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Occurrence of trichodinid ciliates (Peritricha: Urceolariidae) in the Kiel Fjord, Baltic Sea, and its possible use as a biological indicator

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Abstract Investigations on the occurrence of trichodinid ciliates from fish caught in the Kiel Bight and Kiel Fjord (western Baltic Sea) were carried out between September 1996 and March 1997. Smears of the gills, fins, and skin of 120 *Gadus morhua* and 92 *Platichthys flesus* caught by fish traps and trammel nets revealed the presence of trichodinid ciliates. According to the fish species and locality, different prevalences and densities of trichodinid ciliates were found. Fish caught in the Kiel Bight revealed a lower prevalence of trichodinid ciliates on their gills (*P. flesus* 74.2%, *G. morhua* 3.8%) in comparison with fish of the same species and size caught in the Kiel Fjord (*P. flesus* 75.0%, *G. morhua* 26.2%). In both areas, *P. flesus* was more heavily infested than *G. morhua*. Seasonal changes in the prevalence of infestation of *P. flesus* between autumn and winter in the Kiel Fjord are proposed to be linked to an increase in bacterial biomass during winter. The fish ecology in combination with the total number of bacteria in the fish environment is discussed as an important factor influencing the abundance of trichodinid ciliates. The present data suggest the use of trichodinid ciliates as an indicator for eutrophication in brackish-water environments.

Introduction

Parasites are an essential part of each aquatic community. The presence of these organisms often becomes evident after a massive development, causing clinical signs in or leading to mortality of infested hosts. Such a situation can be combined with biotic or abiotic changes in the environment (Möller 1987). Knowledge on the biology of the parasite and their host(s), the host-parasite relationship, and the environmental situation can help in the detection of such environmental changes. The use of parasites as biological indicators will be of increasing importance not only for pollution studies but also for ecology and biodiversity studies.

Parasites of various taxonomic groups have been used as biological indicators, for example, for stock separation of *Trematomus bernacchii* (Moser and Cowen 1991), *Scomber scombrus* (MacKenzie 1990), and *Clupea harengus* (MacKenzie 1985). The feeding behavior of fish is also reflected by the parasite fauna (Palm et al. 1998). MacKenzie (1985) has shown that during the 1st year, herring are infected with digenean trematodes; older specimens accumulate other parasites (cestodes) from regions different from the breeding areas. Similarly, different prevalences of *Sphyrion lumpi* (Crustacea) are used to characterize local redfish stocks (*Sebastes marinus*, *S. mentella*) from North Atlantic waters (MacKenzie 1983).

Long-living species (digenean trematodes, cestodes, nematodes) can give information on the seasonal migration of their hosts and the migration habits of different age groups (feeding area/spawning area). Short-living species, combined with a direct life cycle and high reproduction rates (protozoan ectoparasites, monogenean trematodes), can give information on the environmental conditions of the host (Lester 1990). Some free-living ciliated Protozoa (Boikova 1990) as well as acanthocephalans, which specifically accumulate certain heavy metals in higher amounts than does their host, have been discussed as biological indicators of heavy metals (Sures and Taraschewski 1995). Sessiline peritrichous ciliates have been discussed as indicators for eutrophic situations (Rustige and Mannesmann 1994), and mobiline peritrichs have been reported as indicators for petroleum hydrocarbons (Khan and Thulin 1991). In marine coastal areas, human activities directly influence living communities, which can result in a heavier para-

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site infestation as compared with the situation in less polluted sites (Skinner 1982).

Ecological aspects of infestation by parasitic Protozoa and its relationship to environmental factors, such as fish farming, industrial and communal wastewater, and influx of fertilizers from agriculture, are presently beginning to be understood. Recently, mobiline peritrichous ciliates have been experimentally examined for their function as biological indicators of water quality (Voigt 1993). The present study was carried out to examine the trichodinid burden of free-living benthic and benthopelagic fish for evaluation of the possibility of using trichodinid ciliates as indicator organisms for eutrophication within an anthropogenically influenced coastal brackish-water environment.

Materials and methods

During September 1996 and March 1997 a total of 120 *Gadus morhua* (11–66 cm) and 92 *Platichthys flesus* (10–50 cm) were collected using fish traps (controlled after 3 days) and trammel nets (controlled every 24 h) in the Kiel Fjord. In January 1997, trawl fishing was used to obtain fish from Stollergrundrinne, Kiel Bight (Fig. 1). Equally sized smears of skin and fins (7 cm^2) and gill scrapings from the first gill arch were taken from living or freshly killed specimens. Fish caught by trawl fisheries were exclusively examined for the presence of trichodinid ciliates on their gills. Additionally, 35 market-sized *Oncorhynchus mykiss* (56–64 cm) from an aquaculture facility located in the Kiel Fjord were examined for the presence of trichodinid ciliates in October/November 1996. A detailed description of the three species found is the subject of another communication (Dobberstein and Palm 1999).

Klein's silver impregnation technique (Lom 1958) was used to demonstrate the components of the adhesive disc (Fig. 2) and the adoral ciliary wreath. Air-dried smears were lightly washed with distilled water using a Pasteur pipette for the removal of chloride ions, left to air-dry again, impregnated for 30 min with a 5% aqueous solution of silver nitrate ($AgNO_3$), washed again with distilled water, and, finally, exposed to UV light for 30–40 min. The impregnated smears were examined using a compound microscope (Orthoplan, Leitz). The methods and terminology for measurements have previously been described by Lom (1958). The prevalence (number of infested fish) and the density (number of ciliates per scraping) of infestation with *Trichodina* spp. were ascertained. Parasitological terms are used according to Bush et al. (1997).

For scanning electron microscopy (SEM), gill lamellae with living trichodinids were taken from freshly killed *P. flesus* and fixed in 10% buffered formalin. The gill lamellae bearing trichodinid ciliates were dehydrated by a gradated alcohol series, dried in a critical-point dryer (Balzers CPD 030) using CO_2 as the transitional fluid, and covered with gold-palladium in a sputter-coater (Balzers SCD 004) in an argon atmosphere. Examinations and photomicrographs were carried out with a Zeiss DSM 940 scanning electron microscope operating at 5 kV.

The prevalences of trichodinid ciliates on the gills of *P. flesus* and *G. morhua* from the autumn sample and that on *P. flesus* from the autumn and winter samples were examined by chi-square test.

Results

A total of 93 smears of the gills, skins, and fins of the 247 fish examined revealed the presence of trichodinid ciliates (Table 1). Three different trichodinid species, *Trichodina claviformis*, *T. jadranica*, and *T. raabei* were found (Dobberstein and Palm 1999). *T. jadranica* (Figs. 3–6) and *T. raabei* were commonly found on the body surfaces of *Platichthys flesus*, whereas *T. claviformis* (Fig. 2) was most commonly found on the body surfaces of *Gadus morhua*. Below, prevalence and mean density

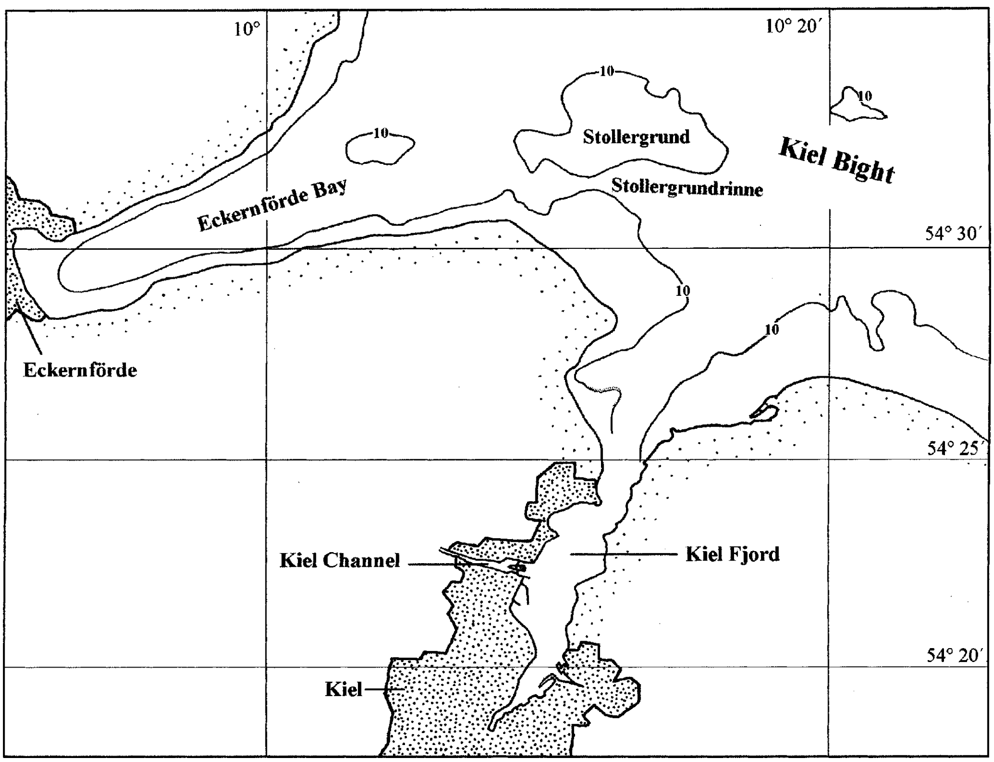
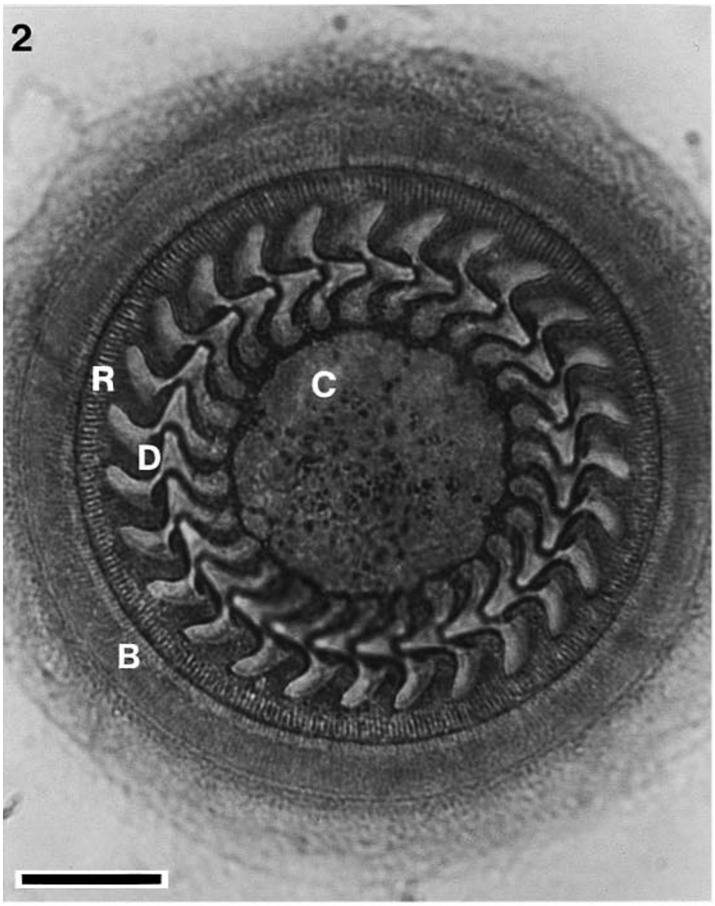


Fig. 1 Map of the study area, showing the areas of sampling: Kiel Bight and Kiel Fjord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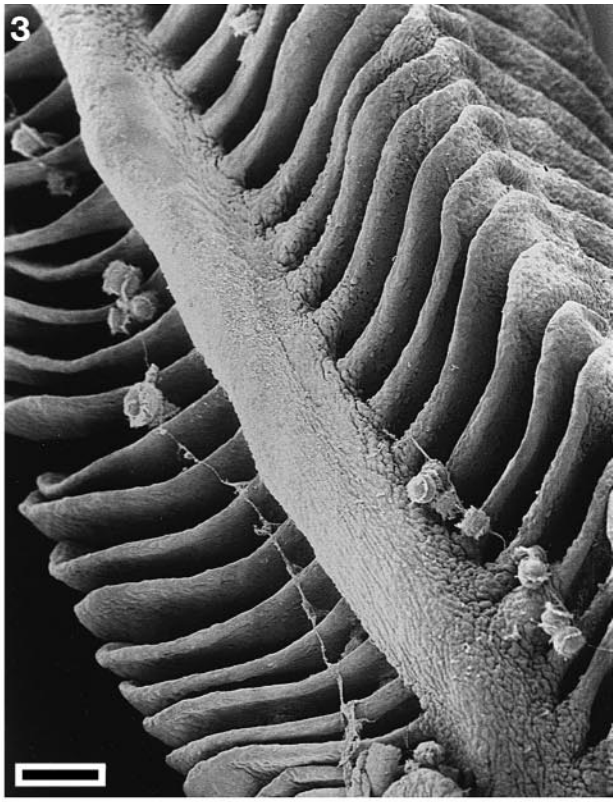
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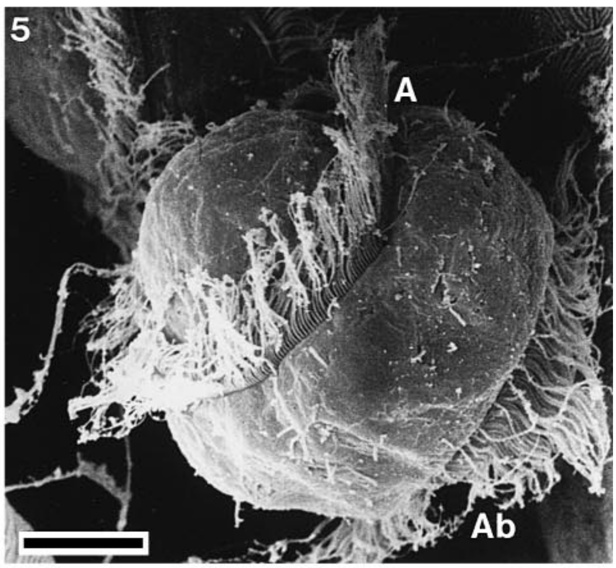
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Fig. 2 *Trichodina claviformis* Dobberstein & Palm, 1999, from *Gadus morhua*; silver-impregnated adhesive disc (*B* border membrane, *C* center of the adhesive disc, *D* denticulate ring, *R* radial pins). *Bar* 20 µm. **Figs. 3–6** Scanning electron micrographs of *T. jadranica* infesting gills of *Platichthys flesus*. **Fig. 3** Infested gill. **Fig. 4** *T. jadranica* between secondary lamellae, different views; adoral ciliary wreath turning counterclockwise into the buccal cavity. **Fig. 5** Lateral view, adoral and aboral ciliary wreath. **Fig. 6** Adoral ciliary wreath plunging into the infundibulum (*arrow*) (*A* adoral ciliary wreath, *Ab* aboral ciliary wreath, *B* border membrane). *Bars:* **Fig. 3** 100 µm, **Fig. 4** 20 µm, **Fig. 5** 10 µm, **Fig. 6** 5 µm

data are given for the genus *Trichodina*. The skin and fins of the fish were infested to a lower extent than the gills; thus, data for the skin and fins are regarded in combination.

Generally, the highest prevalences of trichodinids were observed on the gills of *P. flesus* from the Kiel Fjord (75.0% in autumn and 93.9% in winter, respectively); the skin and fins were found to be less infested (35.7% and 21.2%, respectively). Gills of *P. flesus* from the Kiel Bight were similarly highly infested at 74.2% in the autumn. The mean density of trichodinids infesting common flounder from the Kiel Bight was higher than that observed on flounders from the Kiel Fjord. In contrast to the data obtained from the gills, the prevalence of *Trichodina* spp. on the skin and fins was lower in winter (21.2%) than in autumn (35.7%). The mean density of trichodinids infesting the skin and fins was also lower in winter (6.3) than in autumn (20.7).

Similar to the case of *P. flesus*, the highest prevalences of *Trichodina* spp. were found on the gills of *G. morhua* from the Kiel Fjord (26.2%), whereas the skin and fins were less infested (9.5%). Gills of specimens caught in the Kiel Bight were also less infested (3.8%). The highest mean density was found on the gills (31.8) of *G. morhua* from the Kiel Fjord. The mean density of trichodinids infesting the skin and fins of *G. morhua* in the Kiel Fjord was similar to that recorded for gill-infesting trichodinids from the Kiel Bight (Table 1).

Common flounder caught in autumn revealed nearly similar prevalences of trichodinids on gills at both localities. In winter, increasing infestation of the gills in terms of prevalence as well mean density as opposed to slightly decreasing infestation of the skin and fins of *P. flesus* from the Kiel Fjord was noted. The infestation by trichodinid ciliates of the gills of *G. morhua* was plainly higher in the Kiel Fjord than in the Kiel Bight, which contrasts with the infestation pattern of *P. flesus*, which revealed a similar prevalence at both localities (Table 1).

In the autumn sample the prevalence of the trichodinid infestation of the gills of *P. flesus* did not differ significantly between the two localities, whereas the prevalences in autumn and winter differed significantly in the Kiel Fjord ($P = 0.001$). The trichodinid infestation of *G. morhua* differed significantly ($P = 0.001$) between the two localities. In agreement with the findings of Halmetoja et al. (1992), who examined the *Trichodina* fauna of freshwater fish from Finnish lakes, no pattern of age dependence for the infection was observed during the present study.

Oncorhynchus mykiss revealed the lowest prevalence of trichodinid ciliates among the fish examined. In two smears of gills and skin from two specimens, single cells were found (also see Dobberstein and Palm 1999).

Discussion

Three species of trichodinid ciliates, *Trichodina claviformis*, *T. jadranica*, and *T. raabei*, were found on *Gadus morhua* and *Platichthys flesus*. According to the fish species and the locality, different prevalences and intensities of trichodinid ciliates were found. The total number of bacteria in the fish environment is considered to influence the abundance of these epibionts.

P. flesus from the Kiel Bight and the Kiel Fjord was the most infested species, the gills being the most preferred habitat (Table 1). The present data are comparable with those reported by Calenius (1980) for the eastern Baltic Sea (Åland Islands), where *Trichodina* sp. infested *P. flesus* to a rate of 88.9%, and those reported by Lüthen (1989) for *P. flesus* from the southern Baltic Sea (Arkona-

Table 1 Prevalence and mean density of *Trichodina* spp. on the gills, skin, and fins of *Platichthys flesus*, *Gadus morhua*, and *Oncorhynchus mykiss*

Host (season)	Kiel Fjord					Kiel Bight		
	n	Skin/fins		Gills		*n*	Gills	
		Prevalence (%)	Mean density (range)	Prevalence (%)	Mean density (range)		Prevalence (%)	Mean density (range)
P. flesus (autumn)	28	35.7	20.7 (1–74)	75.0	8.6 (1–32)	31	74.2	81.7 (1–420)
P. flesus (winter)	33	21.2	6.3 (1–36)	93.9	35.6 (1–126)	–	–	–
G. morhua (autumn)	42	9.5	4.0 (1–11)	26.2	31.8 (1–350)	78	3.8	7.3 (2–10)
O. mykiss (autumn)	35	2.85	1.0 (1)	2.85	1.0 (1)	–	–	–

Basin), which were infested at rates of up to 100%. Both authors examined the prevalence of *Trichodina* sp. on the gills. In agreement with the present study, Calenius (1980) identified *T. borealis*, *T. raabei*, and *T. domerguei* subspecies and Lüthen (1989) identified *T. jadranica* and *T. raabei*. Parasitological surveys on *P. flesus* from different parts of the eastern Baltic Sea revealed prevalences of 72.4% for *T. borealis* (Rokicki and Morozinska 1994), 90% for *T. jadranica*, and 20% for *T. raabei* (Vismanis and Kondratovics 1994). Distinctly lower prevalences were observed for *G. morhua* in the Kiel Bight and the Kiel Fjord. Similar to the case of *P. flesus*, the gills were the most infested organ, which contrasts with a study on *G. morhua* from North Atlantic waters, which harbored *T. murmanica* and *T. cooperi* on their skin and fins (Poynton and Lom 1989). The authors reported a 26% prevalence on the skin and fins, whereas during the present study the prevalence was 9.5%.

Seasonal fluctuations in the prevalence of trichodinid ciliates were found on the gills *P. flesus* from the Kiel Fjord (75% in autumn, 93.3% in winter), combined with increasing densities. Lom (1961) observed a similar increase in the *Trichodina* population density in freshwater environments in winter, which he attributed to a decreasing condition of the fish, low oxygen contents, and other unspecified factors. The natural repellent feature of the fish surface is decreased, leading to a mass multiplication of these normally harmless ciliates, which primarily feed on small algae and bacteria from the free water column (Lom 1995).

However, the situation found within the Kiel Bight and the Kiel Fjord appears to be different. Rumohr (1996) detected a direct connection between the seasonal fluctuation in primary production as well as primary consumers and the abundance of benthic organisms in the Kiel Bight. More than half of the primary production in the free water column is directly available for benthic organisms due to the shallowness of the water. In winter the predation of fish leads to a decrease in the abundance of benthic invertebrates. As eroding macrophytes, wastewater, and partly sedimented products of primary production serve as nutrients for bacteria and the populations of benthic omnivores and bacteria grazers are reduced (see above), the number of bacteria multiplies during this period. Such a large bacterial biomass supports the development of trichodinid ciliates, as the occurrence of these filter-feeding peritrichous ciliates is primarily related to a sufficient availability of their food particles. This is similar to their closest related group, the sessiline peritrichs, which are known as effective bacteria filterers in activated sludge in the reprocessing of sewage (Guhl 1985) and as biological indicators for the classification of saprobic indices in freshwater environments (Rustige and Mannesmann 1994). Thus, during winter the condition for trichodinid ciliates increases, whereas the environmental condition (food resource) for the fish decreases. The high trichodinid burden in combination with a low food supply acts as a stressor on the fish immune system. We propose that the increasing bacterial biomass results in a multiplication of trichodinids on the fish, which is supported by the high densities of infestation of *P. flesus* observed during winter within the Kiel Fjord.

On a transect from the Kiel Fjord to the Kiel Bight, steadily decreasing total bacteria numbers and saprophyte numbers have been reported for the free water column (Schiewer and Gocke 1996). Both values are known as eutrophication indicators. In contrast to the similar prevalences observed in the case of *P. flesus* in the Kiel Bight and the Kiel Fjord, *G. morhua* reveals different prevalences and intensities in these adjoining areas (Fig. 7). These differences might also be explained by the decrease in the total number of bacteria along the transect. On the other hand, especially juvenile cod

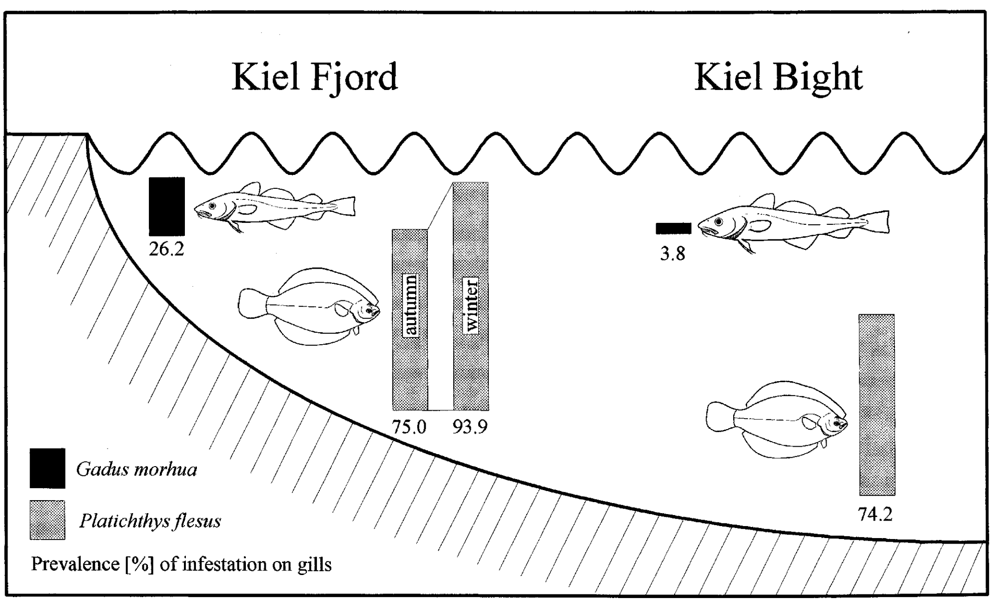

Fig. 7 Schematic illustration of the dependence of marine *Trichodina* spp. on host ecology and environment (for explanation, see Discussion)

feeding on benthic organisms in sublittoral areas are more heavily exposed to an environment containing high numbers of bacteria, which facilitates the occurrence of trichodinid ciliates. Older cod leave their littoral feeding habitat to feed on benthic invertebrates and pelagic fish in offshore waters. The lower prevalences found on cods from the Kiel Bight suggest that trichodinids react promptly with decreasing prevalences when their hosts migrate into waters containing a smaller bacterial biomass. However, no relationship could be observed between the abundance and the fish length.

The lower density of trichodinid infestation of *G. morhua* as compared with *P. flesus* can be explained by the different ecology of these fish species. The bottom-dwelling *P. flesus* offers a permanently stable food resource for trichodinid ciliates (see above). More than 90% of microorganisms live linked to surfaces of sediment particles or sedimenting particles (Hoppe 1996). In benthic communities the share of bacterial biomass (1–1000 μg C/g, dry weight) is comparable with the biomass of all other organisms and the maximal microbiotic activity is associated with surfaces in contact zones such as water-sediment, redox-horizon, or organic-biogenic particles (Meyer-Reil 1996). A host of trichodinids, which, according to its ecology, lives in a bacteria-rich environment, should be infested with trichodinids to a higher extent than a host living in the relatively bacteria-free water column. This could explain the nearly identical gill infestation observed in *P. flesus* from the Kiel Bight and the Kiel Fjord (Fig. 7). In contrast, *G. morhua* from the Kiel Bight, which only temporarily feeds on benthic organisms but also feeds in the pelagic environment, consequently harbors a lower trichodinid burden (Fig. 7). This finding is supported by many publications dealing with marine *Trichodina* spp. from bottom-dwelling fish species belonging to the Blenniidae, Cottidae, Gobiidae, Labridae, Syngnathidae, and Pleuronectidae (Raabe 1958, 1959; Lom 1961, 1970; Lom and Laird 1969; Grupcheva et al. 1989), whereas reports of trichodinids from pelagic fish species are scarce. Additionally, the results of Calenius (1980) suggest that benthic fish such as *Gasterosteus aculeatus* (90.3%), *Myoxocephalus quadricornis* (100%), and *P. flesus* (88.9%) have higher prevalences of trichodinid ciliates than do the pelagic living species *Clupea harengus* (0) or *Osmerus eperlanus* (0). We therefore conclude that the availability of bacteria in pelagic environments limits the distribution of trichodinid ciliates, especially as these ciliates live primarily as commensals but occur only at higher densities as parasites on their hosts.

As eutrophic waters contain a large total number of bacteria, they favor the occurrence of trichodinid ciliates. Schiewer and Gocke (1996) have stated that shallow bays and fjords of the Baltic Sea are important areas for transportation, filtering, and slowing down of the seaward flow of nutrients by slow sedimentation. The shallow water column combined with the high oxygen supply allows large heterotrophic and autotrophic production rates. Highly efficient destruents prevent the accumulation of organic material. Thus, the Kiel Fjord offers bacterivorous ciliates more stable nutritional conditions in comparison with the Kiel Bight, which is supported by the present data on trichodinids infesting *G. morhua* (Fig. 7). Interestingly, none of the fish examined showed signs of trichodinosis (extreme mucus production by epithelial cells, inflammation of the fins). This could be interpreted to mean that even the observed highest densities of the ciliates (Table 1) were below the number causing severe injuries to the host.

In summary, the abundance of trichodinid ciliates describes not only the health status of the fish but also the bacterial biomass, which reflects the eutrophication level of the environment. Thus, being (1) differently abundant in various localities, (2) attached to the host in a number that is unlikely to be removed within the short period until examination, (3) easily collected by scrapings of the various body surfaces of the host, (4) easily identifiable to the genus level, (5) capable of reacting directly to changing environmental conditions, and (6) nonpathogenic in free-living host populations, trichodinid ciliates from benthic and benthopelagic fish are proposed for use as indicator organisms for water quality in fulfilment of the six criteria proposed for indicator organisms by MacKenzie (1983). It must be kept in mind that the infestation of *G. morhua* (present study) and *O. mykiss* (Dobberstein and Palm 1999) with trichodinid ciliates was low in the Kiel Fjord. This demonstrates differences in the suitability of individual fish species for trichodinid infestation. Thus, the selection of the preferred hosts for trichodinids within a locality determines on the successful use of these protozoans as biological eutrophication indicators.

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