The effects of Contracaecum osculatum larvae on the growth of Atlantic cod (*Gadus morhua*)

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ABSTRACT

Atlantic cod (*Gadus morhua*) from the Eastern Baltic stock have decreased in numbers and condition since the 1990's. Among several causes, an increased prevalence and intensity of the nematode *Contracaecum osculatum* has been discussed. This increase has been attributed to a population increase of the parasites final host, the grey seal (*Halichoerus grypus*). Other studies have looked at the role of *Contracaecum osculatum* on cod growth and condition on recently caught cod, or done short term experimental studies in lab. This study instead investigated the importance of *Contracaecum osculatum* for cod growth in a sea pen based experiment, where cod were kept and fed in order to monitor growth. The results show that a higher density (number of nematodes per gram liver) decreases cod growth potential. If the number of nematodes exceeded 8 per gram liver cod did not grow in length, even when given generous amounts of food. Accounting for the lack of growth due to *Contracaecum osculatum* may improve stock assessments and increase the possibility to reach management targets.

1. Introduction

Atlantic cod (*Gadus morhua*) is one of the most important commercial fish species in the world and has historically been important also in the Baltic Sea (Ask and Svedäng, 2019). As a predator, cod has been a key species in the Baltic Sea ecosystem (Silberberger et al., 2018). However, the abundance and condition of the Eastern Baltic stock have declined since the 1990's (Eero et al., 2012), along with decreases in growth rate, general size and size at age (Hüssy et al., 2018; Neuenfeldt et al., 2020; Svedäng and Hornborg, 2014). Causes for the declines in cod numbers and condition, other than overfishing, have been suggested to be the result of food limitations, due to changes in prey abundance caused by decreased salinity and oxygen levels (Casini et al., 2016; Neuenfeldt et al., 2020), and density dependent mechanisms affecting productivity (Svedäng and Hornborg, 2014). Furthermore, hypoxia reduces the metabolic performance of cod, which in turn decrease the energy available for growth and feeding (Chabot and Claireaux 2008; Plambech et al., 2013). In addition to these causes, an increase in intensity of *Contracaecum osculatum* infection has resulted in investigations of potential effects of *C. osculatum* on cod physiological condition (e.g. Mohamed et al., 2020; Ryberg et al., 2020; Zuo et al., 2018).

Infection with the nematode *C. osculatum* has increased in cod, in both prevalence and intensity, in the southern Baltic Sea (ICES SDs 24 and 25, Fig. 1) during the last decades (Haarder et al., 2014; Horbowy et al., 2016; Zuo et al., 2018). The primary final host of *C. osculatum* is the grey seal (*Halichoerus grypus*) and they have shown a strong population increase during the last decades (Haarder et al., 2014), especially in the southern Baltic (Galatius et al., 2020). This increase occurred simultaneously with the increase in occurrence of nematodes in cod (Haarder et al., 2014; Zuo et al., 2018).

Contracaecum osculatum belongs to the Anisakidae family which encompasses species with life cycles that are often complicated, including several transport hosts. *Contracaecum osculatum* has a free-living stage and normally three obligate hosts required for the parasite to complete its life cycle. After eggs are excreted through seal faeces, the eggs hatch and small crustaceans ingest the larvae as a first transport host. Thereafter, it is eaten by larger crustaceans or smaller fish, in which the parasite waits to be eaten by either a larger fish or its final host, the seal (Køie and Fagerholm 1995). Nematodes accumulate in the fish hosts and further up in the food chain. The life expectancy of an anisakid nematode in cod tissue is assumed to be several years (Hemmingsen et al., 1993) which means fish accumulate these parasites over

a long time. Infection levels have shown to increase with cod length, up to about 70–80 cm (Horbowy et al., 2016) and there is a negative association between condition (Fulton's condition factor) and intensity of infection (e.g. Horbowy et al., 2016; Mehrdana et al., 2014 (slightly negative association), Ryberg et al., 2020) and muscle mass and intensity of infection (Mohamed et al., 2020; Ryberg et al., 2020). A decrease in numbers of larger cod was seen already around 2013–2014 (Eero et al., 2015), which coincided with a period when *C. osculatum* prevalence and infection increased in the southern Baltic Sea (Horbowy et al., 2016). Horbowy et al. (2016) suggested that the decrease of infection in the larger cod (>70–80 cm) indicates that there is an increased mortality of large and heavily infected cod.

A parasite may induce energetic costs for the host by limiting resources for reproduction and body growth, both because of using host energy, but also because the host uses energy to resist infection (e.g. McElroy and de Buron, 2014; Timi and Poulin 2020). *Contracaecum osculatum*, which are found in the fish livers, may affect the cod's main energy reserve, in the form of liver lipids (Ryberg et al., 2020). In situations of starvation the first source of energy used are the liver lipids, then muscle and hepatic glycogen, and finally muscle proteins (Guderley et al., 2003). However, the effects of *C. osculatum* are likely more complex and extensive than those mentioned above. Genes related to metabolism, immune function and growth have been shown to be expressed differently in livers of infected cod compared to non-infected cod (Marnis et al., 2019). A recent study revealed that cod with high infection densities had decreased nutritional condition and depressed energy turnover, meaning maintenance costs are reduced in the heavily infected cod (Ryberg et al., 2020). The same study also showed a reduced albumin to globulin ratio with increased intensity of infection suggesting impaired liver function (Ryberg et al., 2020).

As lack of food is one of the major concerns for the cod stock in the Eastern Baltic Sea (e.g. Bryhn et al., 2022; Casini et al., 2016; Neuenfeldt et al., 2020) and the negative effects of *C. osculatum* may be more pronounced when the host is deprived of nutrients (as discussed in Mohamed et al., 2020) this study aimed to investigate the importance of *C. osculatum* infection on the growth of cod when food is abundant and no longer a limiting resource. The study was conducted in conjunction to another study aimed at increasing the profitability of a local commercial fishery by keeping wild-caught cod and feeding them (Lunneryd et al., 2022). The hypothesis was that heavily infected cod, even when given generous amounts of food, would grow less (or die) compared to cod with fewer or no *C. osculatum*.

2. Materials and methods

2.1. Study area and sea pen set up

The study was conducted as a sea pen trial on the coast outside Karlshamn, southern Sweden in ICES SD 25, in collaboration with a local fisherman (Fig. 1). Cod were caught with pots, specifically designed for cod, in order to minimize the stress and injuries on the fish caused by the gear. Only cod >35 cm were used in the experiment as that is when liver parasites become more common (Horbowy et al., 2016, Zuo et al., 2018). Cod with injuries, and/or signs of infection were excluded from the experiment. Cod were transported to the sea pens in a one cubic meter round tank fitted with a water pump in order to ensure a constant exchange of sea water. Before release into the sea pens cod were anesthetised in a 50 mg L^{-1} solution of Tricaine methanesulfonate (MS222) and sea water. Thereafter they were measured for total length, weighed and individually tagged with T-tags in the dorsal fin. Cod were then carefully transferred to a sea pen where recovery was observed. The experiment was conducted during five trials/occasions to cover potential differences in infection and growth between seasons: in spring 2018 to 2020 and autumn 2018 and 2020. Each sea pen harboured a maximum of 60 cod. Cod sizes, the number of cod per sea pen and the number of sea pens in each trial were dependent, and limited, on cod catches and thus varied during the study, however this was later accounted for in the data analyses (see Table 1 for details per sea pen). During each trial one to five sea pens were used. Each sea pen was 2.5 × 2.5 m and 4 m deep with 30 mm polyethylene 3/6 mesh size. A 40 kg/m lead line was sewn in along the edges on the bottom of each sea pen, and a ~10 kg weight was added in each of the four bottom corners of the pens to keep them standing against currents. Each pen was covered by a net to prevent bird predation and disturbance. The number of days per experiment varied between trials and between sea pens, depending on weather conditions (Table 1). Temperature was logged every 10 min
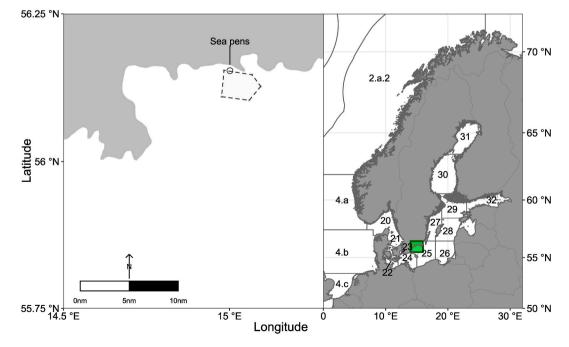

Fig. 1. Map of the study area. The circle marks the position for the sea pen trial and the larger dashed area is where the fish were caught for the trial. The numbers in the map refers to ICES subdivisions (SD).

Table 1
Each row represents the outcome in a trial/pen. The number of fish in each sea pen, total feed during the trial, the number of fish that died within the first ten days, the number of fish that died after the first ten days but before the end of the trial (Dead during trial) and the number of fish that survived to the end with the max and min number of days in trials. (Observe that some pens have a total number of fish exceeding 60 and the reason is replacements due to early deaths). Fish unknown is either fish that escaped or for some reason were not retrieved for data analyses. Average temperature on four meters depth is presented with mean and max.

Year	Season	To trial day/ month	Trial/ Bin	No fish	Total feed (kg)	Dead first 10 days	Average Start Fulton K	SD	Dead during trial	Average Start Fulton K	SD	Fish unknown	Survived	Average Start Fulton K	SD	No days in trial min	max	Temperature Average	min	max
2018	spring	1/5	1/A	59	39	4	0.874	0.087	14	0.879	0.050	1	40	0.901	0.084	54	56	14.1	5.8	20.2
2018	spring	8/5	1/C	53	32.5	4	0.873	0.039	13	0.919	0.067	1	35	0.921	0.068	54	55	15.6	6.2	27.0
2018	autumn	18/9	2/A	46	26	2	0.678	0.007	1	0.620	NA	17	26	0.791	0.151	70	71	8.0	5.1	12.4
2018	autumn	29/9	2/B	52	44.1	0			2	0.698	0.149	0	50	0.794	0.101	57	59	7.9	6.1	11.8
2018	autumn	7/10	2/C	50	24	1	0.672	NA	3	0.753	0.058	7	39	0.803	0.095	65	70	7.5	1.0	14.7
2019	spring	16/4	3/A	55	21.8	2	0.928	0.088	5	0.839	0.067	0	48	0.829	0.073	43	56	15.3	13.2	25.1
2019	spring	16/4	3/B	51	34.4	0			5	0.898	0.114	0	46	0.794	0.061	57	58	15.3	13.2	25.1
2019	spring	17/4	3/C	54	30.3	1	0.684	NA	5	0.816	0.035	1	47	0.826	0.061	46	70	15.5	3.2	25.1
2019	spring	29/4	3/D	55	32.9	0			24	0.830	0.058	2	29	0.812	0.095	46	58	14.3	3.2	24.4
2019	spring	29/4	3/E	52	23.5	0			12	0.836	0.079	2	38	0.818	0.062	57	58	14.3	3.2	24.4
2020	spring	6/5	4/A	64	14.5	10	0.887	0.108	25	0.938	0.073	3	26	0.915	0.083	35	48	12.6	8.0	17.0
2020	autumn	22/9	5/A	68	17	37	0.825	0.095	4	0.848	0.039	4	23	0.811	0.085	63	72	12.0	7.5	16.2
2020	autumn	23/9	5/B	68	17	38	0.807	0.073	8	0.818	0.098	4	18	0.811	0.092	63	71	11.9	5.9	16.2
2020	autumn	23/9	5/C	51	17	21	0.832	0.086	4	0.868	0.019	4	22	0.848	0.054	73	74	11.7	5.9	15.3
2020	autumn	30/9	5/D	45	16.1	5	0.809	0.055	9	0.830	0.119	3	28	0.807	0.140	66	67	11.5	5.9	15.3
Total				872	390.1	125			137			84	526			35	74			

with a Tinytag Aquatic 2, placed at the bottom of the pens (approx. 4 m below the water surface) for the full duration of the trials.

2.2. Feeding procedure

Cod were fed manually every other day with 1–2 cm pieces of thawed herring, caught by local fishermen in the vicinity of the sea pens. The total amount of herring per sea pen was noted, though individual consumption could not be controlled for. Rather, they were fed until uneaten herring pieces were seen at the bottom of the sea pen, as a means of allowing all fish the opportunity to feed and thus decreasing the influence of competitive social behaviour. In total, cod were fed approximately 0.7–1.3 kg herring per kg cod (estimated using total cod weight at the start of trials and total feed supplied during the experiment). By freezing the herring before using it to feed the cod, parasites were killed to prevent further infection. Thus, it was assumed that the cod had the same liver parasite abundance at the end of the experiment as when caught (Hemmingsen et al., 1993). However, the infection prior the trial could not be controlled for and therefore, the time of infection was unknown for all of the trials. Note that time of infection varies naturally between individuals and can be assumed to be randomized between all cod within the experiments.

2.3. Contracaecum osculatum extraction and confidence in identification

At the end of a trial fish were rendered unconscious with a blow to the head, quickly weighed and measured and then killed by cuting the throat. Liver, gonad and gutted weights were noted along with gender. Sagittal otoliths were collected for age analysis (method according to the guidelines recommended in TACADAR Final report, 2006) and livers were frozen for later investigation. In the lab, livers were thawed and put in a pepsin digestion; a solution of 10 g pepsin and 30 ml hydrochloric acid to 1 L of tap water. Livers in the solution were kept at room temperature for approximately 24 h. If not digested enough after 24 h, livers were put on a heat plate (48–50 °C) with a magnetic stirrer for a few minutes to speed up the process of digestion of liver tissue in order to extract nematodes (this process induced damage to the nematodes and therefore was only used if the livers were not sufficiently digested after the 24 h period). Once digested, the samples were poured into a 250 μm mesh sieve and nematodes were collected and counted under a dissecting microscope.

An unpublished study on the use of a molecular technique to quantify the amount of nematode DNA in cod livers, as an alternative to counting nematodes for monitoring, used 19 livers from cod caught in the same time period and place as cod included in trial 1 in this study. The sequence outputs were compared to NCBIs genbank entries using blast (Altschul et al., 1990) in order to extract taxonomic information of the obtained sequences. The results showed that 18 out of 19 cod livers contained only *C. osculatum* sequences (with both subtypes A and B sensu strictu present). Though *C. osculatum* dominated, one sample also contained sequences from *Pseudoterranova decipiens* and/or *P. krabbei* and *Anisakis simplex* (Ovegård et al., unpublished). Sokolova et al. (2018) used morphometric characteristics and genetic analyses to identify nematodes in cod livers, collected from south of Gotland to Skagerrak, and identified all from the central and Eastern Baltic as *C. osculatum*. Mehrdana et al. (2014) morphologically identified *C. osculatum* in livers from cod caught East of Bornholm and confirmed their id with genetic analyses. Another example is Haarder et al. (2014) that, by molecular identification, confirmed that nematodes from cod caught east of the Bornholm basin were *C. osculatum*.

Though each of the studies mentioned above used a small sample size, results from other studies makes us confident that the presence of other species was low also in this study (and may even been caused by contamination from outside the liver). The time-consuming task of identification was therefore de-prioritized and all nematodes found in livers are hereafter called *C. osculatum*.

2.4. Data analysis

Fish that died within the first ten days were excluded from analyses to minimize the risk that death was caused by the catching- or sedation procedures, (after ten days cod had stabilized and were feeding properly). The first step was then to determine if *C. osculatum* had a role in why some cod died after ten days but before the end of the trial. This was done with General Linear Models (GLMs) using library "Stats" in R 3.6.0 (R Core Team 2019). GLMs were used due to sample size differences and non-normally distributed data.

C. osculatum abundance (as the number of *C. osculatum* in a liver) and *C. osculatum* density (calculated as the number of *C. osculatum* per gram liver; similar to calculations in Ryberg et al., 2020 but not extracting *C. osculatum* weight as the pepsin digestion solution may impose an error in *C. osculatum* weight) were used as response variables for the analysis, survive or not survived as factor to account for differences in survival, and the length or condition of the cod at the start of the experiment were used as covariates, giving the four models:

C. osculatum abundance = α (status$_i$) + Cod start length$_i$, family = gamma

C. osculatum abundance = α (status$_i$) + Cod start condition$_i$, family = gamma

C. osculatum density = α (status$_i$) + Cod start length$_i$, family = gamma

C. osculatum density = α (status$_i$) + Cod start condition$_i$, family = gamma

Where status are the two factor levels survived or not survived cod.

The cod condition factor was calculated using Fulton's K (K = 100 x (WL^{-3}) where W is the weight of the fish (g), and L is the total length (cm)). The next step was then to investigate whether *C. osculatum* abundance and density affected the condition of the surviving fish. Thus, only data on cod that survived to the end of the experiment were included in the subsequent analyses. First, end condition was modelled as a function of age (instead of length) and parasite abundance/density using GLMs resulting in two models:

Cod end condition = α (Age$_i$) + *C. osculatum* abundance$_i$, family = Gaussian

Cod end condition = α (Age$_i$) + *C. osculatum* density$_i$, family = Gaussian

Then Generalized Additive Mixed Models (GAMMs) were used to model cod growth as a function of parasite density and other variables (see full model below) using a Gaussian distribution with an identity link function. GAMMs were developed using library "gamm4" (Wood and Scheipl 2020). Mixed models were used in order to take into account the nested experimental design with multiple sea pens used during multiple trials, while the Generalized Additive Model framework was used in order to account for non-linearities between the response variable and the explanatory variables.

Weight and length gain per day, as growth indices, were used as response variables in different models to account for differences in the number of days cod were kept in the experiment (i.e. reflecting the amount of food given) and were calculated as:

Weight gain per day = W$_e$ – W$_b$ / d

Length gain per day = L$_e$ – L$_b$ / d

Where W is the weight, L is the length, *b* is time at the beginning of the experiment, *e* is time at the end of the experiment and *d* is the number of days in the experiment.

Response variables (growth indices) were modelled together with several combinations of explanatory variables. GSI is the gonadosomatic index, which was included to account for differences in growth due to energy put into gonadal development, and was calculated as:

Gonadosomatic Index (GSI) = (W$_{gonad}$ / W$_{gutted fish}$) x 100

The fixed part of the full model explored for each response variable was:

Response variable = β(season) + s$_1$(*C. osculatum* per gram liver (density)) + s$_2$(start condition) + s$_3$(GSI) + s$_4$(start length/weight) + ε

Where *β* is a season specific intercept, s$_i$ are natural cubic splines and *ε* is an error term.

Note that start length or start weight was included in the models as an explanatory variable in order to account for such differences. No multicollinearity was found between explanatory variables of both models as the variance inflation factors (VIF) were always lower than 1.2 (Zuur et al., 2009). All models included a nested random structure with sea pen nested within trial to account for differences in growth of fish between trials and sea pens. Since fish were fed in groups and not individually, there was no possibility to account for the amount of food per individual, but they were fed until all fish stopped eating. Model selection for the randomized part was done by performing likelihood ratio tests on models with a simpler random structure compared to our full model (Zuur et al., 2009). Model selection for the fixed part of all models was done through a backward stepwise elimination process based on statistical significance (Wood, 2006). After fitting the best models, predictions were made to explore the effect of *C. osculatum* density or abundance on the growth of cod under optimal food conditions.

3. Results

On average 16% of the fish that survived the first ten days did not survive to the end of the trial (Table 1). During all trials, there were complications due to weather conditions and temperature. During the spring trials fish experienced a rapid increase in temperature which (and perhaps in combination to feeding to satiation in 2018) caused mortality, especially amongst cod in higher condition. In spring 2019 the fish experienced a period of fin rot (bacteria unknown), though the majority recovered and survived until the end of the trial. During autumn 2018 a storm destroyed some of the sea pens resulting in several fish escaping before analyses (Fish unknown in Table 1). In autumn 2020 the water temperature (15–16 C°) did not decrease as expected and there was a high initial mortality. When the temperature decreased fish were replaced and therefore the number of fish in Table 1 is higher than 60 (which was the maximum number of cod in each sea pen at any given time).

Contracaecum osculatum abundance did not differ between the cod that died and the cod that survived to the end of the experiment (start length as covariate; GLM, p = 0.880 and start condition as covariate; p = 0.419). However, there was, as expected, a significant positive association between abundance and length with more parasites the longer the cod were at the start, i.e. start length (GLM, p = 0.010). Also, *C. osculatum* density did not differ between cod that died and the cod that survived to the end of the experiment (start length as covariate; GLM p = 0.742 and start condition as covariate; p = 0.096).

Of the surviving cod, the prevalence of *C. osculatum* in livers was on average 92% in the trials (ranging from 78 to 100%). Cod had as many as 326 *C. osculatum* (average 41) and as much as 18 *C. osculatum* per gram liver (average 1.5) (Table 2). Start length ranged from 35 to 64 cm (average 41 cm) and ages were between 2 and 7 years. A negative trend can be seen in the change in length (cm), weight (kg) and condition (Fulton's K) in relation to a higher *C. osculatum* density but this trend is not apparent if looking at *C. osculatum* abundance (Fig. 2).

There was no effect on end condition from *C. osculatum* abundance (p = 0.969) or cod age (p = 0.676) (Fig. 3a). However, the results showed a significant positive effect between end condition and *C. osculatum* density (p = 0.001) but not age (p = 0.274). Fig. 3b shows a drop shape (i.e. larger and darker circles at the bottom of the figure) in all age classes, with lower conditioned cod, in the end of the experiment, having higher *C. osculatum* densities.

Likelihood ratio tests performed on both the GAMM model based on length and the one based on weight showed that the full nested random

Table 2
The prevalence of *C. osculatum* per trial/pen, the abundance as the number of *C. osculatum* per liver, with min and max, and *C. osculatum* density as number of *C. osculatum* per gram liver. Also the average change (end-start) in length (cm), weight (kg) and condition (Fulton's K).

Year	Season	Trial/Bin	Prevalence	Abundance (no)			Density (g)			Start lenght (cm)			End lenght (cm)			Average change in		
				average	min	max	average	min	max	average	min	max	average	min	max	length	weight	Fultons K
2018	spring	1/A	97.5%	38.5	0	180	1.1	0.0	3.9	40.8	35.3	57.0	42.9	37.1	58.5	2.110	0.196	0.126
2018	spring	1/C	97.1%	46.0	1	180	1.9	0.0	14.6	41.3	36.0	50.0	43.0	38.0	51.8	1.757	0.146	0.064
2018	autumn	2/A	88.0%	55.0	0	299	1.6	0.0	13.5	41.9	36.9	55.4	44.2	39.3	58.2	2.335	0.255	0.163
2018	autumn	2/B	92.0%	27.8	0	134	0.7	0.0	10.5	42.4	36.7	60.5	44.8	38.0	60.8	2.446	0.368	0.272
2018	autumn	2/C	87.2%	35.2	0	181	1.3	0.0	18.1	44.1	38.0	61.2	45.7	38.0	63.6	1.621	0.190	0.098
2019	spring	3/A	91.7%	21.7	0	100	1.2	0.0	6.8	37.7	35.5	42.1	38.8	35.6	43.4	1.029	0.110	0.119
2019	spring	3/B	100.0%	76.4	4	297	2.7	0.1	14.3	42.9	39.7	53.6	44.2	39.7	53.8	1.215	0.188	0.151
2019	spring	3/C	97.9%	33.2	1	131	1.5	0.0	5.7	37.8	35.1	41.4	39.1	35.5	42.3	1.302	0.125	0.125
2019	spring	3/D	100.0%	61.7	2	292	1.9	0.0	16.2	46.6	40.7	63.8	47.8	41.5	63.8	1.217	0.192	0.116
2019	spring	3/E	89.2%	31.2	0	92	1.4	0.0	9.6	38.9	36.6	41.1	40.1	36.6	42.8	1.153	0.135	0.131
2020	spring	4/A	78.3%	39.7	0	248	1.7	0.0	13.2	41.4	35.5	49.7	42.8	37.4	49.7	1.356	0.123	0.063
2020	autumn	5/A	87.0%	47.8	0	326	1.1	0.0	6.5	42.6	35.4	56.4	45.4	35.8	58.4	2.791	0.263	0.117
2020	autumn	5/B	77.8%	60.7	0	289	1.3	0.0	7.6	42.6	35.4	57.5	46.2	38.5	64.0	3.539	0.323	0.148
2020	autumn	5/C	86.4%	30.6	0	226	1.5	0.0	10.8	42.1	35.4	56.5	45.0	35.4	60.6	2.891	0.218	0.060
2020	autumn	5/D	96.3%	32.3	1	197	1.9	0.0	11.0	41.8	36.2	64.0	44.0	36.4	64.8	2.129	0.151	0.048
Total			92.3%	41.3			1.5									1.795	0.194	0.127

structure was preferable compared to a simplified one. The GAMM model based on length showed that *C. osculatum* density was the only explanatory variable that was significant, and thus the only variable included in the final model (Table 3). However, the model based on weight showed that season was the only non-significant variable, and thus the only variable removed from the final model (Table 3). Visual inspection of the residuals revealed slight departures from the model assumptions on homogeneity of variance, but the overall quality of the residuals was considered to be satisfactory (Supplementary material Figures S1 and S2). There was a slightly positive association between *C. osculatum* density and cod growth per day for values smaller than around two *C. osculatum* per gram liver (both for length and weight), indicating that cod with infection densities below two are growing while cod with infection densities above two showed negative growth (Supplementary material Figures S3 and S4). The negative effect on growth due to *C. osculatum* density appears to increase linearly up to around 8 *C. osculatum* per gram liver for growth in length and 5 for growth in weight (Supplementary material Figures S3 and S4). The negative effect seems to plateau at higher *C. osculatum* densities (note that the confidence intervals increase and sample size decreases the higher densities). The effect of GSI and start weight on the growth in weight per day is linear and positive for values higher than around 10 and around 0.6 kg respectively, while the effect of start condition is linear and negative for values higher than around 0.8 (Supplementary material Figure S4).

Predictions show that cod, on average, do not increase in length when having 8 or more *C. osculatum* per gram liver (though the confidence interval are relatively wide; Fig. 4). Of the surviving cod, 2.6% had 8 or more *C. osculatum* per gram liver. The predictions of growth in weight per day for a hypothetical cod with a GSI of 5 and a starting weight of 0.5 kg show, on average, no growth in weight when having 10, 5 and 3 or more *C. osculatum* per gram liver at different levels of start condition (0.5, 0.8 and 1 respectively) (Fig. 5).

4. Discussion

This work shows that cod, offered feed ad libitum, and that died during the trial did not obviously die because of a high *C. osculatum* abundance or density, as those factors did not differ between cod that died and cod that survived. The majority of cod that survived were able to grow in length, weight and condition regardless of parasite abundance. The study shows that it is rather a high parasite density, than abundance, that is posing a problem for cod growth, even under favourable food conditions (Fig. 2). Other studies have shown relationships between high numbers of *C. osculatum* in fish and decreased condition, but these have analysed parasite abundance directly on fish caught in the wild, without feeding (Horbowy et al., 2016; Mehrdana et al., 2014; Wąsikowska et al., 2018). However, our results show that, if cod is provided with food, these patterns are not as apparent. The cod in lower end condition had generally, in all age groups, higher *C. osculatum* densities but not necessarily a higher abundance (Fig. 3) (note that liver weights are post-experimental and expected to be larger after feeding than at the start of the experiment). An interesting note is that *C. osculatum* abundance increased with cod length but not with age (Fig. 3). As the number of *C. osculatum* theoretically should accumulate with age, our finding either implies that the growth patterns of cod in the Eastern Baltic Sea is disrupted (i.e. if cod take time to grow in length it will later, than normal in life, predate on larger prey, which theoretically should have more parasites) or age readings are difficult. Both reasonable explanations as length at age have decreased since the early 2000's in the Eastern Baltic cod stock (Hüssy et al., 2018) and otolith readings have since then been proven difficult as yearly rings are not as apparent (Hüssy 2010).

The results from GAMM models showed that cod with higher parasite densities generally grew less in length and weight per day during the trials. If more than 8 *C. osculatum* per gram liver were present, cod did not grow in length (Fig. 4). The picture was a little more complicated for
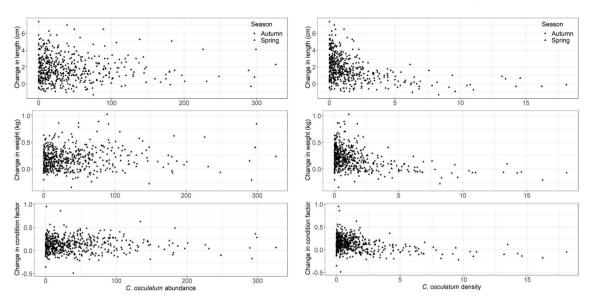
Fig. 2. Plotted change (end-start) in cod length, weight, and condition as Fulton's K against *C. osculatum* abundance (the number of *C. osculatum* in a liver) to the left and *C. osculatum* density (the number of *C. osculatum* per liver weight (gram)) to the right, separated by season. Data represents the cod that survived the trials.
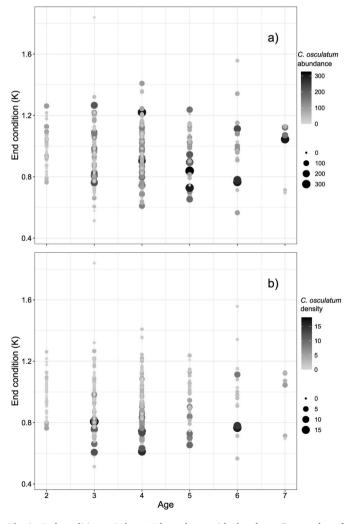

Fig. 3. End condition as Fulton's K by cod age; with abundance (i.e. number of *C. osculatum*) in a) and *C. osculatum* density (number of *C. osculatum* per gram liver) in b). Darker and larger circles means higher abundance or density.

Table 3
Parameter estimates and approximate significance of smooth terms from the final GAMM model of both growth in length and weight per day. Degrees of freedom are denoted by "edf". The full model was: Response variable = β(season) + s_1(*C. osculatum* per gram liver) + s_2(start condition) + s_3(GSI) + s_4(start length/weight) + ε

Response variable: Growth in length per day				
Parametric terms	**Estimate**	**Standard error**	**t-value**	**p-value**
Intercept	0.031	0.0033	9.448	<0.001
Smooth terms	**edf**		**F-value**	**p-value**
Number of *C. osculatum* per gram liver	2.971		39.86	<0.001

Response variable: Growth in weight per day				
Parametric terms	**Estimate**	**Standard error**	**t-value**	**p-value**
Intercept	0.032	0.0004	7.79	<0.001
Smooth terms	**edf**		**F-value**	**p-value**
Number of *C. osculatum* per gram liver	2.549		41.35	<0.001
Start Condition	1		16.22	<0.001
Start Weight	1		21.83	<0.001
GSI	1		16.76	<0.001

growth in weight per day (Fig. 5) as it also was dependent on GSI, start weight and start condition. Cod with a higher initial weight had the potential to grow more compared to cod with lower initial weight, but with the same density of *C. osculatum*. This was probably a reflection of longer/heavier cod being in lower condition than the smaller cod in the experiment as the opposite is shown for cod with higher initial condition growing less than cod with a lower initial condition, but with the same density of *C. osculatum* (Fig. 5). This has probably no connections with issues derived by *C. osculatum*, but rather a matter of recovery or compensatory growth i.e. fast growth if fed after a period of slow growth due to starvation (Pedersen and Jobling, 1989). Cod having higher
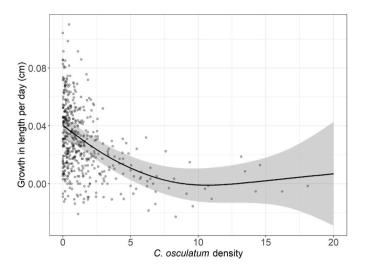
Fig. 4. Prediction of growth in length per day depending on *C. osculatum* density (the number of *C. osculatum* per liver weight (gram)) with confidence intervals. Below zero on the y-axis represents no growth. Dots represents observations.
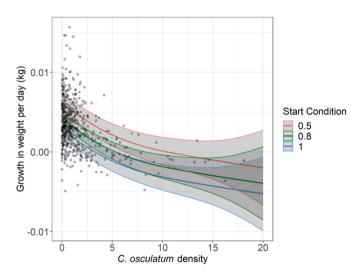

Fig. 5. Prediction of growth in weight per day depending on the number of parasites per liver weight (gram) with confidence intervals for a model cod with a GSI of 5 and a starting weight of 0.5 kg at different levels of start condition (medium condition is represented by Fulton's K 0.8, below 0.8 is considered low condition and >1 is considered high (Marteinsdottir and Begg 2002)). Below zero on the y-axis represents no growth. Dots represents observations.

initial weight may have been longer to start with but in low condition and thus able to put on weight easier than the cod in better condition. Also note that gonad development contributed to the increase in weight per day, regardless of season. To summarise, not only parasite density affects cod growth but also a fat cod in high condition grow less in weight per day than a cod starting out thin and in low condition.

In contrast to growth in weight per day, growth in length per day didn't seem to be dependent by condition, GSI (Gonadosomatic Index) and initial length, only on parasite density. The question is why? Maybe because growth in length is more robust to differences in condition, GSI and initial length, and therefore these effects are hidden, or growth in length is slower than growth in weight so based on the duration of the experiment it might be harder to get contrasting data based on the other variables. Alternatively, high parasite density affects physiological traits (see Timi and Poulin 2020 for a review) reducing growth in length. If the last scenario is correct, parasites may be a contributing factor to the

reduced length at age in the Eastern Baltic cod stock.

It's important to note that other pathogens could have interfered in this study as a range of other parasites are present in Baltic Sea cod (Setyawan et al., 2020), which was not controlled for. Competitive behaviour cannot be ruled out and some cod may not even have eaten during the trials and therefore starved and used lipids from livers, and thus increased *C. osculatum* density. It's also possible that cod in the trials had livers with too low functional liver mass before the trial started, which may have been caused by starvation in combination with *C. osculatum* causing reduction in liver function or even chronic liver disease (Ryberg, 2021), and thus not able to recover from pre-experimental conditions. Even though cod experienced varying environmental situations in the experiment we are confident in the result, as most could be accounted for in the models and the models were able to identify patterns.

With the knowledge that a higher *C. osculatum* density results in reduced growth and even lack of growth, and probably in the long run also death (when condition factor, Fulton's K, gets below 0.06 (Lambert and Dutil 1997)), *C. osculatum* should not be ignored as a possible contributing factor for the situation of the Eastern Baltic cod stock. Further work is however needed to establish the broader impacts on cod stocks. From 2021 it is obligatory during the Baltic Sea International Trawl Surveys (BITS) to, amongst length, weight, age etc., also monitor cod liver weight and visually assign a liver category, based on the number of nematodes visible on the liver surface, (ICES, 2021). This only gives a rough estimate of *C. osculatum*, but it is a cost-effective method compared to digesting livers to count the number of *C. osculatum*. A way forward is to investigate the use of these categories in relation to the results from this study. Specifically, how it can be used to improve stock assessments by accounting for the lack of growth due to *C. osculatum*, which in turn may increase the possibility to reach management targets.

Declaration/conflict of interest

None.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2022.08.006.

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