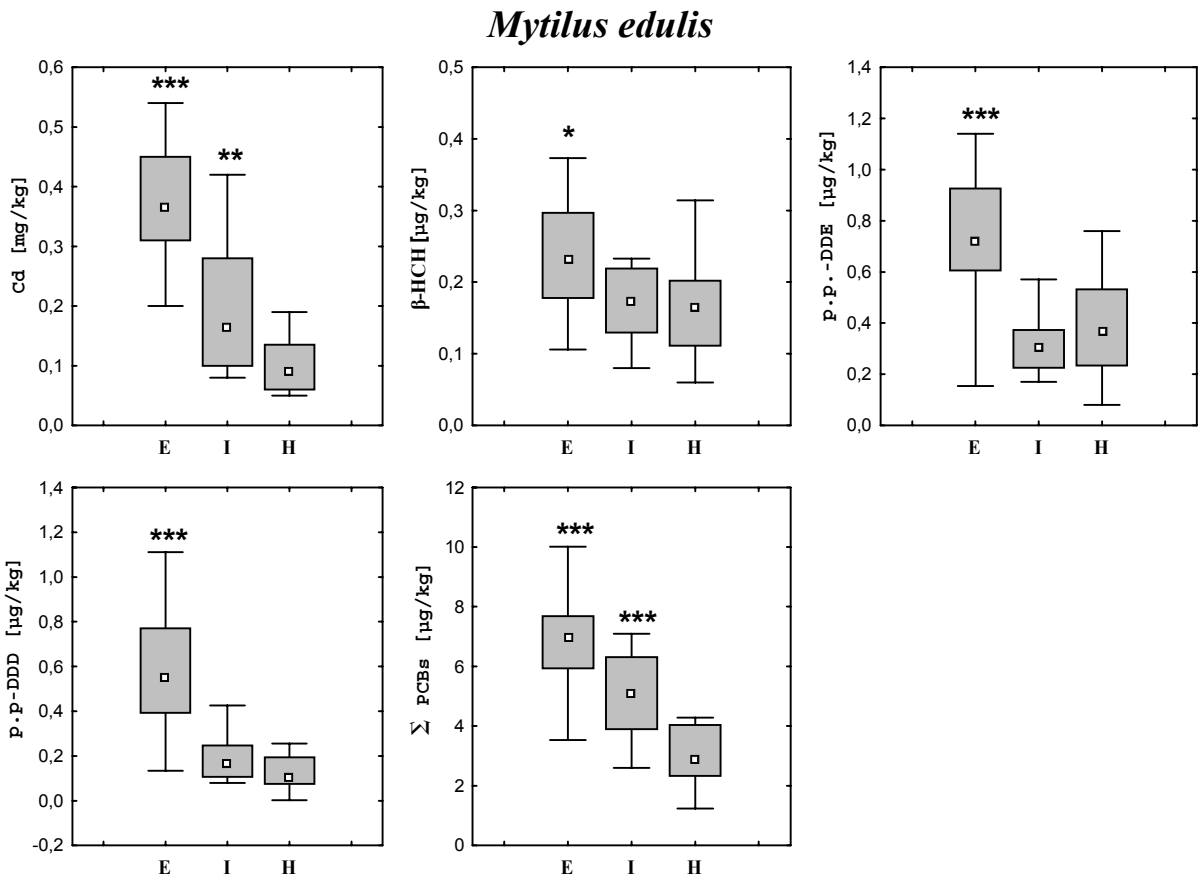


Figure 1: Concentration of selected contaminants in blue mussel tissue (*Mytilus edulis*) at three sampling sites in the German Bight in the years 1999-2000. Given are box plots with median values (□), 25 and 75 percentiles (box) and range without outliers (whisker). E= Elbe, I= Inner Eider, H= Helgoland. Stars above box plots indicate significant differences from that station to all other stations [n= 20 / location , * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$]

Flounder muscle

The results of the correlation analyses between parasitological data and residues in flounder muscle are given in table 4. As for sediment and mussel residues, most of the parasitological parameters were negatively correlated with flounder muscle residues, only the abundance of *Trichodina* spp. showed positive correlations. An exception was the relation with mercury, where *Trichodina* spp. showed a negative, the other parasitological parameters a positive correlation. This was in contrast to the results found for sediments, where most of these parameters were negatively correlated with mercury (table 3).

Table 4: **a)** Correlation analyses between parasitological data and residues in muscle of male and female flounder [n= 185] and **b)** between selected biomarkers and residues in male flounder muscle [n= 93] from the years 1999-2000. Given are correlation coefficients and the level of significance * p<0.05, ** p<0.01, *** p<0.001. Significant correlations are additionally marked in bold. ∑ PCBs (PCB 52, 101, 118, 138, 153, 180). For abbreviations see table 1 and 2.

a)	Tricho	Acan	Lep	Lern	Meta	Zoog	Cuc	S	N	Hsp	H'	1/D
Mercury	-0.01	**0.18** *	**0.23** **	**0.30** ***	0.16	0.06	**0.18** *	**0.27** **	**0.28** ***	0.15	0.11	0.05
HCB	**0.26** ***	**-0.49** ***	**-0.37** ***	**-0.43** ***	**-0.21** **	**-0.17** *	**-0.27** ***	**-0.29** ***	**-0.50** ***	**-0.22** **	**-0.30** ***	**-0.21** **
p.p-DDE	0.13	**-0.29** ***	**-0.22** **	**-0.21** **	**-0.24** **	-0.11	**-0.18** *	**-0.17** *	**-0.34** ***	**-0.17** *	**-0.21** **	**-0.15** *
p.p-DDD	**0.17** *	**-0.37** ***	**-0.19** **	**-0.37** ***	**-0.26** ***	**-0.19** *	**-0.21** **	**-0.22** **	**-0.47** ***	**-0.20** **	**-0.16** *	-0.08
OCS	0.07	**-0.33** ***	-0.09	**-0.15** *	-0.07	-0.13	**-0.21** **	**-0.26** ***	**-0.20** **	**-0.18** *	**-0.21** **	**-0.17** *
∑ PCBs	0.10	**-0.21** **	**-0.18** *	**-0.22** **	**-0.17** *	0.04	-0.04	-0.03	**-0.29** ***	-0.08	-0.09	-0.05

b)	MAA	MAM	LY1	LY2	EROD	ChE-f	DNA-f	Vitellogenin	Pin	ROS	ROS-PMA	Lys
Mercury	-0.11	0.15	**0.21** *	**0.28** **	-0.10	-0.04	**-0.21** *	-0.15	0.06	-0.11	0.10	-0.04
HCB	-0.002	-0.16	**-0.27** **	**-0.58** ***	**0.52** ***	-0.08	0.04	-0.04	0.09	-0.17	-0.14	-0.20
β - HCH	-0.15	**-0.26** *	-0.07	**-0.27** **	**0.22** *	-0.10	0.20	0.06	0.07	-0.14	-0.12	**-0.22** *
p.p-DDE	-0.03	-0.04	-0.002	**-0.35** ***	**0.47** ***	0.11	-0.19	-0.12	**0.26** *	**-0.26** *	-0.15	-0.12
p.p-DDD	-0.10	-0.11	-0.16	**-0.44** ***	**0.38** ***	0.01	0.11	0.09	0.18	-0.14	-0.13	-0.08
OCS	-0.18	-0.20	-0.09	**-0.43** ***	**0.29** **	-0.16	0.05	0.02	0.10	-0.17	-0. 05	-0.20
∑ PCBs	-0.11	0.15	0.14	**-0.23** *	**0.37** ***	**0.23** *	**-0.35** ***	**-0.24** *	**0.32** **	-0.20	0.09	-0.04

In general, correlation coefficients were much lower, than observed in correlation analyses with site-specific contamination. Lowest correlation coefficients were found for *Trichodina* spp. and the digenean *Z. viviparus*. Highest significant coefficients for the majority of parasitological parameters were found with the content of mercury (Hg), HCB, p.p.-DDE, p.p.-DDD and OCS, especially for copepods and the number of macroparasite individuals (N) (table 4a). While in sediment and mussel analyses, no significant correlations were found with *L. pectoralis*, *L. branchialis* and the undetermined metacercaria, in the analyses with flounder muscle these correlations reached highest significances of all. In contrast, the content of PCBs, which was clearly correlated with parasitological data in the analyses of mussel tissue, was not as relevant when individual fish were considered. Although the copepods were significantly correlated with PCB loads, most of the community data, such as species richness (S), the number of heteroxenous species (H_{sp}), Shannon-Wiener's Index of Diversity (H') and inverse Simpson Index (1/D) were not.

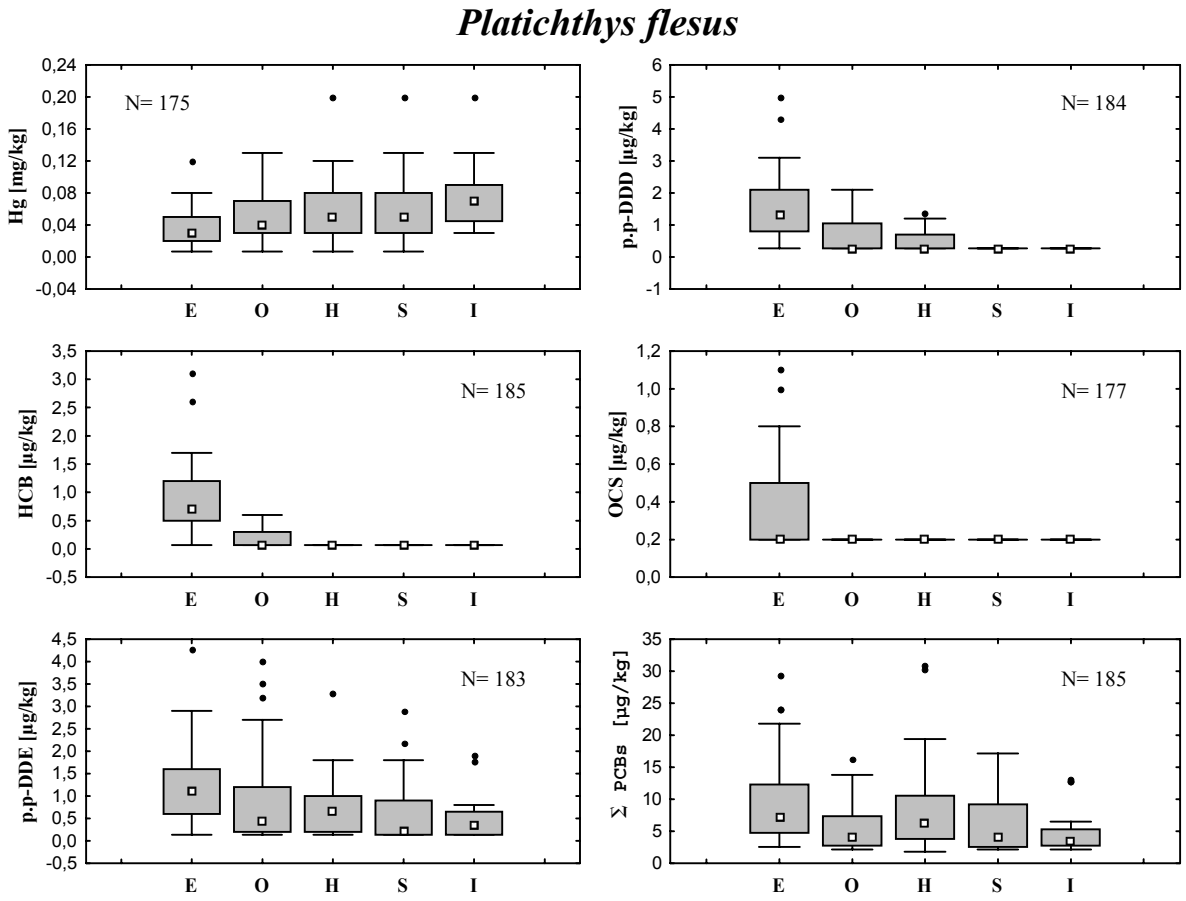


Figure 2: Concentration of selected contaminants in muscle of flounder from five locations in the German Bight in the years 1999-2000. Given are box plots with median values (□), 25 and 75 percentiles (box), range without outliers (whisker) and outliers (•). E= Elbe, O= Outer Eider, H= Helgoland, S= Spiekeroog, I= Inner Eider [n =185]

Most of the contaminants that were correlated with the parasitological data, showed a slight regional trend between the sampling locations, when data were pooled over all sampling campaigns (figure 2). Flounder inhabiting the Elbe estuary presented higher concentrations of HCB, OCS, p.p-DDE and p.p.-DDD than fish from the other sites, whereas mercury (Hg) concentration was lowest in the Elbe estuary. For concentration of PCB, no clear pattern was observed.

Biomarkers

From all biomarkers under study only few were correlated with a greater number of parasitological parameters. The results of these analyses are given in table 5. Best significant correlations were found with EROD, MAM, LY2, ChE-Br and Lys. While correlations with EROD and ChE-Br were mainly negative, correlations with MAM, LY2 and Lys were positive, except of the abundance of *Trichodina* spp., which always exhibited an opposite pattern. Thus decreasing values in parasitological data and increasing abundances of *Trichodina* spp. were related to decreasing values of MAM, LY2 and Lys and increasing values of EROD and ChE-Br. No significant correlations were found with MAA and ChE-f.

For EROD, MAM and LY2, a clear regional pattern was evident, when data were pooled over all sampling campaigns from 1995-2000 (figure 3). EROD was significantly higher in fish from the Elbe estuary than from all other sites, while MAM and LY2 were significantly reduced in fish from Elbe estuary ($p < 0.001$). No significant differences were found for ChE-Br, ChE-f and DNA-F. When only data from 1999-2000 were considered, Lys was significantly lower in fish from the Elbe, Outer and Inner Eider estuary than in fish from Helgoland ($p < 0.001$). In contrast to several parasitological parameters, no gradual differences were observed between the sites for any of the biomarkers (see chapter 3 and 4).

In correlation analyses with residues in flounder muscle, biomarkers such as EROD, MAM, LY2, ChE-f and Lys, which showed highest correlations with the parasitological parameters, were also correlated with the same chemicals as the parasitological data (table 4). Correlation coefficients were higher for biomarkers than for parasites, despite the lower number of fish sampled, but a high coincidence was found between parasites, EROD and LY2. These two biomarkers were correlated to nearly all contaminants with highest correlation coefficients, while the others were only related to selected xenobiotics.

Table 5: Correlation analyses between parasitological data and biomarker responses of male flounder. Given are correlation coefficients and the level of significance * p<0.05, ** p<0.01, *** p<0.001. Significant correlations are additionally marked in bold. The majority of parameters were analysed from 1995-2000, parameters marked with: [1] were taken from 1995-1997, [2] were taken from 1999-2000. For abbreviations see table 1 and 2.

Biomarker													
	n (fish)	Tricho	Acan	Lep	Lern	Meta	Zoog	Cuc	S	N	Hsp	H'	1/D
EROD	441	**0.16** ***	**-0.34** ***	**-0.27** ***	-0.08	**-0.19** ***	**-0.10** *	**-0.10** *	**-0.22** ***	**-0.20** ***	**-0.22** ***	**-0.33** ***	**-0.28** ***
MAM	445	**-0.12** *	**0.23** ***	0.06	**0.24** ***	0.03	**0.18** ***	**0.15** **	**0.24** ***	**0.22** ***	**0.25** ***	**0.17** ***	**0.13** **
MAA	445	-0.06	0.04	0.04	-0.03	-0.05	0.06	0.04	0.03	-0.01	0.07	0.08	0.07
Ly1	444	**-0.19** ***	**0.20** ***	0.08	0.04	0.08	**0.21** ***	0.07	**0.11** *	**0.18** ***	0.06	**0.11** *	0.09
Ly2	444	**-0.21** ***	**0.29** ***	**0.16** ***	**0.13** **	**0.16** ***	**0.12** **	**0.10** *	**0.15** **	**0.22** ***	**0.09** *	**0.18** ***	**0.15** **
ChE-Br [1]	138	0.04	**-0.17** *	**-0.33** ***	**0.25** **	**-0.21** *	0.13	0.03	-0.14	0.14	-0.06	**-0.28** ***	**-0.29** ***
ChE-f	262	0.01	0.12	0.03	0.09	0.03	-0.15	0.04	0.05	0.02	0.02	-0.02	-0.01
DNA-f	262	0.06	-0.12	0.06	0.04	-0.06	**-0.23** ***	**-0.13** *	**-0.19** **	-0.05	-0.10	-0.06	-0.03
Vitellogenin [2]	162	0.01	-0.004	**0.23** **	0.07	-0.14	-0.03	0.003	-0.01	0.002	-0.04	0.03	0.2
Pin [2]	158	0.13	**0.16** *	0.01	0.02	**0.19** *	-0.07	0.06	**0.22** **	-0.01	0.10	0.15	0.14
ROS [2]	165	**-0.16** *	0.10	0.07	-0.01	**-0.19** *	0.11	0.09	-0.07	0.003	-0.07	0.12	0.11
ROS-PMA [2]	165	-0.10	**0.18** *	0.05	0.15	-0.02	**0.20** *	0.12	0.03	**0.18** *	-0.05	0.04	0.02
Lys [2]	181	-0.12	**0.30** ***	**0.34** ***	**0.18** *	-0.13	**0.32** ***	0.08	**0.17** *	**0.24** ***	0.13	**0.21** **	**0.22** **

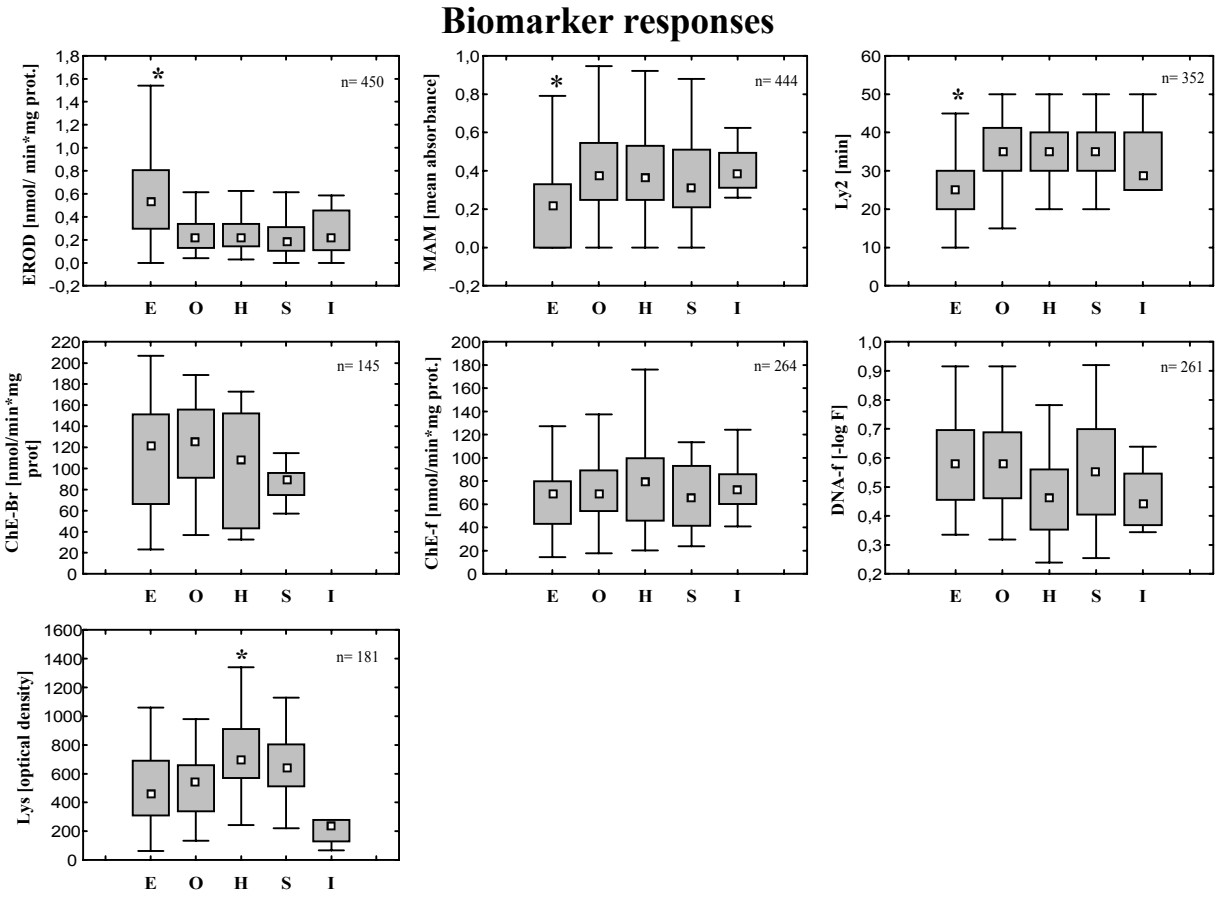


Figure 3: Selected biomarker responses of flounder at five locations in the German Bight in the years 1995-2000. ChE-Br were analysed from 1995-1997 and Lys from 1999-2000 only. Given are box plots with median values (□), 25 and 75 percentiles (box), 10 and 90% percentiles (whiskers). E= Elbe, O= Outer Eider, H= Helgoland, S= Spiekeroog, I= Inner Eider. Stars (*) above box plots of EROD, MAM and LY2 indicate significant differences from that station to Outer Eider, Helgoland and Spiekeroog, and above box plots of Lys from that station to Elbe, Outer Eider and Inner Eider [$p < 0.001$]. Number of fish used for calculations are indicated on the right side of each graph. For abbreviations see table 2.

Discussion

An indispensable prerequisite for biological effect monitoring, which aims at a testing of the effects of pollution, is the proper selection of sampling sites. They should be situated in the same ecological area but differ in their pollution level.

The German Bight, which was taken as an ecological area in the present study, is mainly influenced by the river Elbe, which is the main freshwater input into this area with about 43% of the total freshwater input into the international Wadden Sea. It also holds a key position for the southern North Sea as a pollution source (de Jong et al. 1999). In comparison, the river Eider is only a relatively small freshwater source and moderately polluted. While the Elbe is mainly

affected by industrial pollution like heavy metals, chlorinated hydrocarbons and nutrients, the anthropogenic influence at the Eider estuary is more due to agricultural activities (Schmolke et al. 1999).

Anyway, a strong reduction of the heavy metals influx via freshwater sources from 1980 to 1985 led to an overall reduction of the heavy metal contamination in the Wadden Sea, converging to the natural background of most metals (Anders et al. 1996, de Jong et al.1999). From 1985 to 1996 also the PCB concentration decreased between 45% and 85% (Anders et al. 1996, de Jong et al.1999). Thus, compared to other polluted locations in the North Sea, contamination levels in the German Bight are low (Herut et al. 1999).

Although a great number of chemicals ranged close to or were below the detection limit at all sampling sites (Dizer et al. submitted), a regional pattern could be observed for some "standard" contaminants. Schmolke et al. (1999) confirmed a decreasing contamination gradient for heavy metals in sediments and mussel tissue in the order Elbe> Eider> Helgoland for the years 1996 and 1997. The same gradient was also described for the PCB- content of bird eggs in the Wadden Sea (de Jong et al. 1999). In 1999-2000, this gradient was not as evident in sediments (Dizer et al., submitted), although sediments from Helgoland were less contaminated than those from Elbe or Outer Eider. In contrast, in mussel tissue, a gradient with Elbe> Eider> Helgoland could be confirmed for the content of cadmium (Cd) and PCBs, while concentrations of the remaining contaminants that were correlated with the parasitological data, were significantly higher in the Elbe estuary than at the other stations, when data were pooled over all sampling campaigns of 1999-2000 (figure 1).

All parasitological parameters that were correlated with residue data of sediment and mussel tissue, exhibited gradual differences between the sampling locations in the order Elbe< Eider < Helgoland, *Trichodina* spp. in the opposite order, when data were pooled over all sampling campaigns (chapter 3 and 4). These results reflected very well the contamination gradient found in sediment and mussel tissue, indicating that abundances of selected parasite species, species richness and diversity decreased, while abundances of *Trichodina* spp. increased with increasing contamination levels. These findings were well in line with the working hypothesis of the study.

Whether changes in the distribution of parasites are more related to natural or anthropogenic factors, is one of the most important questions in the use of parasites in environmental pollution monitoring (Möller 1986; Kennedy 1997; Overstreet 1997). Salinity is known as a major natural factor, influencing the distribution pattern of parasites between estuarine and offshore sites (Gibson 1972; Möller 1978; Wichowski 1990; MacKenzie 1995). In the present study, the

distribution of several marine parasite species was affected by salinity, which led to differences in parasite abundances between flounder from the Elbe estuary and from the coastal or offshore sites (see chapter 3). Salinity effects, however, could not explain the gradual differences in parasite abundances on flounder from Elbe estuary, Outer Eider estuary and Helgoland, and it could not explain the differences between the estuarine sites, Elbe and Inner Eider, with comparable salinity characteristics. Residue analysis data from sediments and from mussel tissue, collected during the present sampling program (Schmolke et al. 1999; Dizer et al. submitted) and during other national monitoring programs in the German Bight (de Jong et al. 1999) showed more correlations to the parasitological data than salinity data to the parasitological findings. This suggests that in addition to salinity the distribution pattern of parasites in the German Bight is affected by regional contamination levels.
Further laboratory studies on parasite reactions to water bodies with different contamination levels as well as to tide-dependent, regular changes of water salinity could help to determine the impact of salinity or pollution on parasite distribution.

Trichodina spp. is discussed as a potential biomarker for effects of organic contamination. Recent studies suggest that the presence and the intensity of trichodinids on fishes from a particular site depends on the content of small algae, organic matter and bacterial biomass available in the aquatic environment at the site under consideration (Lom 1995; Yeomans et al. 1997; Palm and Dobberstein 1999; Broeg et al. 1999; Madsen et al. 2000). In the present study, the content of organic matter in the water column and in sediment was not measured, but from national monitoring programs (de Jong et al. 1999) it can be concluded that the nutrient concentration in the Elbe and Eider estuary has decreased from 1985 to 1996, but still remains about 2-10 times above background level. As primary production is high in nutrient rich environments, decomposition processes by bacteria are high as well. Both estuaries therefore seem to provide excellent feeding conditions for trichodinids. High prevalences and infection levels of *Trichodina* spp. in both estuaries (chapter 3) as well as significant correlations with site-specific pollution levels support the use of trichodinids species as indicators of organic pollution.

A regional contamination gradient was not as evident when residues in flounder muscle were considered (Kress et al. 1999; Schmolke et al. 1999; Broeg, pers. comm.). At all sampling sites flounder individuals with elevated contamination levels were found together with individuals, which harboured low residue concentrations. Schmolke et al. (1999) concluded that the regional

effect, which was described for sediments and mussel tissue, might have been blurred in fish tissue by the migratory activity of flounder from the Elbe into deeper areas of the German Bight. Nevertheless, when pooled contamination data from 1999-2000 were considered, a regional trend was noticed, in a way that at the site in the Elbe estuary more flounder individuals with elevated concentrations of specific contaminants were collected than at the other locations.
The present investigation indicates that the contamination load of an individual fish also had an impact on the parasite community of this particular individual. Muscle residues of contaminants were correlated to all of the parasitological parameters, but these correlations were in general not as prominent as found for the site-specific pollution. In contrast, highest correlation coefficients were found between muscle residues and infection characteristics of ectoparasitic copepods, including two species, which did not show correlations with site-specific contamination levels and which in their infection characteristics did not exhibit gradual differences between the sites. This suggested an additional pollution source, affecting the distribution of parasites in the German Bight. This assumption was supported by the responses of several biomarkers that were correlated with the same contaminants as the parasitological data (table 4). The most corresponding results were found with EROD and LY2, which were correlated with all of these contaminants and which were already recommended as potential methods for general monitoring of the biological effects of contaminants by the ICES Working Group on Biological Effects of Contaminants (WGBEC) (1996, 2002). These biomarkers as well as MAM, Lys and DNA-Br were also correlated with most of the parasitological data.

In the following, the link between biomarkers, which reflect the health status of the test organism (flounder) and parasitological data, which principally reflect habitat conditions, is discussed.
The induction of the cytochrome P 450 dependent monooxygenase EROD is known as a marker of the exposure to lipophilic compounds like polycyclic aromatic hydrocarbons (PAHs), dioxins and coplanar, polychlorinated biphenyl (PCBs) congeners. The integrity of lysosomal membranes in hepatocytes (LY) indicates non-specific, acute and chronic toxic effects. A long time (min.) needed to destabilize the membrane, represents a high membrane integrity and good health of the cell. The stability of lysosomal membranes turned out to represented the most integrative parameter, able to reflect sensibly and in a linear matter, pollution induced alterations in affected fish. (Broeg et al. 1999, 2002).
Other parameters were additionally tested during the present monitoring program and discussed as suitable methods in pollution monitoring, as the activity of acid phosphatase in macrophage aggregates (MAM) and lysozyme level (Lys) as indicators of non-specific, immuno-modulating

chemicals (Broeg et al. 1999, 2002; Skouras 2002) as well as the inhibition of cholinesterase activity in brain (ChE-Br) as a diagnostic tool for the effects of acute neurotoxic organophosphorus and carbamate pesticides (Sturm 1999; Bresler 1999; Dizer et al. submitted) and the increase in DNA unwinding in flounder muscle as an indicator of non-specific, primary DNA damage caused by neurotoxic agents (Bresler 1999; Dizer et al. submitted).

In the present study, EROD activity was highest, stability of lysosomal membranes (LY) and the activity of macrophage aggregates (MAM) lowest in fish from the most polluted Elbe estuary, in most of the sampling campaigns (Broeg et al. 1999, 2002, submitted). In addition to this regional pattern, a temporal pattern was also evident for EROD and LY2. Effects of new pollution events (PCBs, DDT, OCS) that occurred twice during the course of the study, were not only restricted to flounder from the River Elbe but also observed in those individuals inhabiting formerly less pollute areas, after contaminants spread further to the stations Eider estuary and Helgoland (Broeg et al. 1999, 2002).

Activity of macrophage aggregates (MAM) as well as lysozyme levels (Lys) in general can be elevated or reduced under pollution stress, depending on exposure to different stressors as well as to acute or chronic effects. In the present study, lowest MAM activity and lysozyme levels were found in fish from the most polluted site, Elbe estuary, demonstrating a negative influence of a mixture of pollutants on lysozyme level in fish in the field (Broeg et al. 1999, 2002; Skouras 2002).

Short-term effects to specific contaminants, especially expressed by the responses of EROD and LY2 did not correspond to changes in parasite community data. Parasitological data varied between sampling campaigns and sites without demonstrating a clear temporal pattern (chapter 3 and 4). Due to the complexity of processes influencing parasite population and community dynamics, changes in community data are not appropriate to reflect such short-term effects of specific contaminants. Parasitological data were most appropriate to reflect the general contamination gradient of the sampling sites under study in the German Bight at a long-term scale and thus of a general health status of the aquatic environment.

Long-term effects on parasite communities related to disturbed habitat conditions were described by several authors. Studies on parasite populations of *Pseudopleuronectes americanus* in the Atlantic (Burn 1980), *Platichthys flesus* from the Baltic Sea (Sulgostowska 1988) and from various fish, crustacean and molluscan hosts from the Baltic Sea (Kesting and Zander 2000) over decades have shown that species diversity decreased as a consequence of the deteriorating environment due to urban and industrial pollution. The same results were found in long-term

studies on parasite communities of *Siganus rivulatus* from the Mediterranean Sea, as well as of *Liza ramada* and *L. aurata* from the Red Sea (Diamant et al. 2001, Dzikowski et al. 2001). In the present study, which lasted only six years, a continuous decrease in parasite richness and diversity could not be observed. Despite seasonal and annual fluctuation that were found at all sampling sites in the abundance of single parasite species as well as in community data, species diversity remained similar at each of the locations under study, but differed between the sites (chapter 3 and 4). In contrast to the sampling areas described in the studies cited above, overall contamination in the German Bight decreased over the last 15 years. Unfortunately a direct comparison of results of the present study with those of an earlier one from the same area is not possible, because there is no study available, which deals with the parasite community of flounder in the German Bight in earlier decades. Anyway, it seemed that currently, the effects of contamination in the German Bight, which was relatively low compared to other polluted sites in the North Sea, did not cause a strong alteration of the parasite community of flounder. Parasites were regularly introduced from the less polluted sites to the more polluted coastal areas. A lasting effect on the parasite community was mainly observed in the dispersion pattern of parasites in the German Bight, indicating even small differences in the habitat conditions between some of the sampling sites, when long-term data were considered.

Correlations between parasitological data and selected biomarkers, in principal, most likely were related to the regional pattern exhibited by the biomarkers in the "long-term" data, which corresponded partly to that of the parasitological parameters. Especially EROD showed significantly higher, LY and MAM significantly lower values in fish from Elbe estuary than from the other sites, Lys highest values in fish from Helgoland (figure 3). Cholinesterase activity (ChE-f) and DNA unwinding (DNA-f) in flounder muscle either did not exhibited a regional pattern in the pooled data, and were not significantly correlated with the parasitological data or showed only few, not very strong correlations respectively. An exception was the activity of cholinesterase in flounder brain (ChE-Br). For this parameter, no regional trend was observed, but correlations were significant to several parasitological data (table 5). Anyway, these correlations mostly were negative, indicating an opposite response to that one expected by the working hypothesis.

In a multivariate statistical approach to the use of the different bioindicators for the years 1995-1997, Schmolke et al. (1999) formed 2 groups of impaired and unimpaired flounder individuals by a cluster analysis. While both clusters could be separated significantly ($p < 0.001$) by

parameters as EROD, LY2, MAA, MAM and ChE, this was not possible by applying the parasitological data of species richness (S) (p= 0.286).

For an adequate interpretation of these results it has to be considered that biomarkers are characterized by responses to changes in the health status of individual fish, which happen on a relatively short time scale. In contrast, parasites show long- term responses on the population and community level to degradation of the ecosystem. Thus both groups of parameters, biomarkers and parasites, probably do not actively affect each other, but are affected by the same environmental conditions. The approach by Schmolke (1999) was based only on the data of individual flounder, thus parasites were not the appropriate measurement for the indication of differences in the health status of fish.

As the biomarkers EROD, LY and MAM presented acute as well as chronic responses to contamination in the present study, indicating Elbe estuary as the main source of pollution in the German Bight, they could serve as a link between short-term effects of pollution on environmental health and long-term effects, which were reflected by the parasitological findings.

Conclusions

The present study showed that the use of parasite communities in an integrated biological effect monitoring in marine and coastal waters is a valuable tool for the assessment of ecological consequences of environmental deterioration on the population and community level. Corresponding to the working hypothesis, abundance of selected parasite species, species richness as well as parasite diversity of flounder in the German Bight was reduced with increasing levels of site-specific pollution and individual contamination of the host. The abundance of the protozoan taxa *Trichodina* spp., which is discussed as an indicator of organic pollution, was elevated under such conditions. Acute and chronic responses of selected biochemical, histopathological, physiological and immunological biomarkers confirm the impact of pollutants on the fish host and establish a link between mechanistic processes on the sub-cellular level of individual fish and the ecological significance of pollution on the population and community levels of biological organisation, represented by dynamics in the parasite community.

Chapter 6

General conclusions

In the present study, parasites of flounder, *Platichthys flesus* (L.), from different locations in the German Bight, North Sea, representing a contamination gradient, were investigated for its potential use as bioindicators in environmental health monitoring, considering natural as well as anthropogenic influences on the parasite community.

Different approaches were undertaken to evaluate information provided by the parasite fauna of flounder: a) the use of potential indicator species, b) the use of community data such as species richness and species diversity indices and c) an integrated approach which in addition to an assessement of the parasite fauna included chemical data of sediment and biota, and responses of established biomarkers in flounder, which were already recommended by the ICES working group (1996, 2002).

In recent national and international monitoring programs the integration of information on biological communities has been restricted to the planktonic as well as to the bentic organisms, while information obtained by parasite communities has not yet been included (ICES 2002). Kennedy (1997), who compared the suitability of bentic and parasitic communities as pollution indicators, concluded that the use of parasites apparently did not show any advantage over the use of bentic organisms, which already had been established as bioindicators. The present study is one of the first attempts to use parasites in addition to biological effects- monitoring as potential bioindicators on the ecosystem level.

The main advantage of this integrated approach is that all information is obtained from the same fish individual; chemical residue analysis in fish mussel, biomarker responses to pollution exposure in fish on the suborganismal level and pollution effects on the parasitic fauna of fish on the population and community level. The sampling of large numbers of fish individuals or of an additional group of planctonic or bentic organisms appears to be unnecessary, when fish parasites are used for an evaluation of changes in ecosystem integrity. The presence of parasite species, especially of those that need one or more intermediate hosts to complete their complex life cycles, provide extensive information about the invertebrate fauna of the environment, assumed that life cycles of the parasites are fully understood.

This approach also allows a direct comparison of short-term responses of biomarkers to pollutant exposure, which may act as early warning indicators, and long-term effects of xenobiotics in the environment, reflected by changes in the parasite community.

The ICES working group of biological effects monitoring (2002) named several criteria, which potential indicators of biological effects should fulfil for their use in environmental monitoring programs (ICES 2002). The indicator should

- be well defined by a protocol and should have a clear endpoint,
- show a clear dose response,
- be easy to understood by the public and mechanistically,
- have an early warning potential and/ or ecological relevance,
- be regularly and easy to sample,
- be rapid and cost effective,
- be statistically robust for trend monitoring,
- be applicable to either national and international environmental issues

Fish parasites fulfil most of these criteria. Sampling of parasites is generally easy and cost effective, especially when parasites are collected from the same fish individuals, which are used for other biomarker studies as well. Information provided by the study of parasites is statistically robust for trend monitoring and is of high ecological relevance, when long-term data are considered. Nevertheless, processing of parasites is time- consuming and a specialist is needed for the classification of species as well as for the interpretation of results, which require profound biological and ecological comprehension. Also a clear dose response to pollution cannot be expected, because interactions at the population and ecosystem level are complex. Thus, it has to be taken into account that bioindicators, indicating changes on the ecosystem level, cannot be evaluated exactly by the same criteria as biomarkers that respond at the suborganismal level. The higher the level of biological organisation, the higher the complexity of interaction between causes and responses. Despite these "disadvantages" in the use of parasites, they provide information about long- term changes at the ecosystem level, such as changes in species composition or species diversity in a community of organisms over a period of years. These information are not obtained by a separate consideration of biomarker responses alone.

The question whether the use of selected indicator species or the use of community data such as species richness and species diversity indices were more suitable in environmental monitoring programs will be considered as a last aspect. Both indicator species and community data showed advantages as potential bioindicators during the present study. Indicator species might show very specific responses to habitat conditions like, for instance, the ciliophoran taxa *Trichodina* spp. It reached highest infection levels in habitats with high organic pollution. Community data were

found to be less sensitive to biological characteristics of a particular parasite species, such as the susceptibility to changes in water salinity or seasonal fluctuations, and were best suitable to give a general characterization of different locations affected by xenobiotic pollution.

Extensive information on the parasite community of flounder from numerous parts in Northern Europe is available for the last decades and the knowledge on the parasitic fauna of flounder has been continuously increased during the last years based on several studies on the laboratory as well as in the field. As flounder also is used as a sentinel species in monitoring programs in the Baltic Sea (ICES 2002), routine monitoring of the parasite community of flounder in future monitoring studies would provide valuable information on long-term changes on the ecosystem level in marine and coastal habitats of different geographical areas of Northern Europe.

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Zusammenfassung

Der Einsatz von Fischparasiten als potentielle Bioindikatoren für Schadstoffbelastungen in aquatischen Lebensräumen hat in den letzten 20 Jahren stark an Bedeutung gewonnen. In der vorliegenden Studie wurde im Rahmen eines integrierten, biologischen Effekt- Monitorrings die Eignung der Parasitengemeinschaft der Flunder *Platichthys flesus* (L.) aus der Deutschen Bucht als Indikator für die Schadstoffbelastung küstennaher Gewässer untersucht. Die gewählten Stationen Elbmündung, Eidermündung (innerhalb und außerhalb des Sperrwerkes), Küste vor Spiekeroog und die „Tiefe Rinne“ vor Helgoland, unterschieden sich in ihren hydrographischen Bedingungen und wiesen einen Belastungsgradienten auf. Die Parasitenfauna wurde auf verschiedenen Organisationsebenen unter Verwendung etablierter ökologischer Konzepte untersucht; auf der Populationsebene wurden Infektionscharakteristika einzelner Parasitenarten in Hinblick auf ihre Eignung als Indikatorarten bestimmt, auf der Gemeinschaftsebene Artenreichtum und Artendiversität berechnet. Zusätzlich wurden natürliche Faktoren wie biologische und ökologische Ansprüche der Parasiten und ihrer Zwischenwirte, Salzgehalt des Wassers sowie jahreszeitliche und anuelle Schwankungen in die Auswertung der Ergebnisse einbezogen. Der Einfluss anthropogener Faktoren auf die Parasitenfauna der Flunder wurde in einem integrierten Ansatz untersucht, in dem ausgewählte parasitologische Parameter mit der chemischen Belastung von Sedimenten und Lebewesen sowie den Reaktionen etablierter, von ICES empfohlener Biomarker auf Schadstoffexposition korreliert wurden. Von 1995-2000 wurden 1073 Flundern untersucht und insgesamt 30 verschiedene Parasitentaxa aus den Gruppen Apicomplexa, Ciliophora, Microsporea, Myxozoa, Digenea, Monogenea, Cestoda, Nematoda, Acanthocephala und Copepoda identifiziert. Nur 7 Arten konnten als potentielle Indikatorarten berücksichtigt werden. Entsprechend der Arbeithypothese unterschieden sich die Infektionsstärken all dieser Arten signifikant zwischen Flundern aus der Elbe, der am stärksten belasteten Station und solchen aus den weniger verschmutzen marinen und küstennahen Stationen, Helgoland, Außeneider und Spiekeroog. Im Langzeitdatensatz einiger dieser Arten fanden sich zudem graduelle Unterschiede zwischen den Stationen. Artenanzahl und Diversität der Parasitengemeinschaft waren in Elbfischen ebenfalls signifikant niedriger als in Fischen anderer Stationen, graduelle Unterschiede zwischen den Stationen wurden im Langzeitdatensatz ebenfalls beobachtet. Trotz der Abhängigkeit der meisten potentiellen Indikatorarten von der Salinität, dem wichtigsten natürlichen Einflussfaktor auf die Verbreitung von Parasiten und ihrer Zwischenwirte in Küstengewässern, fanden sich zwischen Orten gleichen Salzgehaltes, die aber Unterschiede im Belastungsgrad aufwiesen, ebenfalls signifikante Unterschiede sowohl in den Infektionsstärken dieser Arten als auch in den Werten zahlreicher Gemeinschaftsparameter. Jahreszeitliche Schwankungen waren sowohl in den Kurzzeit- als auch in den Langzeitdaten der meisten parasitologischen Parameter zu finden. Der Einfluss dieser Schwankungen auf orts-spezifische Unterschiede in den parasitologischen Daten war stärker wahrnehmbar in den Infektionsstärken einzelner Parasitenarten als in den Gemeinschaftsdaten wie Artenreichtum und Diversität, die sich weitgehend unempfindlich gegenüber saisonalen Schwankungen zeigten. Dauerhafte Veränderungen in der Zusammensetzung der Parasitengemeinschaft oder ihrer Diversität konnten über den Untersuchungszeitraum von 6 Jahren nicht beobachtet werden. In der integrierten Studie konnte mit Hilfe chemischer Rückstandsdaten aus Sedimenten und Muscheln (*Mytilus edulis*) ein Belastungsgradient zwischen den Stationen bestätigt werden. Dieser korrelierte signifikant im besonderen mit den parasitologischen Daten, die graduelle Unterschiede zwischen den Stationen aufwiesen. Die Reaktionen verschiedener, in den Flundern gemessener Biomarker korrelierten am stärksten mit der individuellen Belastung dieser Fische, welche jedoch nicht mit dem ortspezifischen Belastungsgradienten übereinstimmte. Die Biomarkerreaktionen bestätigten die Anwesenheit und den Einfluss von Xenobiota auf Organismen im Untersuchungsgebiet. Im Langzeitdatensatz fanden sich ebenfalls Korrelationen zwischen parasitologischen Daten, der individuellen Belastung der Flundern und den Reaktionen

einzelner Biomarker. Kurzzeitreaktionen einiger Biomarker auf akute Belastungsereignisse fanden sich in den parasitologischen Daten jedoch nicht wieder. Die Langzeitdaten der parasitologischen Parameter waren hingegen am besten geeignet, eine generelle Charakterisierung der Standorte zu geben.
Die Ergebnisse der vorliegenden Studie zeigen, dass sich die Parasitengemeinschaft der Flunder sehr gut eignet, um Standorte in einem Gebiet wie der Deutschen Bucht gegeneinander abzugrenzen. Natürliche Einflussfaktoren allein konnten die beobachteten Standortunterschiede nicht erklären. Erst die Kombination parasitologischer Daten mit anderen Messverfahren, wie chemischer Rückstandsanalytik und Biomarkerreaktionen in einem integrierten Ansatz zeigte einen klaren Zusammenhang zwischen den parasitologischen Ergebnissen und der Schadstoffbelastung der Standorte. Die Verknüpfung von Biomarkerreaktionen auf suborganistischer Ebene individueller Fische mit den Reaktionen ihrer Parasitengemeinschaft auf Populations- und Gemeinschaftsebene, schafft zudem ein besseres Verständnis der durch Schadstoffe hervorgerufenen, funktionellen Prozesse im Organismus und der ökologischen Relevanz von Schadstoffeinflüssen auf das Ökosystem.

Schlagwörter: Parasiten, *Platichthys flesus*, Nordsee, Bioindikatoren, integriertes Schadstoffmonitorring

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1995 – 1996	Grant of the German Academic Exchange Service (DAAD) for studies of Tropical Biology in Costa Rica, Central America
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