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Baltic cod endohelminths reflect recent ecological changes

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Abstract

We suggest helminthological investigations of cod as a supplement to traditional biological and hydrographical methods for elucidation of ecological changes in the Baltic Sea. It is under discussion if oxygen deficit or seal abundance should explain the present critical situation of Baltic cod. A comparative investigation of endoparasitic helminths in Baltic cod (*Gadus morhua*), captured in the same marine habitat with an interval of 35 years (1983/2018) recorded 11 species of helminths comprising trematodes (*Hemiurus luehei*, *Podocotyle atomon*, *Lepidapedon elongatum*), nematodes (*Contracaecum osculatum*, *Hysterothylacium aduncum*, *Capillaria gracilis*, *Cucullanus cirratus*), cestodes (*Bothriocephalus* sp.) and acanthocephalans (*Echinorhynchus gadi*, *Pomphorhynchus laevis*, *Corynosoma semerme*). Significant prevalence and intensity increases were recorded for third-stage larvae of the nematode *C. osculatum* (liver location) and larvae of *C. semerme* (encapsulated in viscera). Both parasite species use grey seal as their final host, indicating the recent expansion of the Baltic seal population. A lower *E. gadi* intensity and an increased prevalence of *L. elongatum* of small cod (31–40 cm body length) suggest a lowered intake of amphipods (intermediate host) and elevated ingestion of polychaetes, respectively, but no significant changes were seen for other helminths.

Introduction

The distribution and dynamics of fish parasites in the aquatic environment are influenced by biotic and abiotic factors, and the parasitofauna in a host population may, therefore, reflect changes in the environmental conditions (Marcogliese, 2002; Poulin, 2006). Accordingly, several authors have applied parasites in fish as indicators to reflect the biology of fish stocks (Williams *et al.*, 1992; MacKenzie, 2002; Marcogliese, 2002). This approach was also used for Baltic Sea fishes (Reimer, 1970; Køie, 1999; Sobecka *et al.*, 2011; Unger *et al.*, 2014; Mehrdana *et al.*, 2015) addressing salinity variations in the Baltic (Herlemann *et al.*, 2011). The Baltic cod is a subpopulation of the Atlantic cod (*Gadus morhua*) residing in the Baltic Sea since the last glacial age. It performs local migrations within this brackish water area but has limited exchange with other cod populations (Sick, 1965). Due to the low salinity in the Baltic, the cod diet composition is less diverse, but shifts from mainly invertebrates in small cod (below 40 cm body length) to a predominantly piscine diet in larger fish (Zuo *et al.*, 2016). During the latest decade, cod in the eastern part of the Baltic Sea has been in a critical state and a range of biotic and abiotic causes have been suggested to explain poor growth and performance – including oxygen depletion, climate, salinity, food availability and parasite infections (Eero *et al.*, 2015; Horbowy *et al.*, 2016). We performed a parasitological investigation of Baltic cod – caught in 2018 in a specific habitat east of the island of Bornholm in the Baltic sea – and compared the recorded parasite fauna with a similar study on cod from the same area conducted in 1983. By analysing differences and similarities, we discuss if any ecological change over three decades in this part of the Baltic sea may be reflected in the parasite fauna.

Materials and methods

Fish

Baltic cod (*G. morhua*) (body length 31–50 cm) were caught along the east coast of the island Bornholm in the Baltic sea (ICES subdivision 25) both in 1983 (40 specimens) and 2018 (33 specimens). Total fish length was recorded both in 1983 and 2018. All fish were frozen after capture and kept at −20°C until examination. We divided fish into length groups (31–40 cm and 41–50 cm) to minimize size group bias. By necropsy, organs were separated, placed in Petri dishes and inspected under the dissection microscope (magnification × 40–400) (Leica MZ125, Wetzlar, Germany). Endohelminths were isolated, recorded and conserved immediately in plastic vials containing 70% ethanol.

Table 1. Primers applied for the molecular identification of Baltic cod parasites.

Parasite group	Primers	Target region	Sequence	Reference
Acanthocephala	Acanth_F1	18S	5′-AGA TTA AGC CAT GCA TGC GTA AG-3′	Verweyen *et al.* (2011)
	Acanth_R1	18S	5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′	
Nematoda	NC2	ITS (18S, ITS1, 5.8S, ITS2,28S)	5′-TTA GTT TCT TTT CCT CCG CT-3′	Zhu *et al.* (1998)
	NC5	ITS (18S, ITS1, 5.8S, ITS2,28S)	5′-GTA GGT GAA CCT GCG GAA GGA TCA-3′	
Trematoda	Erib1	18S	5′-ACC TGG TTG ATC CTG CCA G-3′	Zilberg *et al.* (2012)
	Erib10	18S	5′-CTT CCG CAG GTT CAC CTA CGG-3′	

Morphological identification

Haematoxylin-stained parasites (ten per species if applicable) were mounted on microscope slides in Aquatex (Merck, Darmstadt, Germany) and studied in the compound light microscope (Leica DM5000B, Wetzlar, Germany).

Molecular identification

A section of the individual parasite was used for molecular analysis (lysis, DNA-purification, polymerase chain reaction (PCR) sequencing) (primers in table 1). Five out of 11 parasite species were genetically identified, whereas six were not due to insufficient parasite material. Lysis was performed with the QIAGEN® DNeasy Blood & Tissue Kit (Ballerup, Denmark), and PCR performed in a 60 µl PCR set-up (Zuo *et al.*, 2018) using predenaturation at 95°C for 5 min; amplification starting with denaturation at 95°C for 30 s, annealing at an assay-specific temperature for 30 s, elongation at 72°C and post-elongation at 72°C (7 min). All PCR products were examined by 1.5% ethidium bromide containing agarose gel electrophoresis, purified using the illustra™ GFX™ PCR DNA Purification Kit (GE Healthcare, Brøndby, Denmark), sequenced (Macrogen, Seoul, Korea) and analysed (CLC Main Workbench v7.9.1, Qiagen, Aarhus, Denmark) by BLAST® (Bethesda, Maryland, USA) analysis at GenBank.

Statistics

The sample sizes (40 and 33, respectively) were relatively small but allowed us to perform significance tests. Prevalence (percentage of hosts infected), mean intensity (mean number of parasites per infected fish), variance-to-mean ratio (reflecting overdispersion if >1) and range (lowest and highest number of parasites in a host) was calculated (Bush *et al.*, 1997). Intensity differences between years were evaluated by the Mann–Whitney U-test. Prevalence differences were evaluated using a contingency table (Chi-square). All tests performed (Graph Pad Prism version 7.2, www.graphpad.com) applied a 5% significance level.

Results

Parasite occurrence

We recorded 11 species of helminths comprising trematodes, cestodes and acanthocephalans (table 2). Low infections due to the *Bothriocephalus* sp., *Hemiurus luehei* and *Podocotyle atomon*, and *Cucullanus cirratus* were recorded in both 1983 and 2018, and no significant changes were evident for these species. We recorded a slight but non-significant increase of infection for the intestinal nematode *Capillaria gracilis* and a lowered *Hysterothylacium aduncum* infection. A dominant helminth in both years was the acanthocephalan *Echinorhynchus gadi*, which occurs in the intestinal lumen of cod, but in 2018 the infection intensity was slightly lower for small cod (31–40 cm body length). Occurrence of the nematode third-stage larva *Contracaecum osculatum* increased significantly during the three decades from almost absence in 1983 to 100% prevalence in 2018, with intensities increasing from one parasite (1983) to more than 200 per fish liver (2018). The larval acanthocephalan *Corynosoma semerme* occurrence increased significantly as well.

Discussion

Communities of certain fish parasites with complex life cycles are dependent on the presence of specific hosts, whereby the parasite fauna in a fish reflects availability of these organisms. The main final host of *C. osculatum* (Rudolphi, 1802) in the Baltic Sea is the grey seal (Køie & Fagerholm, 1995; Skrzypczak *et al.*, 2014; Lunneryd *et al.*, 2015) and its population has exhibited a significant expansion since 1990 (Harding *et al.*, 2007, Haarder *et al.*, 2014; Zuo *et al.*, 2018). Thus, this biotic factor is likely to explain the marked increase of *C. osculatum* infection from a low level in the 1980s and 1990s (Myjak *et al.*, 1994) to present levels (Haarder *et al.*, 2014; Nadolna & Podolska, 2014; Rodjuk, 2014; Sokolova *et al.*, 2018; Zuo *et al.*, 2018). The associated and elevated *C. semerme* infection, also using seal as final host (Sinisalo & Valtonen, 2003), adds to seal signatures in the cod parasitofauna. Generally, the variance-to-mean ratio was higher for all species indicating a large variation of intensity between individual fish. The acanthocephalan *E. gadi*, commonly occurring in Baltic cod (Nordenberg, 1963; Buchmann, 1986; Sobecka *et al.*, 2011), showed a slightly decreased infection level in the smallest cod (31–40 cm) in 2018 when compared to 1983. The intermediate hosts are amphipods such as *Gammarus* spp. (Buchmann, 1986) and a lowered infection may reflect decreased ingestion of this food source. Less prevalent species (and thereby useless as indicators) were the cestode *Bothriocephalus* sp., the nematode *C. cirratus* and the acanthocephalan *Pomphorhynchus laevis*. Minor non-significant differences were noted for the trematodes *H. luehei* and *P. atomon* using molluscs and crustaceans as intermediate hosts (Køie 1981, 1990), the nematode *H. aduncum* using the isopod *Saduria entomon* (Pawlak *et al.*, 2018) and other crustaceans (Køie, 1993) as first intermediate host. These changes are too limited to draw any strict conclusions about availability of intermediate or transport hosts, but we suggest that future ecoparasitological studies should include these species. Larval

Table 2. Prevalence (% of fish infected), intensities (MI, mean intensity), standard deviation (SD) and variance-to-mean ratio (V/M) of endohelminth infection of Baltic cod (captured along the east coast of Bornholm island, ICES subdivision 25) in 1983 and 2018.

Year of examination	1983				2018			
Size group of fish (cm) (SD)	31–40 (3.5)		41–50 (4.2)		31–40 (3.1)		41–50 (4.4)	
No. of cod in size group	22		18		17		16	
Parasite	No. fish infected	Prevalence %	No. fish infected	Prevalence %	No. fish infected	Prevalence %	No. fish infected	Prevalence %
GenBank accession number		MI (V/M)		MI (V/M)		MI (V/M)		MI (V/M)
Identity (similarity %)		SD (range)		SD (range)		SD (range)		SD (range)
Trematoda								
Podocotyle atomon	2	9.1	2	11.1	0	0.0	0	0
ND		4 (2)		5 (NA)		0 (NA)		0 (NA)
		2.8 (2–6)		1.4 (4–6)		NA		NA
Hemiurus luehei	0	0.0	1	5.6	0	0.0	0	0.0
KM401883		0 (NA)		1 (NA)		0 (NA)		0 (NA)
95.48%		NA		NA (1)		NA		NA
Lepidapedon elongatum	6	27.3	6	33.3	10	58.8*	2	12.5
Z12600		21 (5.8)		20 (6.8)		10.6 (18.7)		16.5 (16.0)
99.39%		11.0 (9–27)		11.7 (8–38)		14.1 (1–42)		NA
Cestoda								
Bothriocephalus sp. (larva)	0	0.0	1	5.6	1	5.9	0	0.0
ND		0 (NA)		1 (NA)		1 (NA)		0 (NA)
		NA		NA (1)		NA (1)		NA
Nematoda								
Hysterothylacium aduncum larva	0	0.0	3	16.7	1	5.9	0	0.0
JQ934881		0 (NA)		1 (NA)		1 (NA)		0 (NA)
100%		NA		NA (1)		NA (1)		NA
Hysterothylacium aduncum adult	3	13.6	3	16.7	0	0.0	0	0.0
JQ934881		5 (0.8)		10 (5.7)		0 (NA)		0 (NA)
100%		2.0 (3–7)		7.6 (3–18)		NA		NA
Capillaria gracilis adult	1	4.6	1	5.6	3	17.7	4	25.0
ND		3 (NA)		3 (NA)		18.3 (12.8)		6 (3.7)
		NA (3)		NA (3)		15.3 (1–30)		4.7 (2–11)

(*Continued*)

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Table 2. (*Continued.*)

Year of examination	1983				2018			
Size group of fish (cm) (SD)	31–40 (3.5)		41–50 (4.2)		31–40 (3.1)		41–50 (4.4)	
No. of cod in size group	22		18		17		16	
Contracaecum osculatum larva	0	0.0	3	16.7	17	100.0*	14	87.5*
KM273050		0 (NA)		1 (0)		22.4 (12.3)*		48.6 (82.3)*
99.90%		NA		0 (1)		16.6 (1–51)		63.5 (1–205)
Cucullanus cirratus adult	0	0.0	1	5.6	1	5.9	0	0.0
ND		0 (NA)		1 (NA)		1 (NA)		0 (NA)
		NA		NA (1)		NA (1)		NA
Acanthocephala								
Echinorhynchus gadi adult	22	100.0	18	100.0	15	88.2	16	100.0
AY218123		44 (22.6)		37 (43.5)		15.3 (13.9)*		11.3 (9.0)
99.88%		31.5 (5–120)		40.1 (4–122)		14.7 (1–41)		10.2 (1–34)
Pomphorhynchus laevis adult	0	0.0	1	5.6	0	0.0	1	6.3
ND		0 (NA)		1 (NA)		0 (NA)		1 (NA)
		NA		NA (1)		NA		NA (1)
Corynosoma semerme larva	0	0.0	1	5.6	4	23.5*	5	31.3*
ND		0 (NA)		1 (NA)		6.51 (8.3)*		6.0 (11.9)*
		NA		NA (1)		7.3 (1–17)		8.5 (1–21)

ND, molecular identification was not performed due to limited samples or negative PCR (in these cases, ID is based on morphological characters only); NA, not applicable.
*Significant differences between years, $P < 0.05$ (Mann–Whitney U-test for intensity and Chi-square for prevalence).

parasites are expected to increase their numbers in cod during growth, but rarely occurring species (*Bothriocephalus* sp., *H. aduncum*) occurred sporadically more often in smaller cod, probably due to chance effects. The prevalence of the pyloric digenean *Lepidapedon elongatum* in smaller cod increased from 27.3% in 1983 to about 58.8% in 2018, but no significant difference of the intensity was recorded. The trematode uses the prosobranch snail (*Anoba aculeus*) and polychaetes as first and second intermediate hosts, respectively (Køie, 1985). Hence, the study suggests a higher availability of these invertebrates on the feeding grounds of Baltic cod. Likewise, the increased *C. gracilis* infection does not indicate any decrease over the study period in availability of invertebrate intermediate hosts. Chironomids, oligochaetes and sand goby serve as intermediate hosts (Køie, 2001), suggesting presence of these organisms on the feeding grounds. When evaluating ecological differences in the Baltic Sea between the years 1983 and 2018, as reflected by parasites in cod, the increasing population of grey seal (*Halichoerus grypus*) (Harding *et al*., 2007; Haarder *et al*., 2014; Zuo *et al*., 2018) comes up as the main influential biotic factor because the seal is the final host of the nematode *C. osculatum* and the acanthocephalan *C. semerme*. The present investigation found no significant indications on extirpation of invertebrate intermediate hosts when compared to 1983, but we suggest to record the presence of endohelminths in cod in future ecological studies as they may serve as indicators for these populations.

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Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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