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# Spatial patterns in infection of cod *Gadus morhua* with the seal-associated liver worm *Contracaecum osculatum* from the Skagerrak to the central Baltic Sea

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**ABSTRACT:** Fish serve as transport hosts to a range of parasites, with potential negative effects on fish health. In the Baltic Sea, the grey seal *Halichoerus grypus* (Fabricius) population has increased markedly since the early 2000s. *H. grypus* is the main final host to the liver worm *Contracaecum osculatum* (Rudolphi, 1802), a parasitic nematode to which cod *Gadus morhua* (Linnaeus) is one of several transport hosts. Recent investigations have shown a marked increase in prevalence and abundance of infection of this parasite in livers of *G. morhua* inhabiting the central Baltic Sea. Yet no recent knowledge exists on levels of *C. osculatum* infection in *G. morhua* in adjacent areas. We investigated spatial differences in prevalence and abundance of this parasitic nematode in livers of *G. morhua*, covering a transect consisting of 9 areas from the Skagerrak to the eastern part of the central Baltic Sea. We further provide survey data of local abundances of *H. grypus* and harbour seal *Phoca vitulina* (Linnaeus) throughout this transect. Prevalence and abundance of *C. osculatum* sensu stricto in *G. morhua* livers differed significantly between east and west, with highest levels of infection occurring in the low-salinity central Baltic areas. Fish in the east had significantly lower condition than their westerly conspecifics. Spatial differences in local seal abundance and seal species, salinity and feeding ecology may explain the observed differences in *C. osculatum* infection between eastern and western *G. morhua*.

**KEY WORDS:** Parasitic nematode · Species interactions · Salinity · Transport host · Condition factor · Feeding ecology · Grey seal · Harbour seal

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## INTRODUCTION

Fish serve as transport hosts to a range of parasites, which often possess complex life cycle strategies. These parasites may negatively affect fish health, which facilitates transmission to the subsequent host (Barber et al. 2000). At times, the parasite-induced effects act conjointly with other stressors (Marcolli & Pietrock 2011). However, usually the ulti-

mate consequences of specific parasites on the health of fish stocks are difficult to assess.

In the Baltic Sea, grey seals *Halichoerus grypus* (Fabricius) are among the top predators, and their role in the ecosystem and potential effects on local fish stocks are presently heavily debated. Their population has increased markedly since the beginning of the 2000s to around 30 000 individuals counted on the haul-out sites in 2017 (Hårding et al. 2007, ICES

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WGMME 2018). In the Baltic region, *H. grypus* is the main final host to the liver worm *Contracaecum osculatum* (Rudolphi, 1802), a parasitic nematode to which cod *Gadus morhua* (Linnaeus) is one of several transport hosts (Valtonen et al. 1988, Lunneryd et al. 2015, Zuo et al. 2018). Recent investigations have shown a marked increase in the prevalence and intensity of infection for this parasite in *G. morhua* of the central Baltic Sea as compared to estimates from the 1980s when *H. grypus* abundance was lower and distribution was narrower (Buchmann & Kania 2012, Haarder et al. 2014, Mehrdana et al. 2014, Nadolna & Podolska 2014, Zuo et al. 2018). Larger-sized *G. morhua* are infected with third-stage *C. osculatum* larvae by eating smaller infected fish, e.g. sprat *Sprattus sprattus* (Linnaeus), their main fish prey in the central Baltic (Zuo et al. 2016). When ingested, the larvae penetrate the stomach of *G. morhua* and then migrate to the liver (hence the common name 'liver worm') where they become encapsulated, a typical host reaction towards larger pathogens (Haarder et al. 2014, Dezfuli et al. 2016a,b, Zuo et al. 2017). In some areas, e.g. in the Western Bornholm Basin and along the Polish coast, single livers may contain several hundred *C. osculatum* larvae, causing structural changes to the organ (Mehrdana et al. 2014, Horbowy et al. 2016, Zuo et al. 2018).

*G. morhua* in the Baltic Sea are divided into 2 stocks, i.e. eastern and western. The eastern Baltic stock (inhabiting mainly the central Baltic) thrived in the early 1980s, decreased gradually to low levels over the following decades and showed signs of a recovery in the mid-2000s, which has been attributed to changes in fishing pressure and environmentally mediated recruitment success (Eero et al. 2015, Köster et al. 2017). However, a gradual deterioration of individual nutritional condition accompanied the increase in their numbers, together with a virtual disappearance of larger fish from the stock. It remains unclear if this is due to increased natural mortality, reduced growth or a combination of both (Eero et al. 2015, Casini et al. 2016, Horbowy et al. 2016). The role of seal-associated liver infection of *G. morhua* with *C. osculatum* may play a role in this situation, but major ecological linkages in the system are still unclear. However, considering that the liver controls various essential metabolic functions and constitutes the main energy reserve for fish, the high infection levels are potentially one factor (among others) involved in the current decrease in nutritional status of *G. morhua* in the central Baltic Sea (Eero et al. 2015). Many *C. osculatum* in the liver may ultimately result in increased natural mortality (Horbowy et al.

2016). Notably, increased nematode parasite infection due to spread of infective stages from seals was observed in *G. morhua* in Newfoundland and Labrador (Canada) before the collapse of those stocks (Bratley et al. 1990).

The core distribution area of *H. grypus* is in the central Baltic between latitudes 58°N and 61°N, where 85% of the moulting seals were counted in 2003 (Hårding et al. 2007). With increasing abundance, the distribution has expanded as well, particularly to the south and west (HELCOM 2017, Olsen et al. 2018). Thus, in 2017, approximately 2700 individuals of *H. grypus* were counted south of 58°N in Sweden, Denmark, Germany and Poland (HELCOM unpubl. data). Furthermore, *H. grypus* are very mobile animals and have been observed to move from the Danish Baltic to breeding haul-outs in Estonia (Dietz et al. 2015). Given the re-colonization and expanding range linked to high mobility, these marine mammals may be expected to spread *C. osculatum* throughout the Baltic Sea. While the recent increase of infection with *C. osculatum* in the central Baltic around Bornholm is well documented (Mehrdana et al. 2014, Horbowy et al. 2016, Zuo et al. 2018).

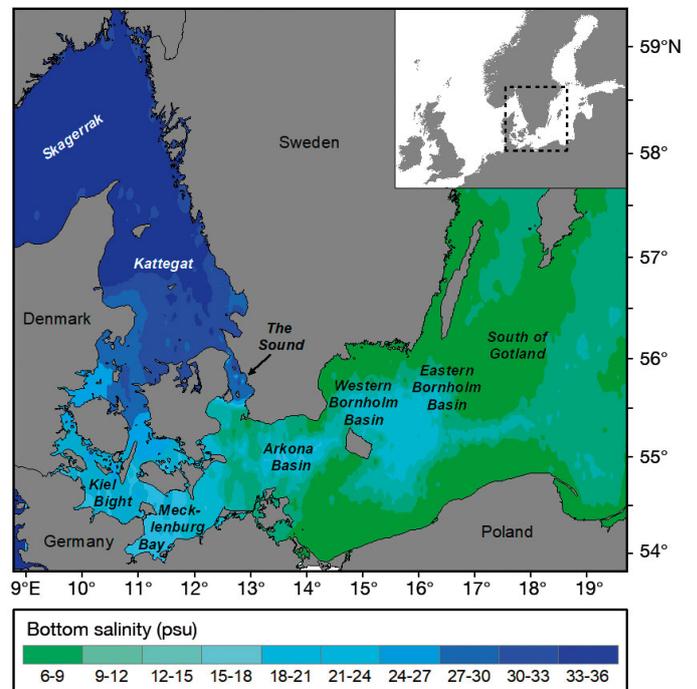


Fig. 1. Nine areas where cod *Gadus morhua* were sampled for analysis of liver worm *Contracaecum osculatum*, covering a transect from the Skagerrak at the northern entrance to the Danish straits to the eastern part of the central Baltic Sea. Distribution of bottom water salinities is shown (based on the ICES Dataset on Ocean Hydrography; <http://ocean.ices.dk/HydChem/HydChem.aspx?plot=yes>)

dana et al. 2014, Eero et al. 2015), it remains presently uncertain to what extent *G. morhua* are infected with *C. osculatum* in adjacent areas towards the west and east. Such knowledge has major implications for our general understanding of factors controlling parasite load and how infection may affect individual fish health and eventually stock status and dynamics.

The aim of the present study was therefore to investigate prevalence and abundance of infection of *C. osculatum* in adult *G. morhua* from 9 different areas, thoroughly covering a transect from the Skagerrak to the central Baltic Sea, including the Danish straits, the western Baltic and the Arkona Basin (Fig. 1). We further compared the condition of *G. morhua* between areas and relate the spatial patterns of infection prevalence and severity to survey data providing local abundances of *H. grypus* and harbour seal *Phoca vitulina*, another known final host for *C. osculatum*. We finally discuss how the differences observed between areas may relate to differences in abiotic (salinity) and biotic factors (seal abundance, differences in prey fields and resulting feeding ecology).

## MATERIALS AND METHODS

### Study areas and fish collection

*Gadus morhua* were caught in 9 selected areas, covering a transect from the Skagerrak to the central part of the Baltic Sea: Skagerrak, Kattegat, the Sound, Kiel Bight, Mecklenburg Bay, Arkona Basin and the central Baltic areas, i.e. western and eastern parts of the Bornholm Basin and south of Gotland (Fig. 1). Fish were sampled in quarter 4 of 2016 (Skagerrak, Kattegat, the Sound, 3 areas in central Baltic) and quarter 4 of 2017 (Kiel Bight, Mecklenburg Bay and Arkona Basin) mainly by bottom trawling during scientific surveys or scientific investigations on commercial vessels, but also via gillnets and pound nets (Mecklenburg Bay, Kiel Bight) (Table 1). To account for the restricted size range of *G. morhua* encountered in the eastern Baltic Sea (*G. morhua* >50 cm total length [TL] are presently rare) and to ensure comparability between areas, only *G. morhua* within the size range of 35 to 50 cm TL were used in the analysis (n = 321 in total). The smallest size of 35 cm was chosen because the proportions of *G. morhua* in the central Baltic infected with *Contracaecum osculatum* increase rapidly in fish above 30–35 cm (Zuo et al. 2016). We also attempted to keep the size range relatively narrow, as infection intensity increases

Table 1. Sampling information of cod *Gadus morhua* sampled from 9 areas for analysis of liver worm *Contracaecum osculatum* (see also Fig. 1), and measured and estimated parameters. Mean  $\pm$  SE values given. TL: total length; W: total wet weight; Prevalence: percentage of infected fish in the sample; Abundance of infection: mean number of parasites per fish, including uninfected individuals; Intensity of infection: mean number of parasites per fish, including only infected individuals; VMR: variance to mean ratio. Poor condition is defined as Fulton's K (based on ungutted individuals) < 0.8

Area	Gear type	Number of livers	TL (cm)	W (g)	Prevalence (%)	Abundance of infection	Intensity of infection	Fulton's K	Fish in poor condition (%)	VMR
Skagerrak	Bottom trawl	9	38 $\pm$ 1	666 $\pm$ 101	33	1 $\pm$ 0.35	2 $\pm$ 0.5	1.13 $\pm$ 0.03	0	1.7
Kattegat	Bottom trawl	38	41 $\pm$ 0.7	753 $\pm$ 35	37	1 $\pm$ 0.1	2 $\pm$ 0.8	1.04 $\pm$ 0.01	0	1.4
The Sound	Bottom trawl	51	40 $\pm$ 0.5	720 $\pm$ 27	51	1 $\pm$ 0.15	2 $\pm$ 0.2	1.08 $\pm$ 0.01	0	1.3
Kiel Bight	Gill net/Pound net	30	45 $\pm$ 0.5	958 $\pm$ 31	0	0 $\pm$ 0.00	0 $\pm$ 0	1 $\pm$ 0.02	0	0.0
Mecklenburg Bay	Gill net	26	44 $\pm$ 1	952 $\pm$ 41	15	1 $\pm$ 1	6 $\pm$ 2	1 $\pm$ 0.02	0	7.3
Arkona Basin	Bottom trawl	46	41 $\pm$ 1	633 $\pm$ 32	72	15 $\pm$ 3	21 $\pm$ 4	0.9 $\pm$ 0.02	15	31.4
Western Bornholm Basin	Bottom trawl	41	39 $\pm$ 0.6	557 $\pm$ 31	100	27 $\pm$ 4	27 $\pm$ 4	0.91 $\pm$ 0.01	10	18.8
Eastern Bornholm Basin	Bottom trawl	40	38 $\pm$ 0.4	447 $\pm$ 18	100	40 $\pm$ 4	40 $\pm$ 4	0.82 $\pm$ 0.02	40	17.0
South of Gotland	Bottom trawl	40	37 $\pm$ 0.2	436 $\pm$ 10	90	29 $\pm$ 5	32 $\pm$ 5	0.89 $\pm$ 0.02	15	30.4

with fish length, at least in the Baltic proper (Horbowy et al. 2016). In this way, a potential effect of differences in fish size among areas was minimized.

*G. morhua* were either (1) immediately frozen whole onboard (Kattegat, the Sound and the 3 central Baltic areas) and then thawed and processed in the laboratory following routine procedures (determination of TL, whole-fish wet weight, sex, removal of liver), (2) transported fresh to the laboratory and processed there (Kiel Bight, Mecklenburg Bay) or (3) processed onboard (Skagerrak, Arkona Basin). Upon processing, individual livers were kept at  $-20^{\circ}\text{C}$  for subsequent analysis of the number and species of nematodes.

#### Analysis of livers for nematodes

All analyses of livers for the presence of parasitic nematodes were performed at the Laboratory of Aquatic Pathobiology, University of Copenhagen (Frederiksberg, Denmark). Individual livers were thawed, placed in plastic bags ( $200 \times 400 \times 0.07$  mm) between 2 glass plates ( $15 \times 15 \times 1$  cm) and compressed to 1 mm thickness by adding gentle pressure to the plates (Buchmann 2007). Livers were examined under a Leica stereo microscope (6.3–40 $\times$  magnification) (Leica Microsystems Germany), and detected nematodes were rinsed in tap water and isolated in Petri dishes. The nematode species identification was based on morphometric characteristics of the caudal and cephalic ends according to Fagerholm (1982). After species identification, nematodes were stored in 96% ethanol (Kemetyl) in V-bottom 50 ml plastic tubes for subsequent molecular identification.

For all areas, prevalence of infection (percentage of infected fish in the sample), abundance of infection (mean number of parasites per host, including both infected and uninfected individuals), and intensity of infection (mean number of parasites per host, including only infected individuals) were calculated according to Bush et al. (1997). The 15 cm size range was not further stratified and means refer to a sampled area. Furthermore, Fulton's  $K$  was calculated as  $F = \left(\frac{W}{L^3}\right) \times 100$ , where  $W$  is the wet weight (g) of the whole fish and  $L$  is TL (cm) (Ovegård et al. 2012). The proportion of *G. morhua* in poor nutritional condition, i.e. with Fulton's  $K < 0.8$  (Martensdottir & Begg 2002) was determined for each area. Finally, the variance to mean ratio (VMR) was calculated as  $\text{VMR} = \frac{\sigma^2}{\mu}$ , where  $\sigma^2$  is the sample variance and  $\mu$  is a mean value of the number of parasites per individual fish (Upton & Cook 2006).

#### Genetic analysis of parasites

For verification of the nematode species and type, PCR and sequencing were performed for the internal transcribed spacer (ITS) region and the cytochrome oxidase subunit II (*COX2*) gene, respectively. A subsample of 24 worms from the Sound, in addition to 5 nematodes from Skagerrak and south of Gotland that were unidentifiable by morphometric analysis) were selected for molecular analysis of the ITS region. Further, a subsample of 9 worms from the Kattegat and 1 worm from Skagerrak were selected for molecular analysis of the *COX2* in order to differentiate between types of *C. osculatum*. Parasite tissue (the middle part of each individual) was incubated in 10 $\times$  lysis buffer (100% Tween20, 0.5 ml EDTA, Trisbase,  $\text{H}_2\text{O}$ , Proteinase K, Qiagen) at  $55^{\circ}\text{C}$ , 500 rpm for 2 h. Complete tissue digestion was subsequently confirmed by light microscopy. Following lysis, Proteinase K was inactivated by heating the sample to  $95^{\circ}\text{C}$  for 10 min.

Species diagnosis was performed by sequencing the ITS and the type diagnosis by sequencing the *COX2*. In the case of ITS, NC5 (5'-GTA GGT GAA CCT GCG GAA GGA TCA TT-3') [10  $\mu\text{M}$ ] was used as forward primer, and NC2 (5'-TTA GTT TCT TTT CCT CCG CT-3') [10  $\mu\text{M}$ ] as reverse primer. The mitochondrial gene *COX2* was amplified using 211F (5'-TTT TCT AGT TAT ATA GAT TGR TTY AT-3') [10  $\mu\text{M}$ ] and 210R (5'-CAC CAA CTC TTA AAA TTA TC-3') [10  $\mu\text{M}$ ] as forward and reverse primers, respectively. Both regions were amplified in a 60  $\mu\text{l}$  reaction volume per sample containing 10 $\times$  PCR buffer, dNTP (4 $\times$  10 mM),  $\text{MgCl}_2$  (1.5 mM),  $\text{H}_2\text{O}$ , polymerase (Bioline) and the template. PCR conditions of the ITS region and the *COX2* gene were the same as reported by Zhu et al. (2007) and Zuo et al. (2018), respectively. PCR products were analysed by agarose gel electrophoresis in ethidium bromide stained 1% agarose gels and subsequently purified using the Illustra™ GFX™ PCR DNA purification kit (GE Healthcare) and sequenced (Macrogen Korea). The obtained sequences were analysed using the software CLC Main Workbench v7.9.1 (Qiagen) and confirmed by a BLAST® search of GenBank.

Maximum likelihood phylogenetic analysis (1000 bootstraps) was performed using the *COX2* gene. The sequence alignment was achieved by ClustalW. The model GTR+G+T was chosen, as this model was the best choice in 3 of 4 tests (Akaike's information criterion [AIC], AIC corrected for small sample sizes [AICc] and the Bayesian information criterion [BIC]). The fourth test (hierarchical likelihood ratio test) had the HKY+G+T as the first choice and GTR+G+T as

the second choice. The sequences obtained in this study were compared to a series of other *Contracaecum* species and their subtypes (*C. osculatum* A, B, D, sensu stricto [s.s.] and E; GenBank accession nos. EU477203–7, respectively) together with *C. radiatum* (EU477213), *C. omorhini* (EU477211), *C. margolisi* (EU477212), *C. moroungae* (EU477213), *C. rudolphii* A (EF535570) and *C. rudolphii* B (EU852349). The 2 nematodes *Pseudoterranova ceticula* (DQ116435) and *Ascaris suum* (X54253) were used as the outgroup.

### Genetic analysis of *G. morhua* from the Arkona Basin

*G. morhua* from the Arkona Basin were analysed with genetic markers to identify population of origin (Eero et al. 2014), as this geographical region is an area of mixing between eastern and western Baltic cod populations (Eero et al. 2014, Hüssy et al. 2016). Here, we used 187 single nucleotide polymorphisms (SNPs) that provided high statistical power for assigning fish to either of the 2 Baltic populations (Nielsen et al. 2012). We used fish collected at spawning time from eastern and western Baltic populations as base lines and assigned fish from the Arkona region through a Bayesian approach (Rannala & Mountain 1997) implemented in the program GeneClass2 (Piry et al. 2004). Individuals were assigned based on maximum assignment scores (ratio of assigned sample likelihood to the sum of all likelihoods), and 2 individuals with assignment scores below 99% were excluded from the analyses.

### Abundance of *Halichoerus grypus* and *Phoca vitulina* in the Baltic Sea

Haul-out counts during the respective moulting seasons of *H. grypus* and *P. vitulina* were used as proxies for seal distribution. Seals may be distributed differently between seasons, and haul-out use during the moulting season does not translate directly to distribution patterns at sea. However, we assume that haul-out counts constitute an approximation of at-sea distribution of seals, at a level of accuracy that is adequate in the context of this study, where parasite life cycle completion depends on a significant presence of suitable final hosts in the area. Haul-out counts of seals were collected following Galatius et al. (2014). To avoid bias caused by fluctuations during a single year, data from 2015–2017 were used. For Sweden, 2015–2016 estimates were used since data for 2017 were not yet available.

For *H. grypus* and *P. vitulina*, during the moulting season each locality was counted 1–3 and 2–3 times, respectively. The average of the 2 highest counts for a given locality in a given year (or the single count) was used to calculate the average, to minimize the effects of, e.g., disturbance of the haul-out prior to the count.

The study area included *H. grypus* from 2 populations, which overlap, but have different moulting and breeding seasons (Fietz et al. 2016). Data from Fietz et al. (2016) indicate that seals originating from the North Sea make up the majority of *H. grypus* in the Kattegat, while in the Danish Baltic, most *H. grypus* are of Baltic origin. Thus, survey data collected during the North Sea moulting season in March–April were used for the Kattegat, while data from the Baltic moulting season in May–June were used elsewhere. *P. vitulina* surveys were conducted during their moulting season in August.

*H. grypus* individuals are not counted during their moulting season along the Swedish west coast, so the absence of this seal species along this coastline in our data does not mean that *H. grypus* are absent from that area. However, data on *H. grypus* presence and abundance in this area were collected during moult surveys for *P. vitulina* in August.

### Statistical analysis

An ANOVA of the linear model (Eq. 1) was used to examine if there was any difference in fish length ( $L_i$ ; cm) between areas, where area ( $\beta_{\text{area}_i}$ ) was used as a descriptive variable, and subscripts  $l$  and  $i$  denote the individual fish and different areas, respectively:

$$\widehat{L}_i = \beta_{\text{area}_i} \quad (1)$$

In order to test dependence of abundance of infection ( $\widehat{\mu}_i$ ), 4 different variables were included in the generalized linear model (GLM) of negative binomial family: Fulton's  $K$  ( $F_i$ ) and length ( $L_i$ ) as continuous variables, and sex ( $\delta_{\text{sex}_i}$ ) and area ( $\beta_{\text{area}_i}$ ) as categorical variables:

$$\widehat{\mu}_i = \alpha_1 F_i + \alpha_2 L_i + \beta_{\text{area}_i} + \delta_{\text{sex}_i} \quad (2)$$

Further, the significance of each parameter was tested through a likelihood ratio test. Thus, the final model (Eq. 3) contained  $F_i$ ,  $L_i$  and  $\beta_{\text{area}_i}$  as descriptive variables:

$$\widehat{\mu}_i = \alpha_1 F_i + \alpha_2 L_i + \beta_{\text{area}_i} \quad (3)$$

The differences in abundance of infection between separate study areas were analysed and illustrated through visualization of model predictions.

Examination of the difference in the prevalence of infection ( $\hat{\eta}_i$ ) between areas was obtained using a likelihood ratio test of GLM of the binomial family containing  $F_i$ ,  $L_i$  and  $\beta_{\text{area}_i}$  as descriptive variables:

$$\hat{\eta}_i = \alpha_1 F_i + \alpha_2 L_i + \beta_{\text{area}_i} \quad (4)$$

and the model without area as a descriptive variable:

$$\hat{\eta}_i = \alpha_1 F_i + \alpha_2 L_i \quad (5)$$

where prevalence was *G. morhua* coded as '1' for infected fish and '0' for not-infected fish. The statistical tests were carried out in R version 3.3.2. (www.R-project.org).

## RESULTS

Sex was not significant ( $p > 0.05$ ) when testing for significance of the descriptive parameters (sex [ $\text{sex}_i$ ], length [ $L_i$ ], Fulton's  $K(F_i)$  and area [ $\text{area}_i$ ]) on the mean abundance and prevalence of infection. Hence, sex was removed from the subsequent models (models 3 and 5, respectively).

TL of sampled fish was significantly higher in the western areas (Kiel Bight and Mecklenburg Bay) as compared to the other areas ( $p < 0.05$ ), and there was a significant negative correlation between TL and abundance of infection. In contrast, no significant differences in abundance of infection between male and female fish was found in any of the areas ( $p > 0.05$ ) (Table 1).

The nematodes belonged to the species *Contracaecum osculatum* as determined by sequencing of the ITS region. Further sequencing and phylogenetic analysis of the mitochondrial gene *COX2* revealed that the nematodes were of the *C. osculatum* s.s. type.

### Prevalence and abundance of *C. osculatum*

The prevalence of infection with *C. osculatum* differed significantly between areas ( $p < 0.05$ ). While it was 90–100% in the 3 central Baltic areas, it was 33–51% in the Sound, Kattegat and Skagerrak, and lowest in the Mecklenburg Bay and Kiel Bight (15 and 0%, respectively). The Arkona Basin (72%) was intermediate between the eastern and northwestern areas (Table 1, Figs. 2 & 3A). A similar trend was found for the abundance of

infection (Table 1, Figs. 2 & 3B): it was highest in the eastern areas, i.e. western and eastern parts of Bornholm Basin and south of Gotland, lower in the Skagerrak, Kattegat and the Sound, and lowest in the southwestern Baltic (Kiel Bight and Mecklenburg Bay). Most infected were livers of *Gadus morhua* from the eastern Bornholm Basin, where two-thirds of the livers carried  $\geq 20$  parasites; 32 and 55% of the livers from the western Bornholm Basin and south of Gotland contained  $> 30$  worms (Fig. 2). The abundance in the Arkona Basin was intermediate, being significantly lower than in the central Baltic areas, but significantly higher than in the Skagerrak, Kattegat, the Sound, Kiel Bight and Mecklenburg Bay (Fig. 3B). However, a different picture emerged when *G. morhua* sampled in the Arkona Basin were split into fish of western and eastern origin based on the genetic analyses. Of the 46 fish from the Arkona Basin that could be assigned based on genetics, 13 were assigned to the western and 33 to the eastern stock. Notably, the abundance of infection with *C. osculatum* in their livers was significantly higher for the fish assigned to the eastern stock ( $21 \pm 4$  *C. osculatum*, mean  $\pm$  SE) as compared to the western stock ( $1 \pm 1$ ) ( $p < 0.01$ ).

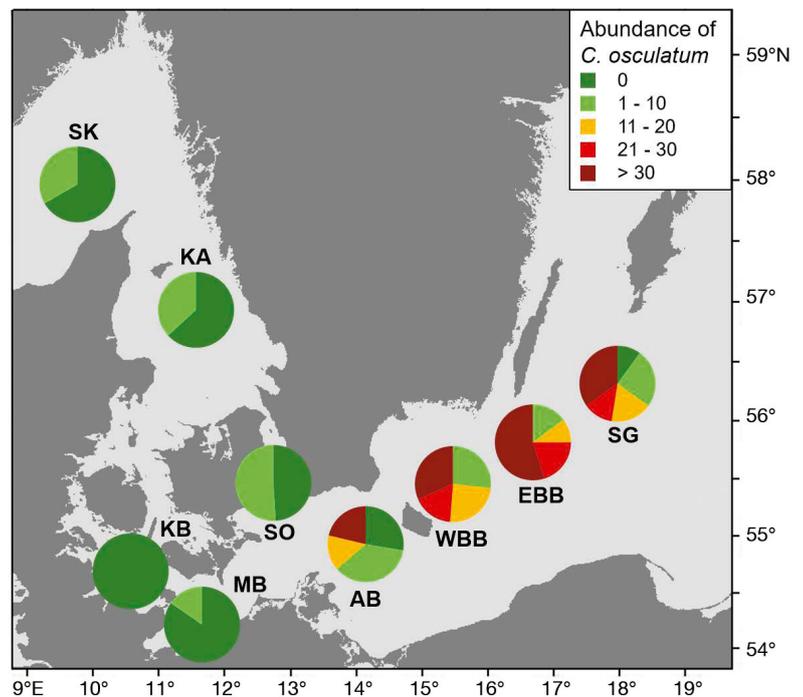


Fig. 2. Abundance of *Contracaecum osculatum* infection (mean number of parasites per liver of *Gadus morhua*, including uninfected individuals) in the 9 study areas: Skagerrak (SK), Kattegat (KA), the Sound (SO), Kiel Bight (KB), Mecklenburg Bay (MB), Arkona Basin (AB), Western Bornholm Basin (WBB), Eastern Bornholm Basin (EBB) and south of Gotland (SG)

### Fulton's condition factor, $K$

*G. morhua* from the western areas with low infection were in good nutritional condition (average Fulton's  $K \geq 1.0$ , no fish with Fulton's  $K \leq 0.8$ ). In contrast, a substantial proportion of the more heavily parasitized eastern conspecifics from the Arkona Basin and eastwards were in poor nutritional condition, i.e. with Fulton's  $K < 0.8$ . Specifically, 15, 10, 40 and 15% of *G. morhua* from the Arkona Basin, the western and eastern Bornholm Basin, and South of Gotland, respectively, had Fulton's  $K < 0.8$  (Table 1). The overall trend in Fulton's  $K$  across areas was opposite to the trends for the area-specific abundance of infection with *C. osculatum* (Fig. 4). For the Arkona Basin alone, *G. morhua* assigned to the east-

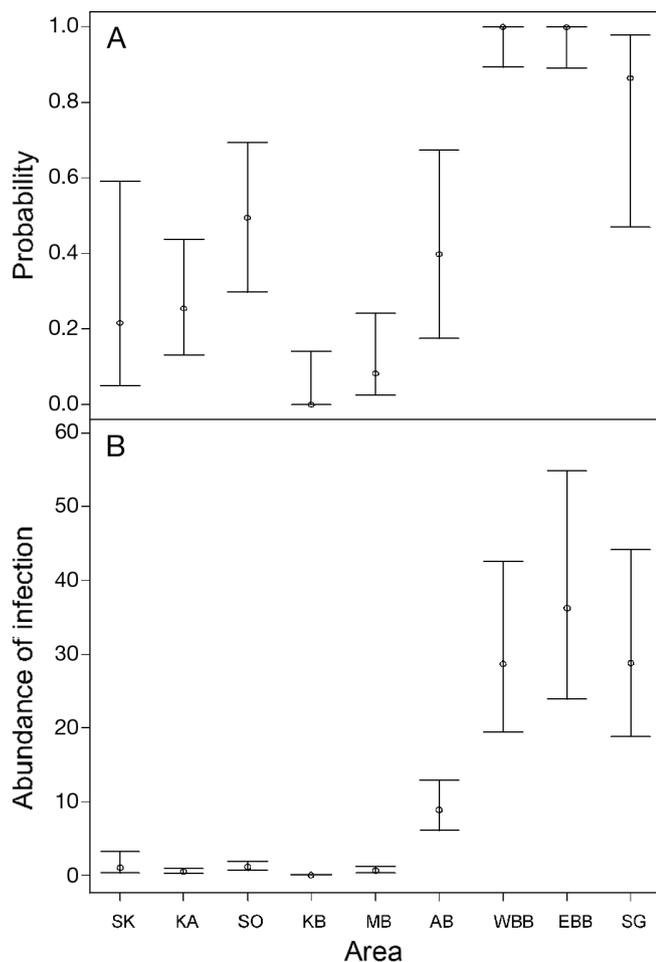


Fig. 3. Generalized linear modelling results from predictions of (A) log prevalence of infection (percentage of infected fish in the sample) in the 9 study areas (output from model 4) and (B) the abundance of infection (mean number of parasites per *Gadus morhua*, including uninfected individuals; output from model 3). Mean  $\pm$  95% confidence intervals are shown. Area abbreviations as in Fig. 2

ern stock had significantly lower Fulton's  $K$  (average = 0.9) than their conspecifics from the western stock (average = 1.0) ( $p < 0.05$ ).

### Frequency distributions of *C. osculatum*

Except for the eastern Bornholm Basin, where the majority of *G. morhua* livers contained 21–30 *C. osculatum*, most fish from all other areas had 0–10 parasites in their livers (Fig. 5). In fact, in Skagerrak, Kattegat, the Sound, Kiel Bight and Mecklenburg Bay, no *G. morhua* carried more than 10 parasites. In contrast, most livers from the 3 areas in the central Baltic contained higher and highly variable numbers of parasites. Some individual livers of *G. morhua* had up to 121–130 parasites (Fig. 5). This resulted in a highly overdispersed frequency distribution and was also reflected in high VMR values (17.0–30.3, Table 1). Also, the Arkona Basin displayed high variability of infection (but at an intermediate level) with 30 out of 47

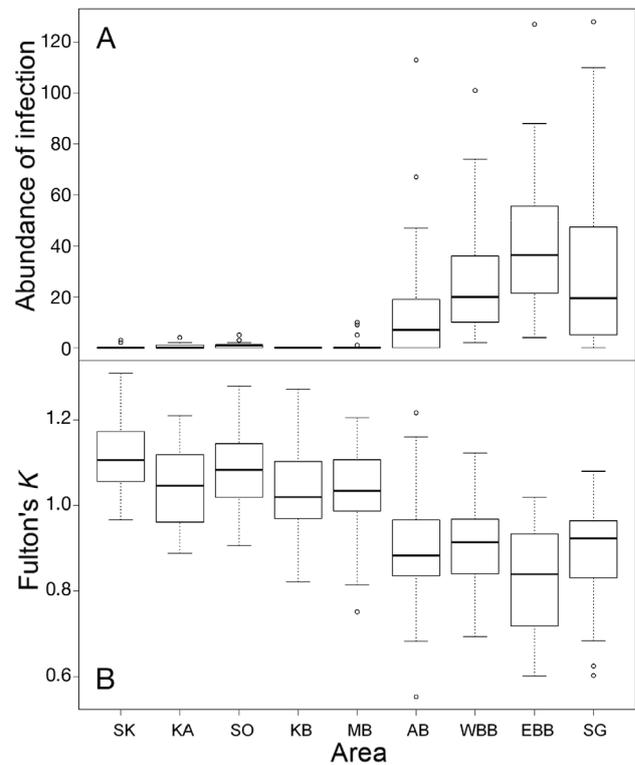


Fig. 4. (A) Abundance of *Contracaecum osculatum* infection (mean number of parasites per liver of *Gadus morhua* including uninfected individuals) in *G. morhua* livers. (B) Fulton's  $K$  of *G. morhua* from the 9 areas. Area abbreviations as in Fig. 2. Solid line: median; box: interquartile range (IQR), where bottom and top are 25th and 75th percentiles, respectively; whiskers: max/min observation if within 1.5 of the IQR, or  $1.5 \times$  IQR. Circles: observations not covered in the area between the whiskers (outliers)

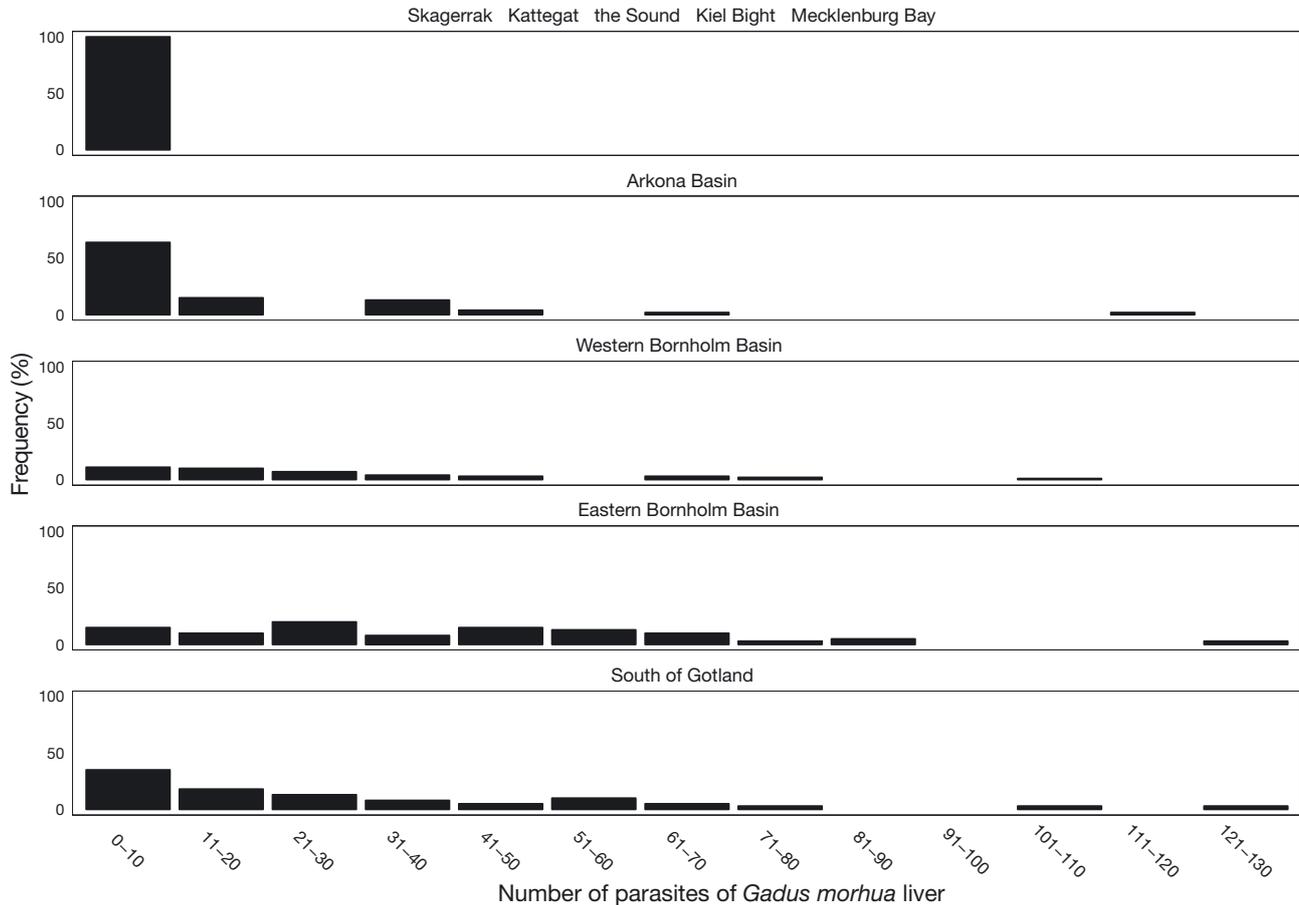


Fig. 5. Frequency distribution diagrams of *Contracaecum osculatum* in *Gadus morhua* livers in the 9 areas

*G. morhua* carrying from 0 to 10 *C. osculatum* in their liver while a single individual carried 113 parasites, resulting in the highest VMR for that area (31.4).

#### Nematodes other than *C. osculatum* in *G. morhua* livers

Of the 3830 parasites analysed in the 3 central Baltic areas (western and eastern parts of the Bornholm Basin and south of Gotland), only 6 specimens were not *C. osculatum* (<0.002%). Three of these could not be identified, 1 was herring or whale worm *Anisakis simplex* (Rudolphi, 1809), and the remaining 2 were *Hysterothylacium aduncum* (Rudolphi, 1802). No parasites were found in the livers of *G. morhua* from Kiel Bight, and only 1 (unidentifiable) parasite besides *C. osculatum* was found in Mecklenburg Bay. The highest number of parasites other than *C. osculatum* (a subsample of 15 was confirmed by sequencing) was found in *G. morhua* livers from the Sound, with 1 *A. simplex*, 2 *H. aduncum* and

6 seal worm or cod worm *Pseudoterranova decipiens* (Krabbe, 1878), which were species confirmed by sequencing, in addition to 8 unidentifiable parasites. In Kattegat, 7 unidentifiable and 3 *A. simplex* were found. In Skagerrak, 2 *A. simplex* and 3 *H. aduncum* (species confirmed by DNA sequencing) were found.

#### Seal distribution

From Skagerrak to the central Baltic Sea, the number of *Halichoerus grypus* increases by orders of magnitude, while *Phoca vitulina* display the opposite pattern (Fig. 6). *P. vitulina* are abundant in the Skagerrak and Kattegat, with around 15 000 individuals along the Swedish and Norwegian coasts and the Danish islands. In the Sound and the Danish straits, *P. vitulina* density is much lower. Further east, they are rare, except for an isolated population in Kalmarsund, in the northern central Baltic.

In contrast, *H. grypus* are rare in the Skagerrak and Kattegat (only 10–25 along the entire Swedish

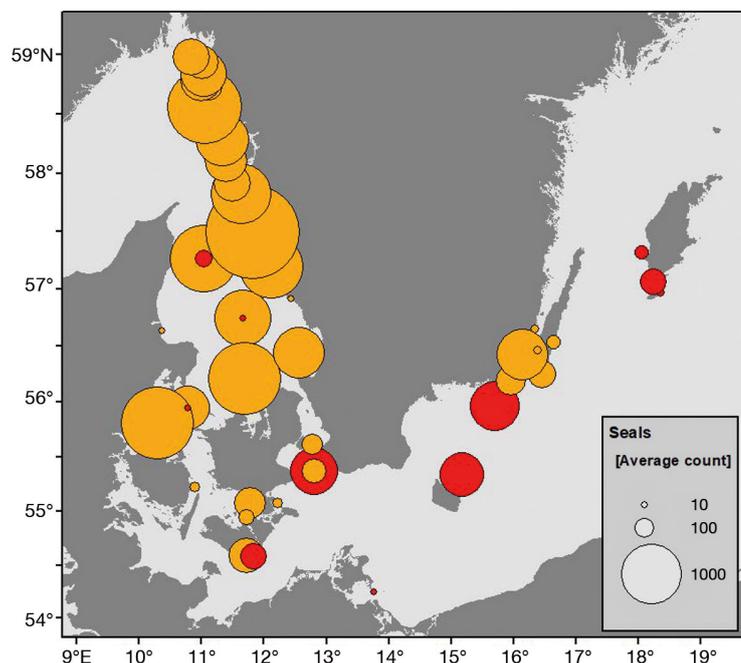


Fig. 6. Distribution of *Halichoerus grypus* (red) and *Phoca vitulina* (orange) on haul-outs based on surveys during their respective moulting seasons. Data from Denmark and Germany are from 2015–2017, data from Sweden are from 2015–2016

west coast north of Skåne, counted outside the grey seal moulting season; M. Ahola pers. comm.), and only around 100 *H. grypus* were counted in the Danish Kattegat. The most important haul-outs of *H. grypus* in the western Baltic are found at Falsterbo at the border between the Sound and the Arkona Basin and Rødsand in the northern Mecklenburg Bay. Further, into the Baltic, there are important haul-outs at Christiansø and Utklippan in the central Baltic as well as off southern Gotland with hundreds of individuals at the haul-outs.

## DISCUSSION

Our study reveals a clear spatial pattern in prevalence and abundance of infection with *Contracaecum osculatum* s.s. in *Gadus morhua* livers sampled along a transect from the Skagerrak to the central part of the Baltic Sea, with highest levels of infection in the low-salinity areas from the Western Bornholm Basin to the south of Gotland. The high prevalence and abundance of *C. osculatum* in *G. morhua* livers in the Western Bornholm Basin are in line with Haarder et al. (2014), whereas we show for the first time that infection levels are equally severe further eastwards in the central Baltic. We also reveal that

*C. osculatum* infection is less prevalent for *G. morhua* in the more westerly areas with higher salinities, comprising the western Baltic, the Sound, the Kattegat and the Skagerrak. As the fish in Kiel Bight and Mecklenburg Bay were considerably larger and almost without *C. osculatum* in the livers, we found (contrary to initial expectations) a negative correlation between TL and abundance of infection when considering the entire data set. When looking solely at fish from the 3 areas in the central Baltic, no correlation between TL and abundance of infection was found (data not shown). Thus, our selected size range of *G. morhua* (35–50 cm) did not introduce any relevant bias to our results.

Notably, *G. morhua* caught in the Arkona Basin showed pronounced inter-individual variation, with an infection pattern between that of the eastern and western areas, i.e. some individuals had very high numbers of *C. osculatum* in the liver whereas others had none or few parasites. The Arkona Basin is a mixing area for the western and the eastern Baltic stocks of *G. morhua*, i.e. individuals from both stocks co-occur in this area (Hüssy et al. 2016). The present genetic assignment results strongly suggest that the difference in infection patterns is linked to the population of origin, and shows that this region comprises a mixture of *G. morhua* stocks with high and low burdens of *C. osculatum*. Thus, on an overall geographical scale, patterns of infection aligned well with the geographical distributions of the 2 main Baltic Sea *G. morhua* populations, with eastern Baltic fish showing higher levels of infection than western Baltic conspecifics.

In addition, the present data are in agreement with similar studies, revealing that a substantial proportion of *G. morhua* from the central Baltic Sea are in poor nutritional state. This pattern of low condition coincides with the highest numbers of *C. osculatum* in the livers of the most eastern fish. However, the degree to which this is a causal correlation is not deducible with the present sampling design, but should be investigated preferably through controlled experiments, or appropriate field sampling designs. Whether fish already in poor condition may be more susceptible to infection also warrants future elucidation.

The clear area effect displayed by increased prevalence and abundance of infection with *C. osculatum* in *G. morhua* livers towards the central Baltic coincides with parallel spatial trends in (1) seal species composition and abundance, (2) salinity and (3) differences in prey fields and resulting differences in the feeding ecology of *G. morhua* and seals. There

may be inter-correlations among these factors, and they may also correlate with other factors that actually influence infection in addition to or instead of the investigated factors. Yet they may, alone or in combination, help to explain the observed differences in infection between east and west.

### Seals

Seals are a prerequisite for *C. osculatum*, as they act as final host for the parasite (Køie & Fagerholm 1995, Zuo et al. 2018). Distribution of *Halichoerus grypus* overlaps to some extent with the areas with greater infection levels and higher abundances of *C. osculatum* in *G. morhua* livers, while the *Phoca vitulina* distribution seems inversely related to infection levels. This minor role of *P. vitulina* may be explained by their suitability as the final host of this parasite species. Studies by Lunneryd (1991) indicated that *P. vitulina* in the area covering Skagerrak, Kattegat and the Baltic carry very few adult *C. osculatum* parasites, whereas *H. grypus* in the Baltic may carry more than 1000 adult individuals (Lunneryd et al. 2015, Zuo et al. 2018). *P. vitulina*, on the contrary, host many *Pseudoterranova decipiens*, the life cycle of which seems constrained by the lower salinities in the easternmost areas of the Baltic (Buchmann & Kania 2012). This complies well with our results, revealing *P. decipiens* in livers from *G. morhua* in the Sound, but not in the easternmost areas.

There are important haul-outs with hundreds of *H. grypus* individuals counted in Mecklenburg Bay and in the Arkona Basin; nevertheless, the infection of *G. morhua* livers with *C. osculatum* in these areas was lower than in the central Baltic. However, telemetry studies of *H. grypus* equipped with GPS tags at these localities have also revealed that movements to the north and west from the haul-outs are limited while the tagged seals make frequent and much longer forays further into the Baltic (Dietz et al. 2015). If occurrence of *H. grypus* is the main limiting factor for local infection levels of *G. morhua* livers by *C. osculatum*, it should be expected that the areas presently showing low infection levels would become more heavily affected in the future. Historical sources and archaeological evidence indicate that *H. grypus* was the most common seal species in the Kattegat and the inner Danish waters and that culling campaigns and hunting shifted the balance in favour of *P. vitulina* (Olsen et al. 2018). In the absence of these pressures, it is likely that *H. grypus* will recolonize these areas in larger numbers.

### Salinity

Salinity may also be a driving factor for the observed spatial differences. Aquatic species that are adapted to and thrive in brackish waters are potentially challenged physiologically in marine waters, resulting in reduced performance and survival (Behrens et al. 2017). The present study areas display a steep salinity gradient, from the brackish central Baltic Sea to the oceanic Skagerrak. The distribution of *C. osculatum* in marine fishes includes fully marine areas in the North Atlantic (Greenland, Iceland) but it cannot be excluded that the present nematode sibling species *C. osculatum* s.s. is favoured by lower salinities as found in the Baltic. This complies with both lower prevalence and abundance of *C. osculatum* in some marine waters. Studies have reported no or low (5–10%) prevalence of *C. osculatum* in the highly saline central North Sea, the Celtic Sea, the Irish Sea and Icelandic waters (Perdiguero-Alonso et al. 2008, Gay et al. 2018). Notably, we only investigated livers in the present study and no other parts of the viscera, and recent investigations have shown that at least in the Barents Sea, different sibling species of *C. osculatum* (*C. osculatum* B) may favour sites other than the liver (K. MacKenzie unpubl. data). However, besides salinity, a series of other ecological factors differ between the North Atlantic and the Baltic, and controlled life cycle studies are required to elucidate the association between salinity and life cycle success of *C. osculatum*. Although the Kiel Bight—with intermediate salinity conditions—was the only area in the present study where no *C. osculatum* was found in *G. morhua* livers, it is also relatively distant from *H. grypus* haul-out areas.

### Prey fields and feeding ecology

Besides seals and salinity, dissimilarities in prey fields of *G. morhua* between east and west and the resulting differences in feeding ecology of the fish may also explain the difference in levels of infection with *C. osculatum*. As mentioned above, the 9 study areas comprise a transition zone between the marine North Sea and the brackish Baltic Sea, with higher salinities in the west and lower salinities in the east. Many species meet their physiological limits along this salinity gradient, resulting in a higher species richness in the west as compared to the east, including both benthos and fish (Bonsdorff 2006, Pecuchet et al. 2016) which constitute the main prey items of *G. morhua*. In fact, this difference is reflected in the

diet composition of the fish, which is rather diverse in the western areas. Here, it includes high shares of benthic invertebrates such as different crustaceans (mainly the common shore crab *Carcinus maenas*, Linnaeus), but also molluscs (mainly bivalves) and Annelida at all depth strata as well as various fish species, while herring *Clupea harengus* (Linnaeus) and *Sprattus sprattus* only constitute a minor share (Funk 2017). In contrast, the feeding ecology of *G. morhua* in the lower saline central Baltic is quite different. Due to physiological constraints at low salinity, *C. maenas* abundance sharply decreases eastwards (Maagard & Rheinheimer 1974, Dries & Adelung 1982). Instead, the isopod *Saduria entomon* (Linnaeus) has historically been an important benthic prey of *G. morhua* in these more eastern areas of the Baltic. However, expanding hypoxic areas in recent years in the central Baltic Sea (Hinrichsen et al. 2011) have resulted in reduced availability of key benthic invertebrate prey species such as *S. entomon*, increasing the reliance of *G. morhua* on abundant piscine prey, in particular *S. sprattus*, and to a lesser extent *C. harengus* (Pachur & Horbowy

2013, Casini et al. 2016). *S. sprattus* was recently identified as a major transport host of *C. osculatum* in the central Baltic, and increasing size-specific infection coincided with *G. morhua* sizes at which increasing shares of *S. sprattus* are included in their diet (Zuo et al. 2016). This may have contributed to higher infection rates of *G. morhua* in the central Baltic.

In addition, even though information about *H. grypus* diets in our study area is relatively limited, there are also indications that the diet of *H. grypus* in the western areas is more diverse than in the eastern areas where the main prey species are *C. harengus*, *G. morhua* and *S. sprattus* (Zrust 2017, Scharff-Olsen et al. in press). In summary and as depicted schematically in Fig. 7, the trophic interlinkages between seals, *S. sprattus* and *G. morhua* are much stronger in the eastern than in the western areas. This may lead to a higher and faster transfer of parasites between the involved species in the eastern areas, which could in turn explain the pronounced area effect in prevalence and abundance of infection observed in the present study.

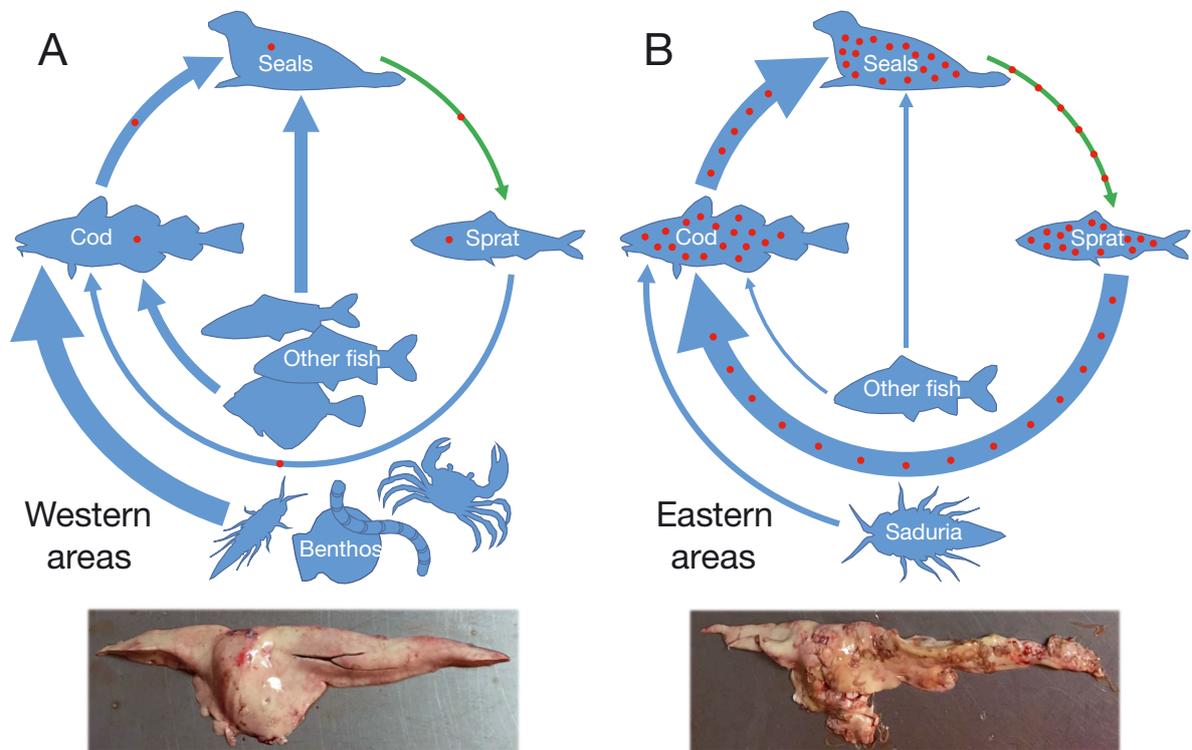


Fig. 7. Potential effects of variable prey composition of *Gadus morhua* and seals on their parasite load in (A) western and (B) eastern Baltic areas. Red dots = parasites, blue arrows = direct predation, green arrows = transport of parasites via indirect predation (i.e. parasite larvae released with seal faeces and ingested first by copepods or other zooplankton taxa and then by sprat). The width of the arrows indicates differences in the ingested proportions of different prey types. Photographs show examples of little (left) and heavily infected (right) *G. morhua* livers from western and eastern areas, respectively (photos by B. Huwer)

## Condition factor

Long-term data reveal a substantial and persistent deterioration of the nutritional state of *G. morhua* in the central Baltic since the 1990s, and in 2011, 15% of the fish (40–60 cm) were in poor nutritional state (Fulton's  $K < 0.8$ ) (Hinrichsen et al. 2011, Eero et al. 2012, Casini et al. 2016, Reusch et al. 2018). Our results comply well with this, as we found on average 20% of *G. morhua* to be in poor nutritional state in the eastern areas. Several factors may explain this poor health status. Firstly, there is a spatial mismatch between *G. morhua* and *S. sprattus*, presently the main prey of *G. morhua* (Eero et al. 2012, Casini et al. 2016, Reusch et al. 2018). Secondly, expanding hypoxia in recent years has led to reduced abundance of benthic prey (Pachur & Horbowy 2013, Casini et al. 2016, Reusch et al. 2018). The expanding hypoxia furthermore places increased energetic demands on the fish when they have to rely, at least partly, on anaerobic energy production when staying in oxygen-poor waters, with a subsequent oxygen debt to be paid (Plambech et al. 2013, Casini et al. 2016, Behrens et al. 2018). Interestingly, however, the decline in the health status of *G. morhua* seems to be most pronounced for the larger individuals (Casini et al. 2016), which are also the ones most heavily infected with *C. osculatum* (Horbowy et al. 2016, Zuo et al. 2016). Horbowy et al. (2016) have recently shown that *G. morhua* containing many *C. osculatum* in their livers have lower condition as compared to conspecifics free of this parasite. Many liver parasites challenge liver integrity, and malfunctioning of this key organ, which aids in digestion and serves as a storage site for fats and carbohydrates (Bruslè & Anadon 1996), may have direct adverse effects on health and condition. Natural mortality is assumed to be higher in *G. morhua* with poor condition (Dutil & Lambert 2000, Swain & Benoît 2015), and a high parasite load may push individual fish towards such poor health status that recovery is impossible (Horbowy et al. 2016). Alternatively, or contributing to this, *G. morhua* with heavy infections (and poorer condition) may exhibit reduced swimming performance, leading to a higher natural mortality (e.g. by seal predation) or higher fishing mortality by trawls. For instance, infection with *P. decipiens* reduced swimming performance in European smelt *Osmerus eperlanus* (Linnaeus) and European eel *Anguilla anguilla* (Linnaeus), leading to increased natural mortality in the wild (Sprenkel & Luchtenberg 1991, McClelland 1995, Rohlwing et al. 1998).

## CONCLUSIONS

Our findings reveal a significant difference in prevalence and abundance of infection with *C. osculatum* s.s. in *G. morhua* livers between the eastern and western areas, with highest levels of infection in *G. morhua* from the central Baltic as compared to the western Baltic, the Sound, Kattegat and Skagerrak. Future studies should investigate if this pattern is consistent independent of season. Highest abundances of *C. osculatum* coincided with lower nutritional condition of the fish. However, whether a causal link between abundance of infection and nutritional condition exists remains to be investigated. Although we cannot rule out other factors, abundance of *H. grypus*, salinity and prey field are potential factors driving the different infection levels. With currently increasing numbers of *H. grypus* in western areas, *G. morhua* here may be prone to higher levels of infection in the coming years. Interestingly, eastern and western Baltic *G. morhua* (as assigned by genetics) mixing in the Arkona Basin had a clear signature with higher and lower *C. osculatum* abundance in their livers, respectively. Combining genetics for population identification and infection to track migration (MacKenzie & Hemmingsen 2015) might be a useful approach in the future.

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