Annual and spatial variability in endo- and ectoparasite infections of North Sea cod (Gadus morhua Linnaeus, 1758) larvae, post-larvae and juveniles

Foojan Mehrdana1*, Qusay Z. M. Bahlool1, Alf Skovgaard1, Jesper A. Kuhn2, Per W. Kania1, Peter Munk3 and Kurt Buchmann1

1Laboratory of Aquatic Pathobiology, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, 1870 Frederiksberg C, Denmark; 2Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries and Economics, University of Tromsø, 9037 Tromsø, Norway; 3National Institute of Aquatic Resources, Technical University of Denmark (DTU), Jægersborg Allé 1, 2920 Charlottenlund, Denmark

Abstract
A parasitological investigation was performed on a total of 5380 Atlantic cod larvae, post-larvae and small juveniles sampled from the North Sea during a period of five years. The copepod Caligus elongatus (Von Nordmann, 1832) and the nematode Hysterothylacium aduncum (Rudolphi, 1802) were found at a relatively high prevalence of infection (4.6% and 5.2%, respectively). The infection by both parasites showed annual and spatial variability. C. elongatus showed a higher prevalence in 1992 compared to the following years, whereas the prevalence of H. aduncum increased from 1992 to 2001. We observed a relation between parasite distribution and parameters such as latitude and water depth. Adult digeneans (Lecithaster gibbosus and Dero- genes varicus) and larval cestodes were also found with lower infection rates. Since changes of infection levels coincided with increasing North Sea water temperature in the studied period, it is hypothesized that temperature may affect parasite population levels. However, it is likely that other environmental factors may contribute to the observed variations. Absence of infection intensities higher than one nematode per fish in small larvae and post-larvae suggests that host survival may be affected by a high infection pressure. The relatively high levels of infection in the younger stages of cod, and the annual/spatial variability of these infections should be considered in the understanding of the early life dynamics of the species.

Keywords
Atlantic cod; North Sea; Hysterothylacium aduncum; Caligus elongatus; growth; temperature; latitude

Introduction
Atlantic cod, Gadus morhua (Linnaeus, 1758), has been reported as host to more than 120 parasite species (Hem- mingsen and MacKenzie 2001), and some of these may have considerable impact on health of both wild and cultured cod (Khan 2004, Heuch et al. 2011). Larval and post-larval stages of fish are considered particularly vulnerable to infections due to their limited body size and the less developed immune system (Chetri et al. 2012), but few investigations have been conducted on parasitism of cod larvae (Karlsbak et al. 2001, Skovgaard et al. 2011). The decline of the North Sea cod population during recent decades has been attributed to overexploitation, recruitment failure and climate change, but should also be investigated in the context of increasing parasitism in the population (Skovgaard et al. 2011). All parasites found in Atlantic cod may represent a threat to the fish depending on the level of parasitism, but some parasite species may be more virulent than others (Heuch et al. 2011). In this context, copepod ectoparasites of the family Caligidae represent some of the most common causes of high-level mortality in marine aquaculture, particularly in salmonid and gadoid culture systems (Johnson et al. 2004, Øines et al. 2006). Different species of parasitic copepods have been reported in Atlantic cod, and Caligus spp. is predicted to become an important pest in cod aquaculture (Johnson et al. 2004, Heuch et al. 2011). These parasites have a direct life cycle with eight growth stages separated by moults. The first stages (nauplius I–II) are free-living, the copepodid is the infective stage and chalimus...
I–IV and adults are parasitic (Piasecki 1996, González and Carvajal 2003). Their attachment and feeding activities can cause localized lesions, which may cause osmoregulatory failure or lead to secondary infections (Wootten et al. 1982, MacKinnon 1993). Likewise, parasitic nematodes of the superfamily Ascaridoidea, which are distributed worldwide due to their low host specificity, may represent another major health threat to cod larvae and post-larvae. Their life cycles include invertebrates and fish species as intermediate/transport hosts (Szostakowska et al. 2005). In order to elucidate the infection level and annual/spatial variations in an important nursery area for North Sea cod, we have studied North Sea cod larval infections during five sampling surveys.

Here we report on the infection patterns of two parasites, *Hysterothylacium aduncum* (Rudolphi, 1802) and *Caligus elongatus* (Von Nordmann, 1832), in cod sampled in the North-eastern North Sea during the years 1992, 1993, 1994, 1999, and 2001. Further, we assessed whether infection with third-stage *H. aduncum* larvae affects the growth rate of North Sea cod post-larvae and juveniles by comparing age/length relations for infected and uninfected fish.

### Materials and Methods

**Sampling and examining of fish**

Atlantic cod larvae, post-larvae and juveniles were collected in the North-eastern part of the North Sea (Nielsen and Munk 2004, Skovgaard et al. 2011), during April/May/June cruises in the years 1992, 1993, 1994, 1999, and 2001 (the sampling sites are shown in Fig. 1). The variables water salinity and

<table>
<thead>
<tr>
<th>Month/Year</th>
<th>Temperature (°C)</th>
<th>Salinity (ppt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>05/1992</td>
<td>7.3–13.0</td>
<td>30.2–35.1</td>
</tr>
<tr>
<td>05/1993</td>
<td>7.3–12.1</td>
<td>25.9–35.3</td>
</tr>
<tr>
<td>05/1994</td>
<td>6.9–9.8</td>
<td>32.9–35.1</td>
</tr>
<tr>
<td>05/1999</td>
<td>5.0–9.8</td>
<td>25.3–35.2</td>
</tr>
<tr>
<td>04/2001</td>
<td>4.5–7.5</td>
<td>28.4–35.0</td>
</tr>
<tr>
<td>05/2001</td>
<td>6.2–11.7</td>
<td>25.0–35.7</td>
</tr>
</tbody>
</table>

Table 1. Abiotic variables ranges at the depth of ≤60 m, measured in geographical areas included in the analyses in each year

![Fig. 1. Sampling sites of cod larvae, post-larvae and juveniles in the North Sea shown in different sampling years](image-url)
water temperature ranges were measured and recorded at sampling sites in each year (Table I).

Fish were sampled using a ring net (2 meters in diameter) and conducting oblique hauls. Samples were immediately preserved in 96% ethanol. Total length of each fish was measured, ranging from 5 to 65 mm, and due to the same fixation method used throughout the study, no correction for length shrinkage was considered (Folkvord 2005). A number of the samples from May 1992 and 2001 were previously reported infected by H. aduncum (Skovgaard et al. 2011). Those samples (n = 927) were combined with new material from June 1992 (n = 80), May 1993 (n = 1568), May 1994 (n = 303), May/June 1999 (n = 665) and April 2001 (n = 1837). Thus, a total of 5380 fish were examined for presence of parasites under a stereomicroscope (Olympus SZX 16, Tokyo, Japan). Skin and fins were scrutinized for presence of parasitic copepods and the body cavities were dissected to isolate nematode larvae. Furthermore, 209 cod larvae and post-larvae, randomly subsampled from May 1994 at a depth of 60 meters (latitude: 56.6°N, longitude: 6.4°E), were scrutinized and examined for the presence of other types of endoparasites located in the gastrointestinal tract (GIT). Recovered parasites were preserved in 70% ethanol for morphological and molecular identification. The occurrence of parasites were characterized by prevalence (number of infected hosts/total number of fish examined), mean abundance (total number of parasites/total number of fish examined), and mean intensity of infection (average number of parasites/number of infected hosts) in the host population. Dispersion of parasite populations was determined as variance to mean ratios of abundance (Bush et al. 1997). Association between fish size and prevalence of nematodes and copepods, and also correlation between environmental parameters, e.g. water depth and latitude at sampling sites, and occurrence of these parasites were tested by applying Spearman’s rank correlation coefficient test using the SigmaPlot software. P values greater than 5% shows no significant relationship between the two variables.

Parasite identification

Parasitic nematodes were diagnosed as H. aduncum according to morphology and molecular identification based on sequencing of the internal transcribed spacer region (ITS-1, 5.8S rRNA gene, and ITS-2) (Skovgaard et al. 2011). Morphological identification of parasitic copepods was based on descriptions by Piasecki (1996) and Kabata (1979). Copepods and recovered GIT parasites were furthermore identified by sequencing the small subunit rRNA gene (SSU). For PCR, individual parasites were lysed in proteinase K as described earlier (Skovgaard et al. 2011). Complete lysis was confirmed by microscopy, and followed by inactivation of the proteinase K at 95°C for 10 minutes. PCR was performed in a Biometra T3 thermocycler (Fisher Scientific) using 60 μL reaction volumes. The reaction mixtures consisted of 6 μL lysate as template, 1 unit of BioTaq DNA polymerase (DNA-Technology), 1 mM dNTP, 1.5 mM MgCl₂ and 1 μM of the two primers. In order to amplify SSU, the primers ERIB1 (5’-ACC TGG TTG ATC CTG CCA G-3’) and ERIB10 (5’-CCT CCG AGT TTG CAG CTA CGG-3’) were used as forward and reverse primer, respectively (Barta et al. 1997). The primers NC5 (forward: 5’-GTA GGT GAA CCT GCG GAA GGA TCA TT-3’) and NC2 (reverse: 5’-TTA GTT TCT TTT CCT CCG CT-3’) were used for amplification of the ITS region (Zhu et al. 2007). The PCR procedure consisted of a pre-denaturation step at 95°C for 2 minutes, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s and elongation at 72°C for 2 minutes, and finally post-elongation at 72°C for 5 minutes. The products were analysed by 2% ethidium bromide–stained agarose gel electrophoresis. PCR products were purified using the illustra™ GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare) and sequenced at Macrogen Inc. (South Korea) using the PCR primers. Sequences obtained were submitted to GenBank [GenBank: JX845129, GenBank: JX845130, GenBank: JX845131].

Fish age versus length

Assessment of age was done through counting daily otolith increments on a subsample of 65 post-larval and juvenile cod. These fish (32 fish infected with 1–2 nematodes and 33 uninfected fish) were sampled in 1993 and 1994 from the same 5 stations. Fish total lengths ranged from 20 to 45 mm. Fish were selected to obtain infected and uninfected fish of comparable lengths. The fish were dissected and lapillus otoliths, hereafter called otoliths, were isolated under a stereomicroscope equipped with a Leica DC300 camera. Both right and left otoliths were removed with dissecting needles and embedded separately in thermo-setting resin on a glass slide, then hand-polished on one surface (using lapping film) until the primordia and growth increments were fully visible (Campana 1983, Secor et al. 1992). During otolith preparation, a total of 11 otoliths were discarded due to breakage during polishing. Counting of the increments was made from hatching check to marginal increment (Campana 1983). All otoliths were photographed and increment rings were counted at least three times for both otoliths (left and right). Since only minor differences were found between the numbers of increments in the two otoliths of the same fish, mean numbers were used for data analysis (Campana 1984). Fish size was then plotted as function of age for infected and uninfected fish, and Analysis of Covariance was employed using the R software to test whether there was any difference in the slopes or intercepts of the two linear regressions.

Results

1. Caligus elongatus

A total of 251 specimens out of 5380 investigated fish were infected by parasitic copepods, mostly in the chalimus stages. Anal and dorsal fins were the main attachment sites (40.7% and 27.44% of all copepods, respectively). Some parasites
Parasites of North Sea cod larvae, post-larvae and juveniles were attached to the dorsal (20.82%) or ventral (7.26%) part of the head, whereas only few copepods attached to the pelvic (1.26%), pectoral (1.26%) fins and opercula (1.26%).

1.1. Parasite identification

Based on morphology, the parasitic copepods were identified as *C. elongatus*. No adult copepods were encountered, and identification had to rely on chalimus IV stages. All SSU sequences obtained from copepods were identical (1711 bp long), and a BLAST search revealed similarity with species of the genus *Caligus*. The sequences exhibited 100% identity with *C. elongatus* [GenBank: EF088408, GenBank: EF088409], confirming the identification based on morphology.

1.2. Occurrence of infection

Overall prevalence of infection with parasitic copepods among all fish examined was 4.6%. The intensity of *C. elongatus* varied from 1 to 6 parasites per fish, and only 6.0% of all infected fish hosted more than 2 parasites (Table II). Prevalence, mean intensity, and mean abundance of *C. elongatus* in each sampling year are shown in Table III, and since samples collected in April and June did not exist in all sampling years, related data were excluded from the dataset in this table. The years 1992 and 1993 had the highest and lowest prevalence of infection, respectively, and there was a tendency of a decline in prevalence of parasitic copepods from 1992 to 2001 (Table III). Prevalence, mean abundance and mean intensity of infection across all sampling years were investigated by grouping fish in 5 mm length classes. The prevalence of infection significantly increased with size of the fish (*r* = 0.8, *P* = 0.006), although a slight decrease was seen for juveniles between 35–44 mm (Fig. 2A). Mean abundance of *C. elongatus* showed the same pattern as prevalence of infection (Fig. 2B). Mean intensity varied between 1 and 1.5 parasites per fish among size groups, peaking in the size class of 40–44 mm (Fig. 2C).

2. Hysterothylacium aduncum

Among 5380 collected samples, a total of 282 fish were infected with nematode larvae, which were almost exclusively

| Table II. Frequency distribution of parasitic copepods, *C. elongatus*, in infected North Sea cod larvae, post-larvae and juveniles in investigated years |
|---|---|---|
| Number of copepods/fish | Number of hosts | % hosts |
| 1 | 207 | 82.47 |
| 2 | 29 | 11.55 |
| 3 | 8 | 3.18 |
| 4 | 3 | 1.2 |
| 5 | 2 | 0.8 |
| 6 | 2 | 0.8 |

**Table III.** Prevalence, mean abundance and mean intensity of infection with *H. aduncum* and *C. elongatus* in the sample population

<table>
<thead>
<tr>
<th>Sample Year</th>
<th><em>H. aduncum</em></th>
<th><em>C. elongatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined fish</td>
<td>No. infected fish</td>
</tr>
<tr>
<td>1992</td>
<td>1568</td>
<td>3.28</td>
</tr>
<tr>
<td>1993</td>
<td>285</td>
<td>1.20</td>
</tr>
<tr>
<td>1994</td>
<td>592</td>
<td>1.10</td>
</tr>
<tr>
<td>1999</td>
<td>470*</td>
<td>0.89</td>
</tr>
<tr>
<td>2001</td>
<td>3572</td>
<td>211</td>
</tr>
</tbody>
</table>

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found free in the body cavity and viscera, and never encapsulated. The nematodes recovered from the investigated fish were all third-stage larvae and the species had previously been identified as *H. aduncum* (Skovgaard et al. 2011).

### 2.1. Occurrence of infection

The intensity of *H. aduncum* varied between 1 to 4 parasites per fish. However, most of the infected fish hosted only one nematode (Table IV). Prevalence, mean intensity, and mean abundance of *H. aduncum* infections in May of each year are shown in Table III (data for samples collected in April and June is excluded). Parasite prevalence fluctuated slightly between 2.3% and 5.1% from 1992 to 1999, but increased markedly to 18.9% in 2001. When samples from different sampling years were combined and fish grouped in size classes, a significant positive relationship between fish size and the prevalence of infection was found ($r = 0.76, P = 0.01$).
The overall mean abundance of infection (combined for five years) was 0.06 parasites/fish, and it increased with fish size, peaking in the size class of 35–39 mm (Fig. 3B). Mean intensity of infection did not differ considerably among the different years and also size groups, varying between 1 and 1.5 parasites per fish (Table III, Fig. 3C). Co-infections with endo- and ectoparasites were rarely observed (Table V).

### 2.2. Otolith analysis

No significant difference between infected and uninfected fish growth rates was found ($P = 1$, Analysis of Covariance). However, there was a significant effect of fish size on infection ($P \leq 0.001$), and infected fish were slightly larger than uninfected fish at equivalent age (Fig. 4).

Based on this test, the intercepts of infected and uninfected fish regression lines were also significantly different ($P \leq 0.001$).

### 3. Prevalence of parasites in relation to geography

A trend for a decreasing prevalence of *C. elongatus* with increasing latitude, i.e. lower prevalence towards north, was found in all years investigated (Fig. 5). However, there was not a statistically significant correlation, except for the year 1999 ($r = -1, P = 0.002$).

Other habitat parameters, such as water depth and proximity to the coast, did not show any significant relationship with copepod infections. Similarly, prevalence of *H. aduncum* showed no statistically significant dependence on water depth at sampling stations. Nonetheless, highest prevalence was found at stations with a depth of 30–50 m, whereas lower prevalence was seen at shallow-water and deep-water stations (Fig. 6). Prevalence of nematodes showed no significant association to latitude.

### 4. Other endoparasites

A subsample comprising 209 fish (9–39 mm in total body length) was examined for additional GIT parasites and a total of 10 cod were diagnosed infected with trematodes or cestodes. Five adult hemiurid digenean trematodes were found using light microscopy; *Lecithaster gibbosus* (Rudolphi, 1802) (found in the intestine of the fish), and *Derogenes varicus* (Müller, 1784) (observed free in the stomach).

Five recovered cestodes were all identified as tetraphyllidean plerocercoid larvae. Four of these parasites were firmly attached to the upper intestinal wall, deeply lodged between the villi, and another one was found in the lower intestine. The

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**Table IV.** Frequency distribution of parasitic nematodes, *H. aduncum*, in infected North Sea cod larvae, post-larvae and juveniles in investigated years

<table>
<thead>
<tr>
<th>Number of nematodes/fish</th>
<th>Number of hosts</th>
<th>% hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>245</td>
<td>86.87</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>9.92</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0.71</td>
</tr>
</tbody>
</table>

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![Fig. 4](image_url)  
Comparison of size-at-age of uninfected North Sea cod post-larvae and juveniles with the hosts infected with *H. aduncum* larvae. Age estimates were based on otolith reading (number of daily growth increments).
three SSU sequences obtained from cestodes (2100 bp long) were identical and a BLAST search showed 100% identity with a 430 bp long sequence from *Phyllobothrium* sp. [GenBank: Z98428] and a single nucleotide difference to another sequence (486 bp long) from *Phyllobothrium* sp. [GenBank: Z98429] covering another region of the SSU gene. However, several other species belonging to the subfamily Phyllobothriinae depicted similar identities in these two regions. Highest total similarity (99%) over the entire 2100 bp sequence were found in *Calyptrobothrium* sp. [GenBank: KF685848] and

![Fig. 5. Relationship between prevalence of parasitic copepod, *C. elongatus*, and latitude in different sampling years](image)

![Fig. 6. Relationship between *H. aduncum* prevalence in cod post-larvae and juveniles (10–70 mm) and water depth in the North Sea](image)
Table V. Prevalence and mean intensity of infection with *C. elongatus* and *H. aduncum* in co-infected North Sea cod post-larvae and juveniles in different sampling years

<table>
<thead>
<tr>
<th>Sampling year</th>
<th>Mean length (mm ± sd)</th>
<th>Intensity of <em>H. aduncum</em> (mean ± sd)</th>
<th>Intensity of <em>C. elongatus</em> (mean ± sd)</th>
<th>% co-infected fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>1992*</td>
<td>30.5 ± 7.93</td>
<td>1.0 ± 0.00</td>
<td>1.0 ± 0.00</td>
<td>0.87</td>
</tr>
<tr>
<td>1993</td>
<td>33.0 ± 0.00</td>
<td>1.0 ± 0.00</td>
<td>1.0 ± 0.00</td>
<td>0.06</td>
</tr>
<tr>
<td>1994</td>
<td>26.0 ± 5.65</td>
<td>1.0 ± 0.00</td>
<td>1.0 ± 0.00</td>
<td>0.70</td>
</tr>
<tr>
<td>1999</td>
<td>30.0 ± 5.65</td>
<td>1.0 ± 0.00</td>
<td>2.0 ± 0.00</td>
<td>0.33</td>
</tr>
<tr>
<td>2001*</td>
<td>52.2 ± 7.15</td>
<td>1.2 ± 0.44</td>
<td>1.0 ± 0.00</td>
<td>1.06</td>
</tr>
</tbody>
</table>

Mean length = Mean length of co-infected host
Data for samples collected only in May of each sampling year
*Samples pooled from Skovgaard *et al.* study (2011) into our dataset.

Table VI. Prevalence and mean intensity of infection with parasitic trematodes and cestodes recovered from cod larvae and post-larvae sampled in the North Sea

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Number of parasite(s)</th>
<th>Intensity of parasite (mean ± sd)</th>
<th>Prevalence (%)</th>
<th>Site of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trematoda</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. gibbosus</em></td>
<td>4</td>
<td>1.0 ± 0.00</td>
<td>1.91</td>
<td>Intestine</td>
</tr>
<tr>
<td><em>D. varicus</em></td>
<td>1</td>
<td>1.0 ± 0.00</td>
<td>0.47</td>
<td>Stomach</td>
</tr>
<tr>
<td>Cestoda</td>
<td>5</td>
<td>1.0 ± 0.00</td>
<td>2.39</td>
<td>Intestine</td>
</tr>
</tbody>
</table>

*Crossobothrium longicolle* [GenBank: AF286997] differing at 11 and 17 positions, respectively. Details regarding these parasitic infections are given in Table VI. Due to the low prevalence and abundance of these digeneans and cestodes, no further analysis of their occurrence is presented.

**Discussion**

The present study has documented the occurrence of parasites comprising crustaceans, nematodes, digeneans and cestodes in North Sea cod larvae, post-larvae and juveniles. Previous studies on captive North Sea cod larvae reported a related parasite fauna on a limited number of fish (Karlsbakk *et al.* 2001, Perdiguero-Alonso *et al.* 2008, Heuch *et al.* 2011), but the present survey refers to an investigation of more than 5000 fish from a wild cod population. *C. elongatus* and *H. aduncum* were particularly prevalent and further analyses of these parasites were performed. Frequency distributions and variance to mean ratios (VMR) indicated that these parasite populations were slightly over-dispersed (Table II, III, IV). In a few cases in 1994, a VMR <1 was observed in both nematode and copepod populations (Table III). This could suggest that either parasite-induced host mortality or host immunity may have affected parasite abundance (Kennedy 1975, Anderson and May 1978). Since the larval immune response is generally considered inefficient (Schrøder *et al.* 1998, Chettri *et al.* 2012), parasite-induced mortality is left as a possible explanation (Anderson and May 1978). This hypothesis needs to be investigated under controlled experimental conditions. More than 80% of infected fish hosted only one parasite, and a maximum of 6 caligids and 4 nematodes were recovered from a single host. This could suggest that infection of early stages of cod in the North Sea can impact the cod population. In certain years the prevalence of infection was found to be particularly high (17.5% infected by *C. elongatus* in 1992, and 18.9% infected by *H. aduncum* in 2001), which may lead to increasing pressure on cod survival in these years.

The number of fish infected by both caligids and nematodes was low (prevalence ranging from 0.06% to 1.06% of fish population) (Table V), which would further suggest that survival of cod larvae is influenced by mixed (two species) infections. Host immunity should in this case be regarded as a less likely explanation of the low infection levels, since cod larvae and post-larvae possess inadequately developed immune systems before reaching at least 33 mm in body length (Schrøder *et al.* 1998). This was further supported by observations of nematodes recovered from the fish larvae. In different species of marine fish, serving as transport host for *H. aduncum*, nematode larvae have been found encapsulated by host cells in the body cavity or organs (Berland 1961, Wooten 1978, Fagerholm 1982, Andersen 1993, Koie 1993). This encapsulation process is part of the host defence mechanism in fish which may inactivate the parasite and minimize tissue damage caused by parasite migration (Khan 2012, Buchmann 2012). Therefore, it should be noted that no nematodes recovered from cod post-larvae and juveniles were found encapsulated, which is consistent with the notion of the immune system not being fully developed at this developmental stage of the cod (Schrøder *et al.* 1998).

Prevalence of nematode infections increased with increasing body size of cod, which may reflect that increased food intake over time will enhance the probability of ingesting intermediate hosts carrying infective larvae. In addition, larger cod larvae have increased probability of an encounter with an infective *C. elongatus* larva due to their longer swimming period. However, the suggested negative impact of parasitism at a higher level may be reflected by the low intensity in the larger cod juveniles (>45 mm), indicating low survival if carrying more than one caligid or one nematode per fish. The tol-
erance of the cod juvenile to a single nematode is also inferred by our analysis of the association between fish growth and infection, as no negative influence of a single parasite could be measured. In contrast, the slightly higher fish body length at a certain age of the fish could be interpreted as increased infection probability in more actively feeding fish.

It is noteworthy that latitudinal differences were seen with regard to *C. elongatus* prevalence. Temperature may be the initial explanation, because earlier studies have demonstrated a positive relationship between water temperature and settlement, survival and developmental rate of other parasitic copepods (Conley and Curtis 1993, Hogans 1995, González and Carvajal 2003). However, this connection has been debated by Revie et al. (2002) suggesting that water temperature had no evident effect on mean annual abundance of sea lice, *Lepeophtheirus salmonis*, in farmed Atlantic salmon and could not be a proper explanation for differences in lice levels. Further studies should investigate variability in water salinity, water temperature and other environmental factors due to geographic variations, which may clarify this paradox and explain the variability in sea lice prevalence across sampling years (Zagmutt-Vergara et al. 2005, Khan 2012).

With regard to nematodes, it has been hypothesized that elevated North Sea water temperatures may be favourable for the occurrence of *H. aduncum* (Skovgaard et al. 2011). The present analysis did not show any association between nematode occurrence and latitude, but the infection level increased markedly in the period from 1992 to 2001, during which the North Sea water temperature has increased significantly (Horwood et al. 2006).

We found a slight trend towards elevated nematode prevalence in cod post-larvae and juveniles at water depths around 30–50 m. Different water depths may be associated with distinct habitats in relation to oceanographic conditions (e.g. currents) and plankton community structure including both crustacean parasites and free-living intermediate hosts. No adult stages of the parasitic copepods or nematodes were found in the small cod investigated in this survey, and the infection pressure around the fish larvae is therefore probably based on spreading of infective parasite stages from other organisms hosting the reproductive, adult parasites (Køie 1993, Paisecki 1996). In addition, the diverse habitat structures may provide different opportunities for both free-living (intermediate hosts) and parasitic crustaceans. This advocates for further investigations of the ecology of *H. aduncum* and *C. elongatus* in relation to biotic and abiotic parameters of the North Sea. Knowledge of the associations between salinity, temperature, currents and presence of final and intermediate hosts may eventually lead to a better understanding of cod stock variations in this marine area.

In this study, examination of a limited number of samples for other gastrointestinal parasites revealed the presence of two digenean trematodes, *L. gibbosus* and *D. varicus*, and also cestodes in cod. These trematode species were previously reported from North Sea cod (Karlsbakk et al. 2001, Køie 1984). However, due to the low occurrence of these parasites, no sufficient data were available to conclude about pathogenicity and effects of these recovered parasites on host population.

**Conclusion**

Several parasite types occur in North Sea cod post-larvae and juveniles, but the parasitic crustacean *C. elongatus* and the nematode *H. aduncum* parasitize North Sea cod larvae at a relatively high level, and our analyses suggest that the infections may affect survival especially of the early life stages of cod. The parasite populations and thereby infection pressure in various parts of the North Sea may depend on both biotic and abiotic parameters which indirectly affect the cod population. Therefore, future controlled laboratory investigations are suggested in order to elucidate the pathological impact of *H. aduncum* and *C. elongatus* on juveniles of North Sea cod.

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**References**


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