

Liver worm (*Contracaecum osculatum*) in Eastern Baltic cod: growth, status, effects and biological information - input to management (TESLO & TORVÆKST)

Jane W. Behrens et al.

DTU Aqua Report no. 464-2024



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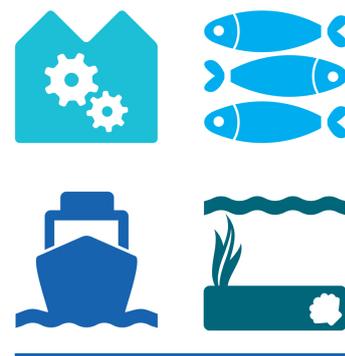
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Preface

This report is financed by the Danish EMFF program “Fiskeri, natur og miljø - Marin biodiversitet”, constitutes the professional delivery of the results of the two EMFF projects "Seal-related liver worm in Eastern Baltic cod: Status, effects and biological input for management (TESLO)" and "Biological press factors limiting the growth of Eastern Baltic cod (TORVÆKST)" with the grant no 33113-B-17-110 and 33113-B-20-161 which, through national and international collaboration between researchers, managers and representatives of the fishery, has contributed new knowledge about how infection load in cod with the parasitic nematode *Contracaecum osculatium* (also known as cod's liver worm) associates with the health status, growth potential and natural mortality of the fish.

Furthermore, these projects have paved the way for implementation (from 2021) of a pan-Baltic, trans-national monitoring of the spatio-temporal occurrence of liver worm in cod, using the BITS surveys as platform, and based on the developed liver category method.

The project leader was Jane W. Behrens, and the project period was from December 2017 to December 2020 (TESLO) and from June 2020 to March 2023 (TORVÆKST).

Moreover, the following persons have contributed to the work and are co-authors of the scientific articles:

- Kurt Buchmann, Department of Veterinary and Animal Sciences, University of Copenhagen
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- Richard Klinger, Institute of Marine Ecosystem and Fishery Science, University of Hamburg
- Christian Jørgensen, Department of Biological Sciences, University of Bergen, Norway
- Virginia Chondromatidou, DTU Aqua, Technical University of Denmark

In Danish the project titles are “Sælrelateret leverorm i Østersøtorsken: Status, effekter og biologisk input til forvaltning (TESLO)” and ”Biologiske presfaktorer som begrænser væksten for østlige Østersøtorske (TORVÆKST)”.

Kgs. Lyngby, August 2024

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Project Summary

The present report describes results of the two EMFF projects "Seal-related liver worm in Eastern Baltic cod: Status, effects and biological input for management (TESLO)" and "Biological press factors limiting the growth of Eastern Baltic cod (TORVÆKST)", funded by European Maritime and Fisheries Fund and the Danish Fisheries Agency.

Knowledge about how parasites influence the health status, growth potential and mortality of fish is extremely limited, likely because it is difficult to quantify and describe, and parasites are rarely monitored. Furthermore, fish in the wild are exposed to various factors (e.g. unfavorable temperature, salinity, oxygen or food conditions), and it is difficult to separate the effects of these factors from those of parasites. Many parasites also have complex life cycles including several hosts, making their infection dynamics difficult to predict.

Grey seal is final host to the parasitic nematode *Contracaecum osculatum* (common name cods liver worm), and concurrent with the recovery of grey seal in the Baltic Sea, high infection levels with this parasite have been observed in cod in the central and southern parts of the Baltic Sea. Cod is one of several transport hosts to this parasite.

Field investigations have shown that infection intensity with liver worm in Eastern Baltic cod coincides with poor nutritional status. As the common name cod's liver worm indicates, this parasite sits explicitly in the liver of the cod. The liver is responsible for many vital processes including nutrient assimilation, bile production, maintenance of metabolic homeostasis and protein synthesis and also serves as an energy reserve and breeding capital for the fish. It is thus intuitive to think that a high liver parasite burden leads to reduced function of the organ with negative effects on the nutritional condition of the infected individual. It however remains unclear to what extent cod that are already thin due to food limitations are more susceptible to becoming infected, or whether the nutritional condition of the fish deteriorates when they have many of these liver parasites. This is especially relevant for Eastern Baltic cod, which has experienced food limitation for decades, and for several years has been a stock in distress. Disentangling potential effects of parasites on their hosts from effects arising in the wake of unfavorable abiotic and food conditions thus demands an interdisciplinary approach combining field and laboratory studies, and bioenergetic modelling.

The projects reveal that cod with many nematodes in their liver generally have a reduced energy status, both in terms of the amount of protein in the muscles and in terms of fat in the liver. These thin cod are possibly more susceptible to liver worms. However, the changed ratio between protein and glycogen energy in the fish found here points to a direct effect of the liver worm on the fish, and not an effect of being starved. In support, we also found that highly infected cod show signs of suffering from a severe liver disease reflected by changes in the blood plasma composition such as decreased plasma albumin and increased globulin levels, resulting in reduced albumin to globulin ratio, as seen in humans with chronic liver diseases. Histopathology revealed that cod with high infection loads of liver worms have pronounced inflammatory reactions and bleedings in their livers, pointing to reduced liver functionality. The growth experiment revealed that even when highly infected cod had access to ample food, they were not able to overcome the negative effects of high liver worm load on their nutritional condition.

Based on an extensive pan-Baltic analysis of liver worm in sprat, a main prey for cod, we show that infection levels of sprat with this parasite is in general low, although with some indications that sprat living adjacent to grey seal colonies have slightly higher infection levels. Yet, the results suggest that cod gets infected by addition prey items besides sprat. Building on these results, and energy content analysis of sprat throughout the year, we parameterized an individual-based bioenergetic model of growth in cod and show that independent of the starting size of the cod, the growth rate and condition factor decreased when the infection rate with liver worm increased. In addition, the model showed that heavily infected cod reached a 'point of no return' where their energy intake was so low that they could no longer get the energy required to cover the basal metabolism; after that the fish starved to death.

The spatial distribution of liver worm in cod will potentially vary over time, depending for example on how the number of grey seals develops in the future in the various areas. Detailed analysis of cod livers where the total number of liver worm in each organ is counted is however time-consuming and expensive, and we therefore developed the so-called 'liver category method' where individual livers are assigned a category between 0 and 4, depending on how many liver worms can be observed on the surface of the liver. We further developed area-specific models which can be used to predict the total number of nematodes based on these categories. Based on this, and that results from the present projects combined clearly point to that infection load with liver worm is needed as an additional indicator of the health status of cod in the Baltic Sea, ICES made it a mandatory part of the routine sampling protocol on Baltic monitoring surveys from 2021 to assign a liver category to individual cod livers.

Background and outline of the project

The Eastern Baltic cod stock is in a remarkably poor state. The stock biomass has declined to the lowest level on record (ICES 2022), and the fish are small, suffer from low body condition and mature at a small size (Eero et al. 2015t, 2023; Casini et al. 2016b). Growth of the fish has declined (Mion et al. 2021), natural mortality has increased (ICES 2022) and during the latest decade, concurrent with an increasing abundance of grey seals *Halichoerus grypus*, an increasing load of the parasitic nematode *Contracaecum osculatum* has been observed in cod livers, where large fish have highest infection loads (Haarder et al. 2014; Sokolova et al., 2018). Cod functions as a transport host for this parasite within the Baltic Sea and gets infected when eating infected prey such as e.g. sprat, and the parasite accumulates with time in the cod liver as the fish eats infected prey. Grey seal is the final host for *C. osculatum*. Several field studies have shown a relationship between high infection load and poor nutritional status (i.e. condition factor) of cod. A high parasite burden might lead to reduced function of the liver with potential negative effects on the nutritional condition of the infected individual, potentially leading to mortality. Yet, in the field several factors (alone or in combination) may influence the nutritional status of the fish, and fish which are already skinny due to e.g. food limitations may be more susceptible to infection.

Combining controlled laboratory experiment with field data and bioenergetic modelling, our aim in the present projects is to provide new knowledge on how high loads of *C. osculatum* affects the physiology and nutritional condition of cod, and how this may relate to natural mortality. More specifically, we investigated the energy content and composition of cod in relation to liver worm load, also including analysis of plasma composition (section 1) and did histopathology of cod livers to describe the state of the organ in relation to *C. osculatum* loads (section 2). To evaluate the likelihood of cod getting infected by sprat (the main prey of cod in this stock) and model the bioenergetics of cod growth in relation to energy intake and infection risk via sprat (section 4), we determined the spatial distribution of *C. osculatum* in sprat across the Baltic Sea in different seasons and determined the energy content of this prey fish for cod throughout the year (section 6). We also tested experimentally if cod when fed preferred prey *ad libitum* can grow despite high liver worm load (section 5). Finally, we tested if the newly implemented pan-Baltic monitoring of liver worm load in cod using the so-called 'liver category method' could be used to reliably predict total number of parasites in individual livers (section 3).

1. The physiological condition of Eastern Baltic cod infected with liver worm

1.1 Energy composition and content of Eastern Baltic cod infected with liver worm (TESLO)

Authors: Marie Plambech Ryberg, Peter V. Skov, Niccolò Vendramin, Kurt Buchmann, Anders Nielsen and Jane W. Behrens

Background

The liver of cod is responsible for processes related to the metabolism of food and protein synthesis. In addition, the liver is very lipid rich and functions as an energy reserve ('lunch box') for the fish (Hinton et al., 2017). The parasitic nematode *Contracaecum osculatum* migrates specifically to the liver of cod following ingestion of smaller infected prey – hence its common name cod's liver worm (Figure 1.1.1.)

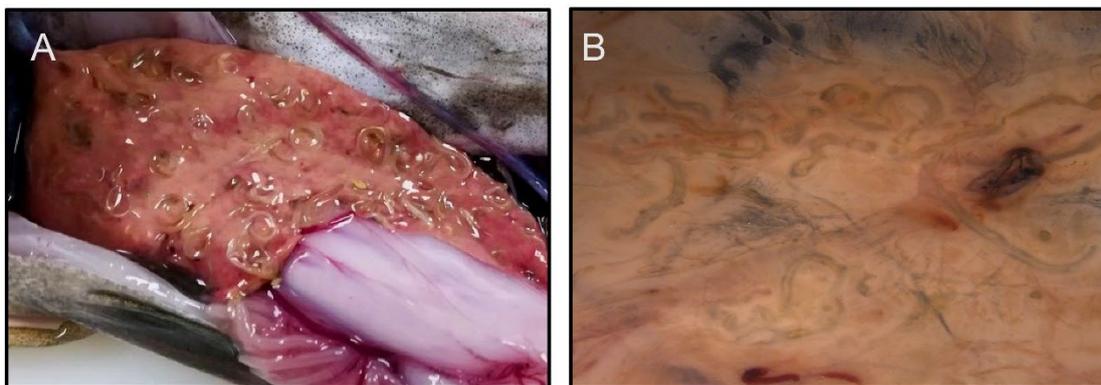


Figure 1.1.1. A) Infections with *C. osculatum* in an Eastern Baltic cod liver (photo: Marie P. Ryberg). B) Several *C. osculatum* found close to the major blood vessels in the liver (photo: Heidi Andreassen. Both photos are from Ryberg, 2020.

Because liver worm accumulates in cod liver with time as the fish eats infected prey items, high infection loads are most often seen in larger cod (Horbowy et al., 2016; Zuo et al., 2016). A high parasite burden might lead to reduced function of the liver with potential negative effects on the nutritional condition of the infected individual. This can be reflected by changes in the energy content and composition of the fish. In the present study we investigated the relationship between infection density (number of parasites per gram liver) with *C. osculatum* and energy content and composition in Eastern Baltic cod.

Material and Method

Cod start to get infected with liver worm when they reach a size of approx. 30cm, and own previous studies have shown that a range of infection levels are seen in fish between 35 and 50 cm (Ryberg, 2020). Based on this, thirty-three Eastern Baltic cod between 35 and 50 cm were caught east at Bornholm and killed directly thereafter, and subsequently transported to DTU Aqua in Hirtshals where they were kept at -20 degrees until analysis. After thawing, the liver was dissected out from the fish and number of parasites was determined for each liver. For

each fish, data was also collected for length and weight of the whole fish, as well as liver weight. To determine protein, fat, glycogen and water content and composition of the fish, and fat and water content of the liver, each fish and liver were there after homogenized separately. Based on this, the energy content of each fish was also determined. Protein content of the fish, and lipid content of fish and livers, were determined using the Kjeldahl (Foss Kjelttec 2200, Hillerød, Denmark) and the Bligh and Dyer methods (Bligh and Dyer, 1959), respectively. To obtain dry matter and water content of the fish and liver, samples were dried for 24 h at 105°C, and weight loss was determined (Memmert UN110, Büchenbach, Germany). Glycogen content of the fish was calculated as the difference between the initial dry weight and the sum of the crude protein, fat, and ash weights (Saint-Paul, 1984). The energy density of dry cod tissue was determined from dried tissue samples and subsequently converted to energy density per gram of wet body mass (kJ g⁻¹) (Schloesser and Fabrizio, 2017).

Results

Number of liver worms in individual livers varied between 0 to 104, and 85% of the livers had nematodes. All nematodes were determined by their morphology to be *Contracaecum osculatium*, the nematode known as cod liver worm. Overall, the results showed that the composition of the fish changed when the cod had many nematodes (Figure 1.1.2.). More specifically, the protein and energy content (Figure 1.1.2A.) of the fish decreased significantly while water content increased significantly (Figure 1.1.2B.) with an increased number of nematodes. The glycogen content was significantly higher in the most heavily infected cod. Overall, this meant that the decrease in the total energy content of the fish that had many nematodes could be explained by their energy source being changed from being primarily protein energy in the fish that had a high energy content, to primarily becoming glycogen energy in the fish that had a lower energy content.

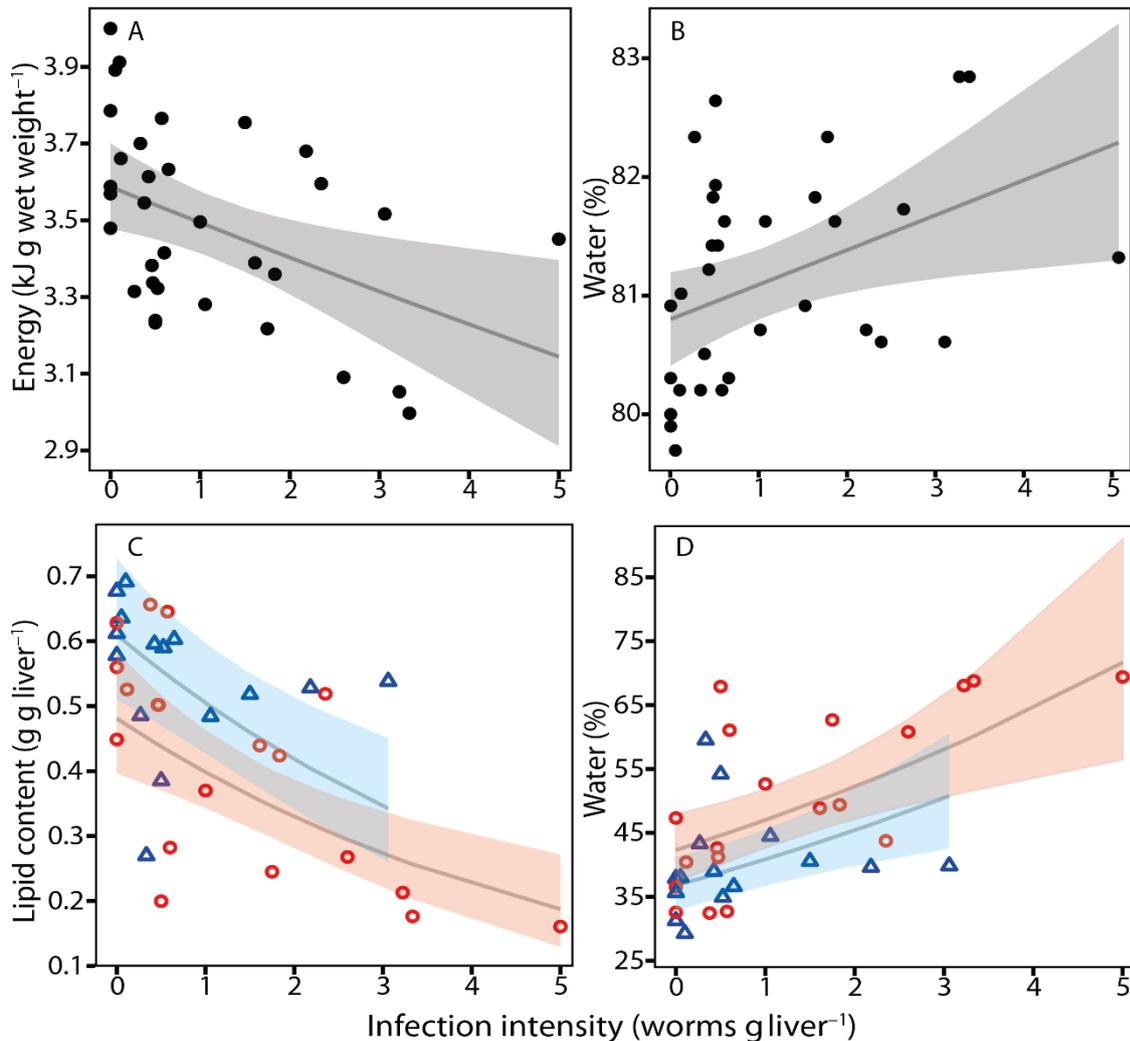


Figure 1.1.2. (A) Total energy content ($\text{kJ g wet-weight}^{-1}$) of the whole fish excluding the liver, (B) water content (%) of the whole fish, (C) lipid content of the liver (g per gram liver) and (D) water content of the liver (%), all in relation to changes in infection density as described by the thin grey lines. Both the total energy of the fish and lipid content of the liver decreased significantly with increasing infection density while water content increased significantly in both the fish and the liver with increasing infection density. In C and D, colours and symbols represent: blue and Δ = female, red and \circ = male. Grey, blue and red areas represent 95% confidence intervals. Figure text and figure from Ryberg et al., 2020.

The lipid content of the livers was significantly lower in the fish that had the highest infection levels (Figure 1.1.2C.). These livers also contained more water than livers from cod with low infection levels (Figure 1.1.2D.). Regardless of the number of nematodes, female fish had fatter livers than the male fish (Figure 1.1.2C.).

Conclusions

Together, the results suggest that cod with many nematodes in their liver generally have a reduced energy status, both in terms of the amount of protein in the muscles and in terms of fat in the liver. The reason for this may be that the liver tissue is destroyed when many nematodes enter the liver, whereby the function of the organ is compromised. It should however be noted that Eastern Baltic cod have also experienced limited food availability over several years, why

fish in this stock in general are thinner than cod in other stocks. These thin cod are possibly more susceptible to liver worms. Furthermore, food restricted cod may also have reduced fat content in the liver, as well as less protein in the meat. However, the changed ratio between protein and glycogen energy in the fish points to a direct effect of the liver worm on the fish, and not an effect of being starved. These results are published in Ryberg et al., 2020, Conservation Physiology <https://doi.org/10.1093/conphys/coaa093>

1.2 Blood plasma in relation to infections with liver worm (TESLO)

Authors: Marie Plambech Ryberg, Peter V. Skov, Niccolò Vendramin, Kurt Buchmann, Anders Nielsen and Jane W. Behrens

Background

To complement the findings in the study of energy content and composition in relation to infection density with *C. osculatum* in Eastern Baltic cod (section 1.1.), we examined potential changes in blood plasma of infected cod by studying the plasma protein composition. Changes in blood plasma composition reflects the health status of the fish, just as in humans where such tests are often performed upon suspicion of certain diseases. In fish, the albumin to globulin ratio (A/G ratio) has been used to reveal different physiological effects of specific pathogens (Aydin et al., 2001; Osmani et al., 2009). We hypothesized that highly infected cod would reveal clinical signs of suffering from a liver disease when their liver tissue is destroyed by high numbers of *C. osculatum*.

Materials and methods

Eighty Eastern Baltic cod were caught by trawling east of the coast of the island Bornholm in the Baltic Sea. Fish were kept in tanks at Nexø Klækkeri for 2 weeks before being transferred to the fish stable at DTU Aqua by professional animal transporters. Here, fish were kept in two circular tanks (2000 L each) with air-saturated recirculated water with constant temperature (10° C) and salinity (10 ppt) throughout the whole experiment. Fish were fed with boiled blue mussels (22KJ/g ash free dry weight) 3 times a week (around 2% of their body mass per week) to ensure no transfer of parasites or other diseases from the food. Besides for the present purpose of relating health status of fish using blood plasma composition and relating this to varying degree of infection, sixty of these cod were also used in another experimental work where we examined how the infections affected the metabolism of the cod (Ryberg et al., 2020). Following these metabolic experiments fish were stunned by a sharp blow to the head; blood was immediately sampled by caudal puncture with a lithium-heparinized 21-gauge hypodermic needle, and fish were euthanized by spinal transection (Figure 1.2.1). Blood samples were centrifuged at 1610G for 5 min, and the plasma fraction was stored at -18°C (Houston, 2002). We analysed different relevant blood parameters related to liver diseases such as total blood protein and plasma protein fractions (i.e., pre-albumin, albumin and 5 different globulins). Total blood protein content (g L⁻¹) was determined using an ADVIA 1800 Clinical Chemistry System (Siemens), while the separation of plasma protein fractions into pre-albumin, albumin and the globulins (alpha-1, alpha-2, beta-1, beta-2 and gamma) was done using capillary electrophoresis (MINICAP PRO-TEIN 6, Sebia, Lisses). We then calculated the A/G ratios by dividing individual plasma albumin and globulins values. The A/G ratio is a very important parameter when studying whether an individual is suffering from a liver disease.

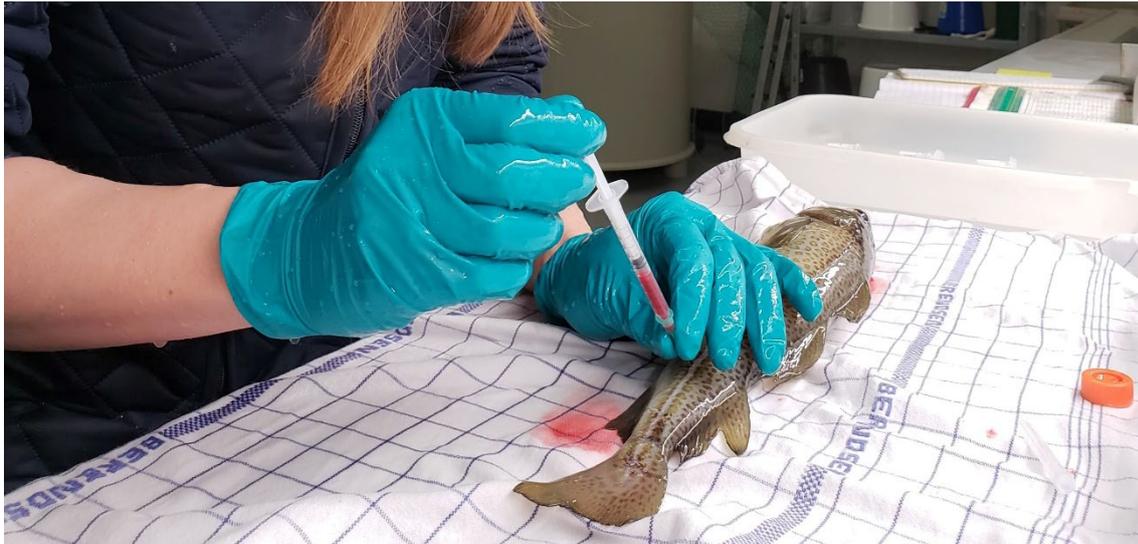


Figure 1.2.1. Blood sampling from the caudal vein of a cod. Photo: Jane W. Behrens.

Results

The total blood protein and the A/G ratio decreased significantly with increase in infection intensity (Figure 1.2.2.). The albumin/gamma ratio could best be described with an exponential decay model (Eq. 1). The best model to describe A/G ratio for all data only included the infection intensity (Figure 1.2.2B.):

$$\frac{A}{G} = e^{(-0.27 \cdot INF + 0.30)} \quad (Eq. 1)$$

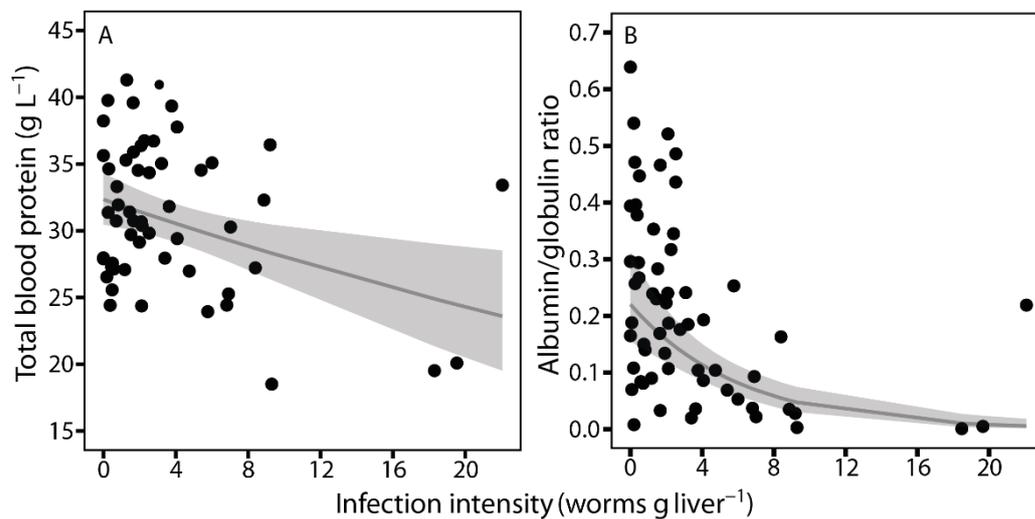


Figure 1.2.2. (A) Total blood protein (g L^{-1}) and (B) albumin/globulin ratio in cod ($n = 60$) in relation to varying degrees of infection densities with *C. osculatum*. Both parameters decreased significantly with increasing infection density, as described by the thin grey lines. The grey boxes are 95% confidence intervals. Figure from Ryberg et al., 2020.

The prealbumin, the precursor of the albumin, also showed the same trend as albumin, with a significant decrease when infection intensity increased (Figure 1.2.3.)

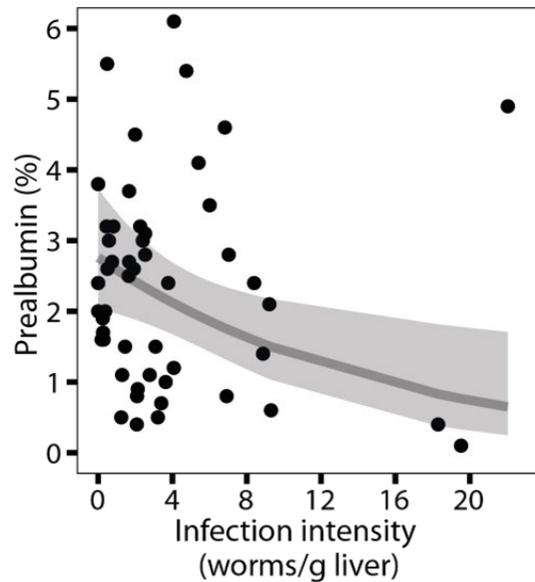


Figure 1.2.3. Prealbumin (%) in cod (n = 60) in relation to varying degrees of infection densities with *C. osculatum*. This blood parameter decreased significantly with increasing infection density, as described by the thin grey lines. The grey boxes are 95% confidence intervals.

Conclusions

Overall, this study revealed that highly infected cod show signs of suffering from a severe liver disease reflected by changes in the blood plasma composition such as decreased plasma albumin and increased globulin levels, resulting in reduced albumin to globulin ratio. Albumin is the main protein of the blood plasma and in humans reduced albumin to globulin ratios are seen in individuals with chronic liver diseases (McDonald and Milligan, 1992; Garcia-Martinez et al., 2013). In support of our findings genes related to immune response are overall affected by infections with *C. osculatum* in Eastern Baltic cod (Marnis et al., 2019). In conclusion we argue that the significant decrease in the albumin to globulin ratio (caused by concomitant changes in plasma albumin and gamma globulin) that occurs in the highly infected cod reflects that these individuals show signs of suffering from a severe liver disease. These results are published in Ryberg et al., 2020, Conservation Physiology <https://doi.org/10.1093/conphys/coaa093>

2. Liver histopathology of cod infected with liver worm (TESLO)

Authors: Jane W. Behrens, Marie Plambech Ryberg, Virginia Chondromatidou and Tine Moesgaard Iburg

Background

As mentioned in earlier sections (1.1. and 1.2.), the parasitic nematode *Contracaecum osculatum* infects the liver of cod. The liver is a highly vital organ that is involved in several physiological functions, many related to growth and health status of the individual (Hinton et al., 2014). For the liver to function optimally, it is important that its structure is intact. Hence, structural damage and/or bleeding in the liver tissue may result in reduced functionality of the organ (Roberts & Ellis, 2012). Considering that liver worm accumulates in the cod liver over time, and that single livers may obtain very high numbers of this parasite (up to several hundred), it can be speculated that physical damage to the tissues of highly infected livers may occur. The parasite may also elicit immune reactions, being a 'foreign body' in the liver. Together, this will in all probability affect the functioning of the organ and result in reduced health status of the infected individual. We know from previous studies (including the findings in sections 1.1. and 1.2., published in Ryberg et al., 2020) that high infection levels in cod with *C. osculatum* lead to impaired physiology of the cod, shown by depressed energy turnover, reduction in the digestive organ masses along with changes in the plasma, body and liver composition and fish energy source. Especially the findings in section 1.2. (i.e., "Blood plasma in relation to infection with liver worm") reveal clear signs that highly infected cod suffer from a severe liver disease (Ryberg et al., 2020). Previous studies have shown that *C. osculatum* becomes encapsulated in the liver of cod as part of an inflammatory reaction (Buchmann & Mehrdana, 2016; Zuo et al., 2017), but no one have studied in detail what actually happens to the liver tissues in infected cod. In the present study, we therefore aimed to examine the relation between the degree of inflammation and other changes in the liver tissue with infection level of liver worm in Eastern Baltic cod. For this, we used histological examinations to categorise the degree of inflammation associated with different levels of infection with *C. osculatum*. With this investigation, we provide new knowledge on the histopathological effects of *C. osculatum* in Eastern Baltic cod.

Materials and methods

Eastern Baltic cod (n = 69) were caught by trawl in the Baltic Sea east of Bornholm, close to the harbour of Nexø in October 2018. Following evisceration of the fish and examination of the surface of their livers for number of visible nematodes, 30 livers were chosen to ensure a high variation in infection load of the livers included in the study. Three healthy, well-fed, and non-infected cod from the national aquarium 'The Blue Planet', Copenhagen, Denmark, were used as baseline for histopathological comparison. These cod have mainly been fed boiled blue mussel throughout their life in captivity. Data on length (cm), wet weight (gram), and liver weight (gram) of the 30 Eastern Baltic cod and 3 control cod were noted (Table 2.1.).

Table 2.1. Information of cod sampled east of Bornholm (30) and control cods from the Danish National Aquarium 'The Blue Planet' (3) for histopathology of their livers in relation to infections with *Contracaecum osculatum*. Table from Behrens et al., 2023.

| # | TL (cm) | TW (g) | LW (g) | Fulton | HSI | Preval. (%) | Intensity |
|----|----------|--------------|------------|-------------|-------------|-------------|------------|
| 30 | 38 ± 0.9 | 454 ± 34.8 | 17 ± 2.3 | 0.82 ± 0.03 | 3.66 ± 0.33 | 90 | 35.5 ± 7.0 |
| 3 | 63 ± 2.9 | 3396 ± 300.2 | 161 ± 66.4 | 1.34 ± 0.06 | 4.58 ± 1.64 | 0 | 0 |

Note: Values are mean ± SE unless otherwise stated.

Abbreviations: #, number of fish; HSI, hepatosomatic index; LW, liver weight; Preval., prevalence: percentage of fish infected with liver nematodes; Intensity: mean number of liver nematodes per fish, including only infected individuals; TL, total length; TW, total wet weight.

Samples were taken from each of three predetermined sites in the liver (left lobe, middle lobe and right lobe) for histopathological examination. Following the removal of the samples, the livers were frozen at -20°C for subsequent estimation of the total number of nematodes in the livers. After thawing, individual livers were placed in a plastic bag ($200 \times 400 \times 0.07$ mm) and compressed between two glass plates ($15 \times 15 \times 1$ cm) to a thickness of 1 mm by the addition of gentle pressure to the plates (Buchmann, 2007). Nematode species identification was based on morphometric characteristics of the caudal and cephalic ends according to Fagerholm (1982). For the histological analysis, we embedded (in paraffin), stained and sectioned 30 cod livers with varying degrees of liver worm infection. The sections were then analysed under a microscope, and the changes in relation to the number of liver worms described. Each liver tissue was assigned an 'Inflammation Category' between 1 and 5 (Cat1-Cat5), and this category was then compared with the number of liver worms in the same tissue. The categories are based on the severity of inflammation seen in the liver tissue, where a so-called granuloma (a collection of macrophages) is formed in response to chronic inflammation (Figure 2.1.). In addition to this, we looked at how the amount and shape of fat vacuoles changed in the liver in relation to infection intensity and we examined the presence of other parasites.

The livers from the Eastern Baltic cod were compared with each other in terms of infection intensity (none, low, medium and high infection intensity), as well as with livers from cod that had been housed on the Blue Planet for a period when they had been fed well (i.e. acting as controls). Two generalized linear models (GLM) with negative binomial distribution and a log link function were used to describe how infection density varied with (1) the five categories of intensity of inflammation in the liver and (2) the three categories each reflecting numbers of other parasites (none, few <10, and many >10). All statistical tests were conducted in R (R Core Team, 2016). The model assumptions of normality and independence were subsequently validated by visual inspection of model residuals.

Results

Overall, we found nematodes in 27 of the 30 livers and found in total 960 nematodes all belonging to *C. osculatum*. The results showed that the livers that had the most pronounced inflammation were also the ones that had the most liver worms. The statistical results showed that infection density of *C. osculatum* in the livers increased significantly with increasing inflammation category of the liver, with the infection density being lowest in livers assigned an inflammation category 0, and highest in livers assigned an inflammation category 3 and 4 ($df = 24$, $p = .002$, Figure 2.1. and Figure 2.2.A.). Track lesions were seen in categories 2–4, presumably created by the large nematodes moving around in the liver destroying or pushing liver cells aside (Figure 2.1.E, F).

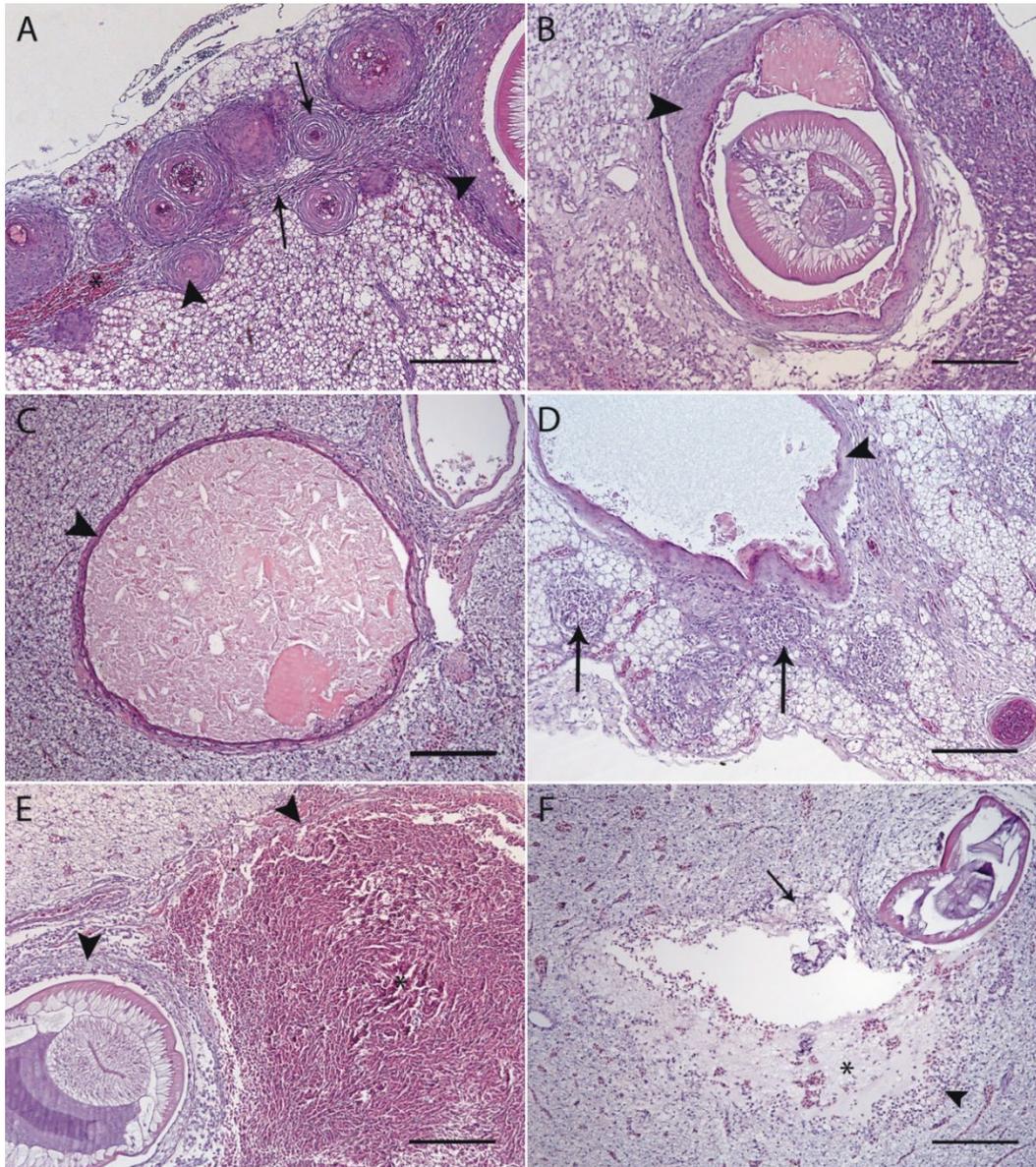


Figure 2.1. Inflammatory lesions in the liver of cod infected with *Contracaecum osculatum*. (A) A row of small granulomas and the edge of a larger granuloma in the right corner. A wall of epithelioid cells (arrowheads) surrounds a centre of parasite or red blood cells or the epithelioid cells fills the centre. The granulomas are surrounded by layers of fibrosis (arrow) and the fibrosis coalesce to form a band of fibrosis with the granulomas (fibrosis). Small haemorrhages are seen (asterix). (B) A large granuloma with a cross section of a nematode centrally and some proteinaceous material surrounded by a wall of epithelioid cells (arrowhead) and a thin layer of fibrosis with lymphocytes. (C) A large granuloma with eosinophilic amorphous material centrally surrounded by a thin wall of epithelioid cells (arrowhead). (D) Loosely formed immature granulomas are seen (arrows) at the borders of a large granuloma with pale basophilic material centrally delineated by epithelioid cells (arrowhead). In the right lower corner, a small granuloma is seen. (E) A track lesion filled with blood (asterix) next to a large granuloma with a cross section of a nematode. Epithelioid cells are seen around the granuloma and the track (arrow heads). (F) A track lesion with a cross section of a nematode in the upper right corner. Proteinaceous fluid is seen in the cavity (asterix) and artefacts are also seen (arrow). The track is surrounded by inflammatory cells (arrowhead). (A-F) Stained with HE. Bar = 176 μ m. Figure and text from Behrens et al., 2023.

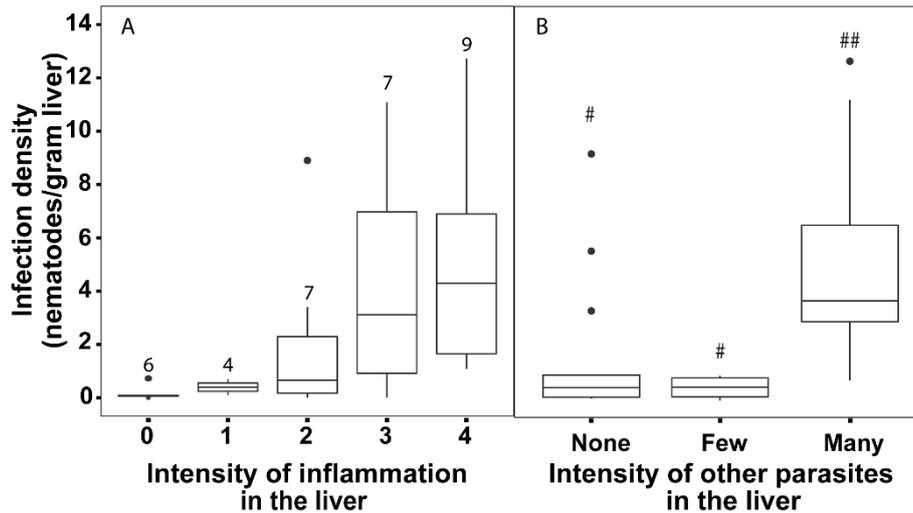


Figure 2.2. (A) Infection density (nematodes/g liver) in relation to the intensity of inflammation. Number on top of each box represent number of cod in each inflammation category. **(B)** Infection density (nematodes/g liver) in relation to the density of other parasites in the liver (none:0, few:1–10 and many: >10). The square symbols relate to the post hoc comparisons. In both figures, the solid line is the median in the box plots and the box is the interquartile area (bottom and top are 25th and 75th percentiles, respectively). Whiskers show either the max/min observation if within 1.5 of the interquartile range or 1.5 times the interquartile range. Figure and figure text are from Behrens et al., 2023.

In terms of the size distribution of the fat vacuoles in the livers, two things were discovered; firstly, the fat vacuoles in the livers of the well-fed cod from the Blå Planet were larger and more well-shaped than the fat vacuoles from the Eastern Baltic cod. Secondly, contrary to expectations, there were no difference in the size distribution of the fat vacuoles between Eastern Baltic cod with no, few, medium or many liver worms. For most of these cod, the fat vacuoles were generally of mixed size (with many very small vacuoles in between), and very irregular in shape. In several livers, another parasite was discovered, which exclusively inhabited the bile ducts (Figure 2.3.). The morphology and location were compatible with a Myxosporidian spp. In connection to this parasite, bile ducts were often dilated, and occasionally very mild fibrosis surrounded the bile ducts. When grouping intensity of infection of other parasites in the liver into none, few or many, the infection density with *C. osculatum* was significantly higher in the groups of livers designated many other parasites (df = 27, $p < .001$, Figure 2.2.B).

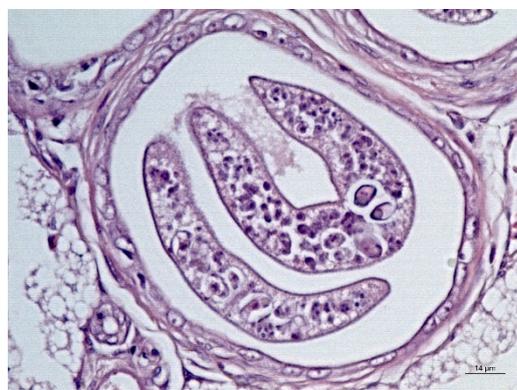


Figure 2.3. Dilated bile duct with flattened epithelium in a cod liver with Myxosporidian parasites. A normal bile duct is seen next to the dilated bile duct. Photo; Virginia Chondromatidou.

Conclusion

The liver of a cod is normally a very fatty organ and can be regarded as the cod's 'lunch box'. Under circumstances where the liver tissue is destroyed, for example by the presence of a high number of foreign bodies like parasites, it can have consequences for the fish's energy status and growth potential. The described histopathological changes in the present study in cod with high infection loads of *C. osculatum* in their livers, including inflammatory reactions and bleedings, supports previous studies (Buchmann and Mehrdana, 2016; Mohamed et al., 2020; Ryberg et al., 2020, 2022) suggesting that high infections loads can result in reduced liver functionality and thus reduced health status, because of damage to the liver tissue. The results from this were published in Behrens et al., 2023, Journal of Fish Disease <https://doi.org/10.1111/jfd.13776>

3. Spatial distribution and liver category of liver worm in the Baltic Sea (TESLO)

Authors: Marie Plambech Ryberg, Bastian Huwer, Anders Nielsen, Jan Dierking, Kurt Buchmann, Maria Sokolova, Uwe Krumme and Jane W. Behrens.

Background

The spatial distribution of liver worm in cod will potentially vary over time, depending for example on how the number of grey seals (which are the main host of the liver worm) develops in the future in the various areas. In other words, the areas where the cod are heavily infected with liver worm can change over time, and it is important to follow this development, to understand which factors may affect the health status of the different cod populations. One major challenge in this respect is that detailed analysis of cod livers where the total number of liver worm in each organ is counted is time-consuming and expensive, and therefore only done occasionally, except in Poland, where continuous analysis of total number of liver worm in individual cod livers has occurred for several years as part of their monitoring routines. As a cheaper and less time-consuming alternative, Denmark and Germany started on a volunteer basis to register liver worm in individual cod on their monitoring cruises, beginning in 2015/2016. These registrations have been done using the so-called 'liver category' method. Here individual livers are assigned a category between 0 and 4, depending on how many liver worms can be observed on the surface of the liver. More specifically, a liver with category 0 has no visible nematodes on the surface, a category 1 liver has 1-10 nematodes visible on the surface, a category 2 between 11-20 nematodes, a category 3 between 21-30 nematodes and category 4 over 30 nematodes (Figure 3.1.).



Figure 3.1. Photo of the five liver categories (0–4) of nematode infection levels of cod livers that is used in the Baltic Sea. A liver category scale is assigned according to the number of nematodes counted on the surface of the liver and the categorical boundaries given by the scale. Photo by B. Huwer and published in Ryberg et al., 2022.

Based on the wish to follow the spatio-temporal development in liver worm load, the aims were to ensure that i) all the countries around the Baltic Sea start recording the occurrence of liver worms in individual cod on their monitoring cruises, ii) that everyone uses the same method for this (whereby data becomes comparable), and iii) that these registrations of liver worms become a permanent part of the countries' data collection protocol. To ensure this, the current project manager (JWB) organized and held a workshop at DTU Aqua in February 2020 for representatives from Germany, Sweden, Poland, Estonia and Latvia, with the aim of discussing the possibility of a joint monitoring of liver worms on the countries' research cruises in the Baltic Sea, as well as agreeing on a protocol for this.

Based on the outcome from the above-mentioned workshop, ICES has subsequently decided that from 2021 it is mandatory to record liver worms in individual cod on all BITS (Baltic International Trawl Survey) surveys in the Baltic Sea (ICES, 2021). It is a great advantage that liver worm registrations based on the liver category method have now become mandatory, but there is one challenge: With the liver category method, only the parasites that are visible on the surface of the liver are counted, and there can be several liver worms inside the organ which are not immediately visible. In other words, the liver worm categories to some extent underestimate the total number of nematodes in livers.

The overarching aim of this study was therefore to test the ability of liver category method to act as a predictor for the total number of nematodes in the liver, and to examine its use as a means of monitoring the spatiotemporal development in infection load at the population level. This was done by testing whether the liver categories are a sufficiently accurate estimate of the total number of nematodes in the whole liver. As a supplement to this we also examined the association between infections and critical individual Fulton condition factor of cod to test whether infections may result in increased natural mortality.

Methods and material

We established a database containing both liver category and counted total number of nematodes for each individual liver assigned a liver category by using the so-called “liver category method” on fresh livers. Altogether, 642 cod from the ICES defined subdivisions (SD) 22 (Kiel Bight and Mecklenburg Bay, western Baltic), SD24 (Arkona Basin) and SD25 (Bornholm Basin) were examined (Figure 3.2.). To account for the potential seasonal variation in condition and in the liver size, sampling was performed in different months between 2017 and 2020.

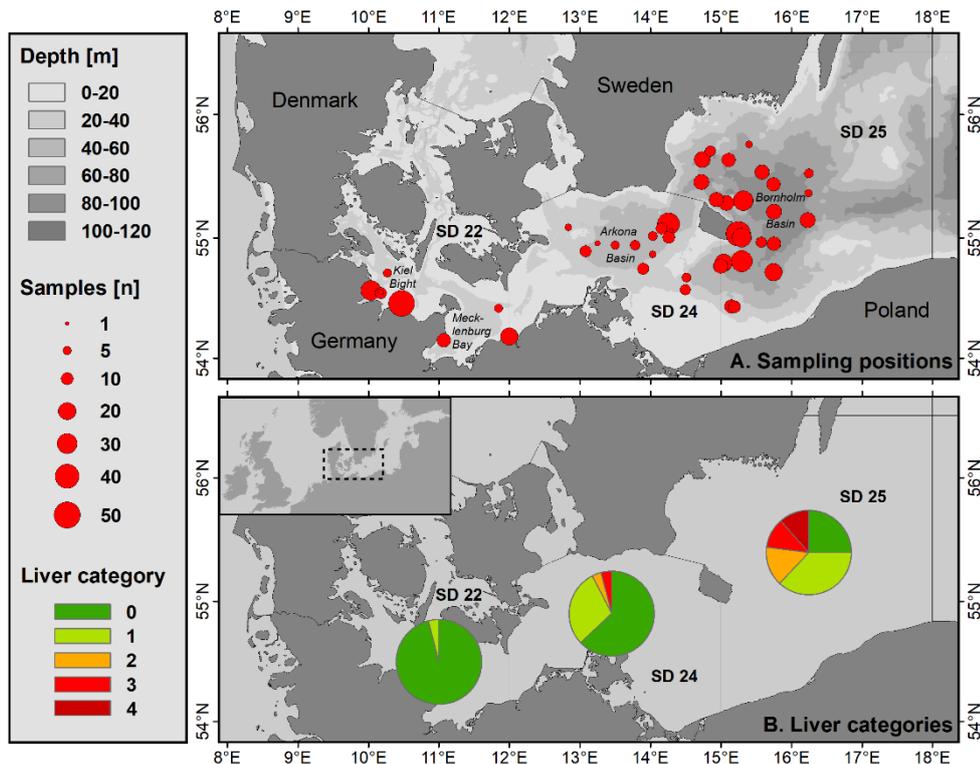


Figure 3.2. (Upper) The study area and the sampling positions within the three ICES subdivisions (SD22, SD24 and SD25) where cod were collected for analysis of liver nematodes: The grey scale indicates water depth of the Baltic, and the size of the bubbles represents the number of samples examined from each position. (Lower) Percentage of the total number of livers assigned to each liver category by subdivision. Figure and figure text from Ryberg et al., 2022.

After assignment of liver categories, individual livers were kept at -20 °C until subsequent analysis of total number of nematodes using the so-called “compression method”. Nematode species identification was based on morphometric characteristics of the caudal and cephalic ends according to Fagerholm (1982).

Four area-specific (for SD 22, 24 and 25 respectively) generalized linear (GLM) models which could estimate the exact number of worms based on the category to which the liver has been assigned were developed for this study. In all four models, the total number of nematodes was defined as the response variable (μ_i), which followed a negative binomial distribution (eq. 1):

$$\log(\mu_i) = \alpha(\text{livercategory}_i) * HSI_i + \beta(\text{livercategory}_i) * TL_i + \delta(\text{livercategory}_i) \quad (\text{eq. 1})$$

Where HSI refers to the liver index (hepato-somatic index) and TL to the total length of the fish. To examine associations between Fulton condition factor and infections with *C. osculatum* in cod, two different analyses were performed on data from SD25. Calculation of Fulton condition factor was based on gutted weight, GW and total length, TL (eq. 2):

$$\text{Fulton condition factor} = \frac{GW}{TL^3} * 100 \quad (\text{eq. 2})$$

The first analysis aimed to examine the association between Fulton condition factor and infection density and the second analysis aimed to estimate the probability of cod with critical condition factor (i.e. below 0.65) in relation to infection density. The critical Fulton condition factor reflects the level where cod are considered dying (Dutil and Lambert, 2000; Casini et al., 2016a). The statistical tests were carried out using R with Rstudio (version 3.4.1.) (R Core Team, 2016) and the four GLM models were fitted with glmmTMB using the package “glmmTMB” (Brooks et al., 2017). Model selection was performed using a stepwise backward selection routine based on a likelihood ratio test for each of the variables included and excluded in the models.

Results

A total of 11352 nematodes were recovered from the 642 livers examined, with 32, 1487 and 9833 nematodes from SD22, SD24 and SD25, respectively. Overall, there were pronounced differences between SD22, SD24 and SD25, both in relation to the number of livers assigned to each liver category, and in the counted number of nematodes within each liver category (Figure 3.3.).

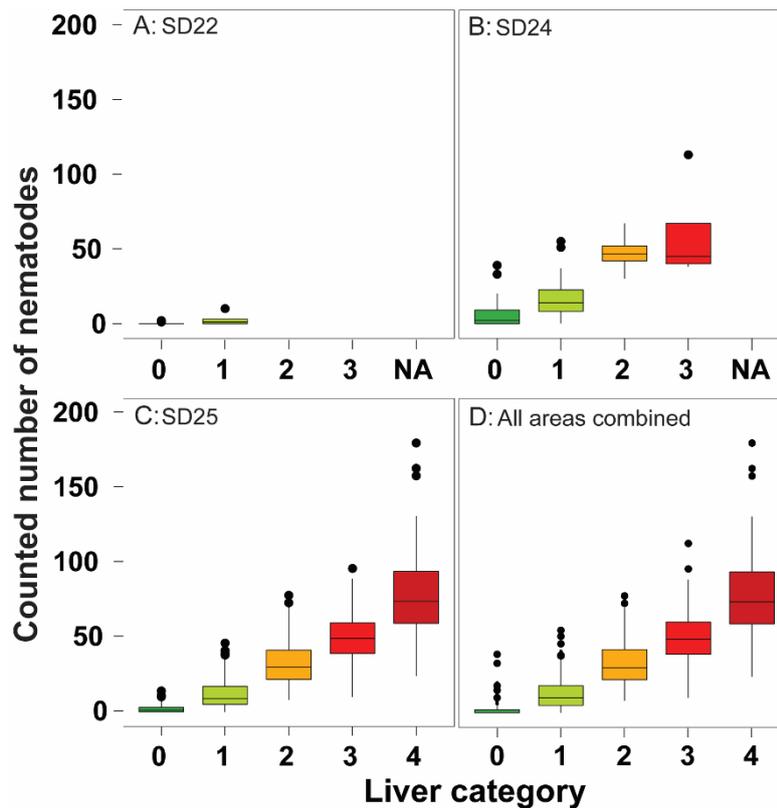


Figure 3.3. Relationship between the assigned liver categories and the counted total number of nematodes inside the livers of Baltic cod sampled in subdivisions (SD) defined by ICES (A = SD22; B = SD24; C = SD25) and all areas combined (D). See Figure 3.1. for visual appearance and a detailed description of the five liver categories. In panels (A) and (B), NA illustrates that to date this category has not been used in the areas SD22 and SD24. For the box plots, the solid line is the median and the box is the interquartile area (bottom and top are 25th and 75th percentiles, respectively). Whiskers show either the max/min observation if within 1.5 of the interquartile range or 1.5× the interquartile range. Black dots illustrate the outliers in the data. Figure text and figure from Ryberg et al., 2022.

In all three area models, the estimated number of nematodes increased significantly with increasing liver category ($p < 0.001$). In SD22, the estimated number of nematodes increased significantly with TL ($\beta = 0.094$, $p = 0.04$) and HSI ($\gamma = 0.331$, $p = 0.04$), whereas there was no effect of HSI and TL on estimation of nematodes in SD24. In SD25, estimation of the number of nematodes increased with TL ($\beta = 0.049$, $p < 0.001$) but decreased with increased HSI ($\gamma = -0.043$, $p = 0.004$). For a 40 cm cod with a category 4 liver (i.e. highly infected) from SD25, the predicted number of nematodes was e.g. 39% lower in fish with highest observed HSI (HSI=14.9; 42 nematodes) compared to cod with a medium HSI (HSI=5.4; 61 nematodes) (Figure 3.4.).

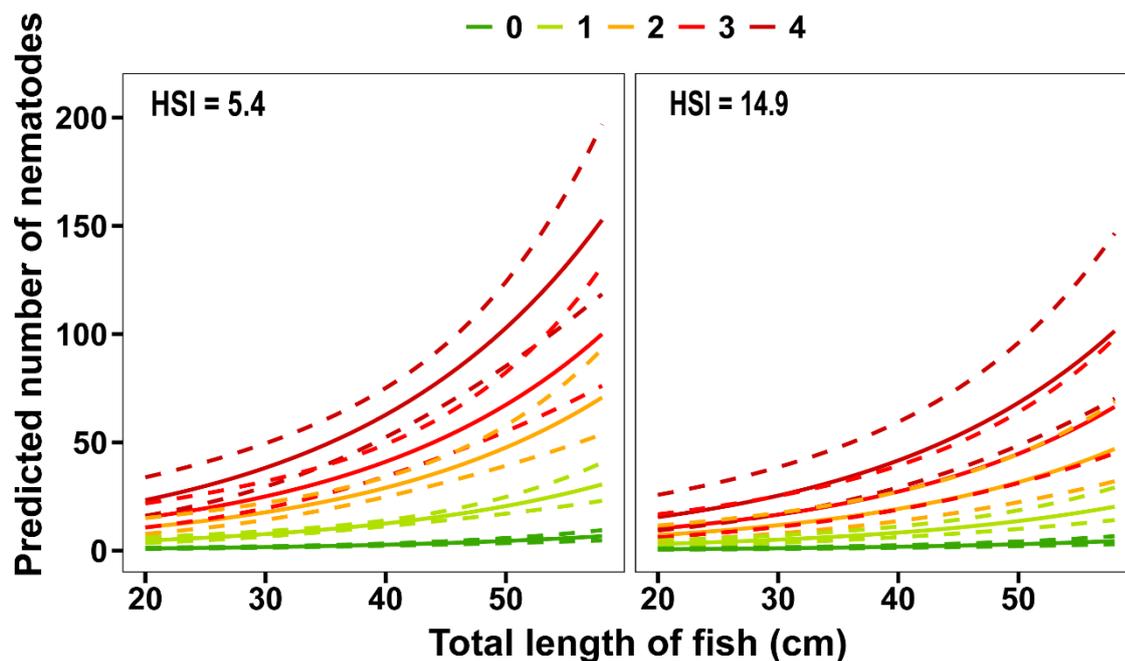


Figure 3.4. Predictions of the total number of nematodes in livers of Baltic cod from area SD25 derived from the final GLM model for different cod sizes with mean hepato-somatic eviscerated index (HSI) = 5.4 (a) and the highest observed HSI index = 14.9 (b). Colours represent the five liver categories 0–4. See Figure 3.1. for visual appearance and a detailed description of the five liver categories. Solid lines: mean predictions of the total number of nematodes, dashed lines: confidence intervals (0.95) of the model predictions. Figure text and figure from Ryberg et al., 2022.

Fulton condition factor decreased significantly with an increase in IFD (likelihood ratio test, $p < 0.001$, $\gamma = \exp(-0.029)$, $SD = 0.002$, $\text{intercept} = \exp(-0.262)$). The subsequent calculation of the tail probability revealed a sigmoid pattern between IFD and the probability of the fish having a critical Fulton condition factor (Figure 3.5.).

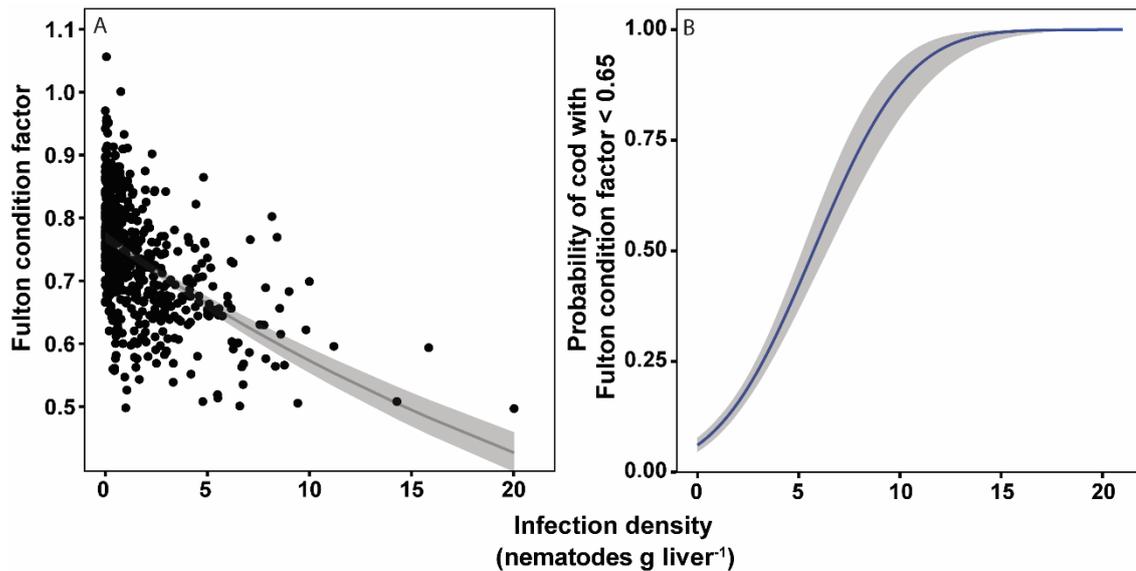


Figure 3.5. A) Model fit (grey solid line) and uncertainty (grey area) of Fulton condition factor in relation to infection density (IFD) for the 594 eastern Baltic cod sampled between 2016 and 2020 from area SD25 and included in the present study. (B) Mean (blue solid line) and uncertainty (grey area) of the probability for cod having Fulton condition factor below 0.65 (i.e., critical Fulton condition factor) for different levels of IFD. The probability model-fit and the uncertainty are calculated based on the predictive output of the model shown in panel A. The level of the critical Fulton condition factor where cod are considered dying is defined in Casini et al., (2016a). Figure text and figure from Ryberg et al., 2022.

Conclusion

It is important to get information about the exact number of nematodes in the liver of cod to calculate the infection density (number of nematodes per gram liver), which is known to relate with the health status of cod (Ryberg et al., 2020). With the present study we for the first time verify the liver category method and its ability to predict the total number of nematodes. We found that the number of nematodes predicted by the models for each liver category was significantly different between all categories. This means that we have demonstrated that the liver category method is a robust proxy for the total number of nematodes in individual livers, i.e., the method is a good and easy supplement to the more detailed analyses of cod livers for liver worms and can be used to follow the spatial and temporal development of liver worm in cod in different areas. Finally, we also showed that infection density is related to an increased probability of cod having a critical Fulton condition factor (below 0.65), believed to lead to mortality of the fish. Altogether our findings show that the liver category is suitable tool to obtain pan-Baltic information on spatiotemporal changes in infection load with liver worm in cod and we hope to inspire others working with disease in fish stocks to include parasite monitoring. The results of this work have been published in Ryberg et al., 2022, *Fisheries Management and Ecology* <https://doi.org/10.1111/fme.12516>

4. Bioenergetics modelling of growth in infected cod (TESLO+TORVÆKST)

Authors: Marie Plambech Ryberg, Asbjørn Christensen, Christian Jørgensen, Stefan Neuenfeldt, Peter V. Skov and Jane W. Behrens.

Background

Parasites are the most common animal lifestyle and universal components of biological systems (Marcogliese, 2004; Kuris et al., 2008). Per definition, parasites exploit their hosts for development and reproduction (Combes, 2001). Yet, infection with parasites is often neglected when describing stressors in wild fish populations (Lloret et al., 2012; Timi and Poulin, 2020) and have so far has been ignored in mechanistically founded bioenergetics models for fish. Building on a previous bioenergetics modelling approach used to study growth and maturation of Northeast Arctic cod (Jørgensen & Fiksen, 2006), we here for the first time assess the effects of parasite load on the energy budget and subsequent growth potential of an infected wild host population. We use the host-parasite system between the Eastern Baltic cod stock and the parasitic nematode, *Contracaecum osculatum*, as a case study. Recent studies have revealed a negative association between parasite load and poor nutritional condition and impaired physiological condition of Eastern Baltic cod infected with *C. osculatum* (Horbowy et al., 2016; Sokolova et al., 2018; Ryberg et al., 2020). The overall aim of the present study was to develop and parameterise the model with data from the literature on Baltic cod, as well as own data generated under the previous EMFF project 'Effect of seal-related liver worm on Baltic cod growth and mortality' (33113- B-16-071) and the studies carried out in section 1.1. and 1.2. Moreover, results from the studies in section 6 (i.e., Spatial distribution of nematodes in sprat and energy content) were used to validate the output of the model. This model is the first bioenergetic model where the effect of parasites on the growth and condition of fish is evaluated.

Methods and material

In brief about the model, the free energy that the cod consumes (via food) can be allocated to either somatic growth (irreversible) and/or storage, where storage is energy that can be used for reproduction, or to pay for metabolism, in situations where the fish is food restricted. This framework of the model is based on the Wisconsin framework (Hewett and Johnson, 1992) that describes how energy in the food is diverted to digestion, excretion and defecation and further cover costs of maintenance metabolism and activity (swimming) before any surplus can be used for growth and reproduction (Figure 4.1.). Changes in growth within the model are seen as changed length/weight/condition. The model is developed from a previously established, state-dependent bioenergetics model that first was developed for the Northeast Arctic stock of Atlantic cod (Jørgensen and Fiksen, 2006) to study growth and fishing induced changes in maturation age and size. In the present study, modifications that describe infections with parasitic nematodes are added to resolve the emerging patterns in growth and condition, and the model is reparameterised for the Eastern Baltic cod.

The surplus energy of the model is the energy intake minus the metabolic cost related to metabolic processes associated with life:

$$\text{surplus energy} = \text{energy}_{\text{intake}} - \text{metabolic}_{\text{cost}} \quad (\text{eq. 1})$$

The key additions in terms of implementation of infections with parasites in the present version of the model are (i) food intake-dependent parasite infection load, and (ii) recursive parasite load effects on cod food intake and energetic requirements. Thus, fish in the model gets infected by eating infected sprat and number of parasites accumulates over time in the model resulting in an increase in infection load over time. Focus was put on the emerging consequences of successively lowered energy intake at progressing parasite infection on cod growth and condition. Four new parameters were implemented to the model to meet the energetic effects of infections with parasites. These were parameterised based on previous studies on the physiological condition of infected cod (Ryberg et al., 2020) and validated based on the findings in section 6 in the current report. For more detailed information about the modelling structure please see a model description in Ryberg et al., 2023. This version of the model was fully recoded and implemented in Python (Harris et al., 2020; Python Software Foundation, 2020), and plots were made in R (R Core Team, 2016).

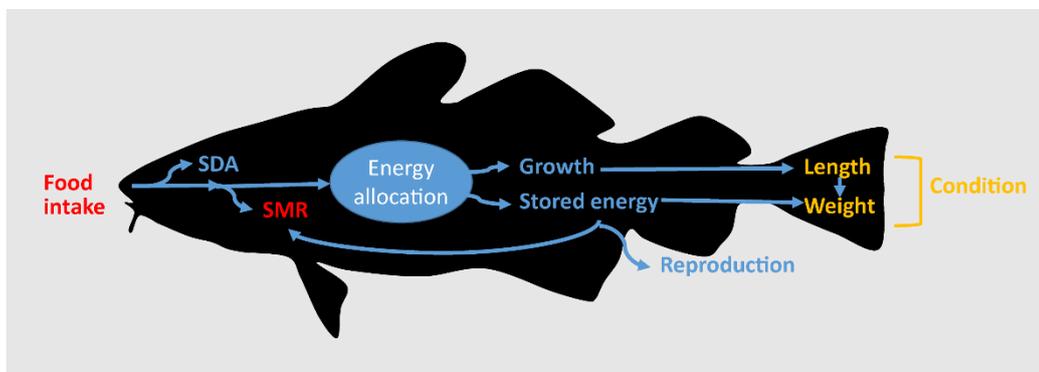


Figure 4.1. Schematic overview of the bioenergetic model examining growth and condition of fish infected with parasites. In the model the parasite causes a reduction (red colour) of maintenance cost (SMR) as well as energy intake. The standard dynamic action (SDA) is covering all energy related to processing of food for use and storage. When the energetic requirements of SMR and SDA are covered, the surplus energy is allocated toward different processes. In the present model, the fish store its energy and use it on growth and/or stored energy where the latter can be allocated to reproduction and SMR depending on the energetic status of the fish (white colour). In the case where surplus energy becomes negative (i.e. reduction in food intake), the fish can start using its stored energy for maintenance of SMR (dashed white line). Length, weight and resultant condition are states of the outcome of the energy allocation (dark grey colour). Figure text and figure from Ryberg et al. (2023).

Results

The results from the model showed that independent of the starting size of the cod, the growth rate and condition factor decreased when the infection rate with liver worm increased (Figure 4.2.).

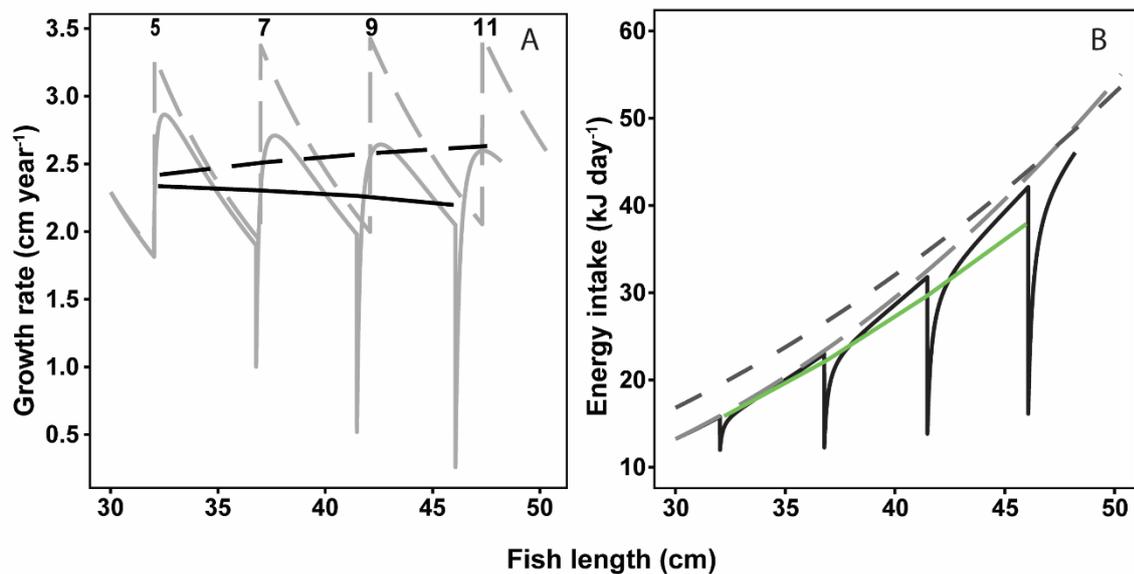


Figure 4.2. (A) Monthly resolved trajectories (grey lines) and annual trends (black lines) of growth rate in individual non-infected cod (dashed lines) and infected cod (solid lines). Numbers above spikes represent the age (years) of the cod in the model. (B) Overall trends of daily energy intake (kJ/day) in relation to body length of infected cod. The four different lines reflect non-infected individuals with either recent satiation level (light grey dashed line) or historic satiation level (dark grey dashed line), and infected individuals with recent satiation level (black solid line) and overall trend (green solid line). The four minimum spikes in both panels A and B reflect the time after spawning where infection density (nematodes per gram liver) is very high due to the use of energy from the liver. The model output for food intake has been validated by comparison with intake levels with previous data from Neuenfeldt et al., (2020). Figure text and figure is from Ryberg et al. (2023).

In addition, the model showed that heavily infected cod reached a 'point of no return' where their energy intake was so low that they could no longer get the energy required to cover the basal metabolism; after that the fish starved to death (Figure 4.3.).

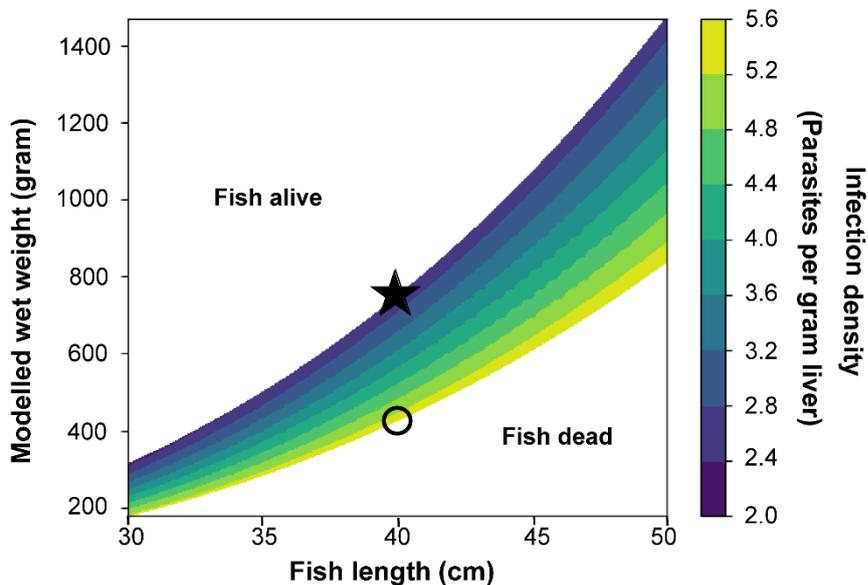


Figure 4.3. The infection level at given length and weight (contour scale) where the energy budget becomes negative in the model and the cod enters a ‘point of no return’ with respect to nutritional condition, i.e. the fish is unable to grow in length because its surplus energy balance is negative. For example, a 40-cm cod with infection density at two enters the vicious spiral when the body mass declines below 750 g (black star) and then dies when it enters below the yellow contour line in the plot (black open circle). In general, the fish is alive in the white area above and dead in the white area below the contour lines. Figure text and figure from Ryberg et al., 2023.

Validation of the model's output based on results from section 6 in the present report showed that the model initially overestimated how many parasites the cod got from its food intake of sprat. This meant that the fish in the model became infected far too quickly with unrealistically high infection rates. With the infection rates for sprat (section 6.1.), a more realistic infection rate of sprat was validated and provided a more realistic output i.e., that the 'model cod' had infection rates that correspond to data from field studies. In addition, the results from the growth trial were used to support the final model output.

Conclusions

In conclusion, building on established bioenergetics concepts, we showed that a broadly applicable modelling approach can be used to complement laboratory and field investigations to unravel effects of parasites on vital rates. This contributes to the understanding of an often-overlooked driver of wild fish populations (and wild animals in general), namely parasites. Thus, with this modelling approach we show how it is possible to bridge between two different worlds, biology and modelling. This concept is important as the modelling framework offers a platform to incorporate candidate drivers of growth and condition simultaneously, for example temperature, hypoxia and prey quantity and quality, to understand their partial and synergistic effects. The results from the present study are published in Ryberg et al., 2023, *Conservation Physiology* <https://doi.org/10.1093/conphys/coad007>

5. Growth potential of infected cod (TORVÆKST)

Authors: Jane W Behrens, Marie Plambech Ryberg, Anders Nielsen, Maria Krüger and Niels Gerner Andersen.

Background

The Eastern Baltic cod stock has suffered from poor individual growth and condition since the early 1990s and the stock is in distress, with historically low productivity (Casini et al., 2016b; Eero et al., 2015; Hüseyin et al., 2018; Mion et al., 2020). Many drivers behind this trend have been debated including changes in feeding levels that recently are very low compared to historic feeding levels (Neuenfeldt et al., 2020). In addition, the Eastern Baltic cod has also suffered from an increase in infections with *C. osculatum* in the last decades (Haarder et al., 2014; Nadolna and Podolska, 2014). Whether growth is negatively or positively affected with a given parasitic infection depends on whether the parasite is a so-called low- or high-cost parasite to its host (Henriksen et al., 2019). Three main factors that can determine the severity of parasitic infections to the host are as follows: 1) the site of infection of the parasite; 2) the immune response of the host; and 3) the condition of the fish when it becomes infected (Ryberg, 2020). If we look at number 3, the high infection loads found in wild Eastern Baltic cod can therefore be a result of the low conditioned cod and thus low feeding levels in nature when cod start to get infected. To gain a better understanding of whether the infections with liver worm in cod also tend to have a direct effect on growth, thus ignoring the food limitations, controlled experiments are needed. In the present study we wanted to investigate the growth potential of non-food-restricted cod with varying degrees of infection loads, to examine the potential effect of *C. osculatum* on cod growth potential. A controlled experiment was carried out, in which we determined growth rates in relation to varying degrees of infection loads in cod fed to saturation.

Method and materials

Wild and naturally infected cod (approx. 200 in total) were sampled by trawl during a Baltic International Survey Cruise (BITS) in the Baltic Sea East of Bornholm in November 2020. The cod were transported by ship to the fish-holding facilities at DTU Aqua in Lyngby. Upon arrival, the fish were treated in a 1:5000 formalin bath for 30 min to kill any ectoparasites, and subsequently held in two circular 2000 l (larger fish) and one 800 l (smaller fish) holding tanks receiving filtered, recirculated, well-aerated (dissolved oxygen 95-98%) seawater at 10 °C, and a salinity of 11 ppt. The fish were fed to satiation every second day with chopped sandeel. After 8 days in the Blue unit, the 161 fish were anesthetized and each individual fish was implanted with a pit tag in order to be able to control every single growth potential growth during the experiment. At the same time, the cod's nutritional status (condition) was calculated by collecting data on length and weight for each individual. Based on length, the fish were separated into three groups divided into three different basin (large fish: n = 37, medium-sized fish: n = 89, and small fish: n = 35) and kept in the above-described tanks for four days to recover before initiation of the growth trials. All experiments were carried out according to the animal welfare regulations of the Technical University of Denmark and EU directive 2010/63/EU for animal experiments. Ethical permit 2017-15-0201-01282 from the Danish Animal Ethics Committee covered all experiments reported here. When there were approx. 4 days into the experimental period, we stopped feeding the fish to ensure that there was no food left in the stomachs that could affect the final weight of the fish. On the last day of the experiment, the fish were euthanized by an overdose of

anaesthetic. Then weight, length and liver weight were recorded, and the liver was subsequently stored in the freezer at minus 20 degrees to be able to later analyse each individual liver for the total number of nematodes (Figure 5.1.).



Figure 5.1. Setup for gaining data on cod after finishing the experiments and collecting data on weight, (left picture) length and (right picture) liver for all cod.

Results

Overall, the results of the controlled growth trial showed that despite unlimited access to food the growth potential (reflected by specific growth rate) was lower in the cod with high infection densities compared to cod with low infection densities (Figure 5.2.).

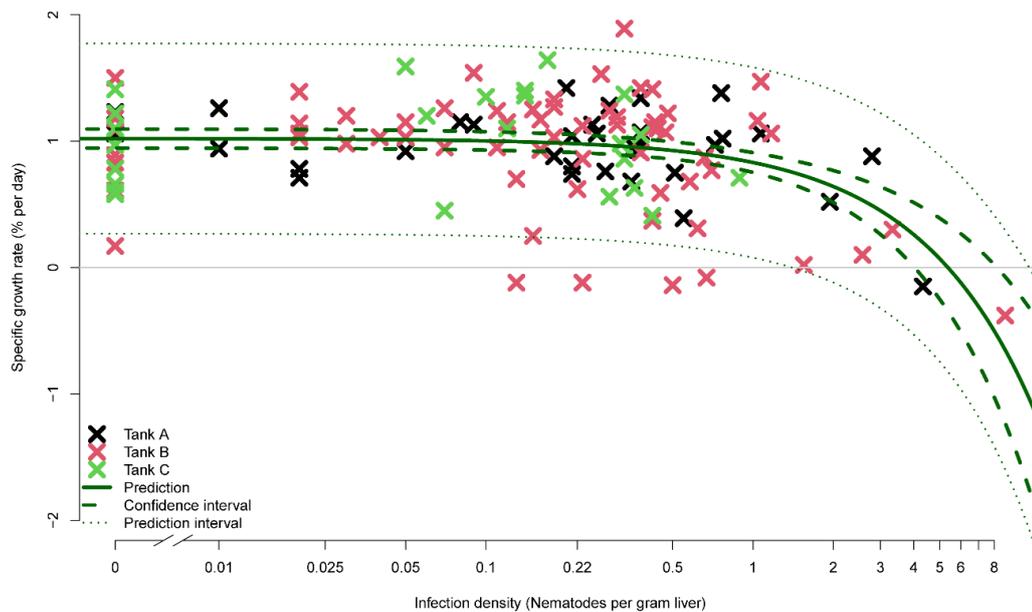


Figure 5.2. Model fit (green solid line), uncertainty of the model fit (green dotted lines) and uncertainty related to observations (light green dotted lines) of specific growth rate in relation to infection density (IFD) for the 119 cod included in the study. Colours represent the three groups of fish defined in the study; small cod (green), medium cod (red) and large cod (black).

In addition, the most infected cod also had a lower liver index (i.e., hepatosomatic index; HSI) meaning a smaller liver size compared to body size (Figure 5.3.) and did not gain as much weight as the non- or low-infected individuals (Figure 5.4.).

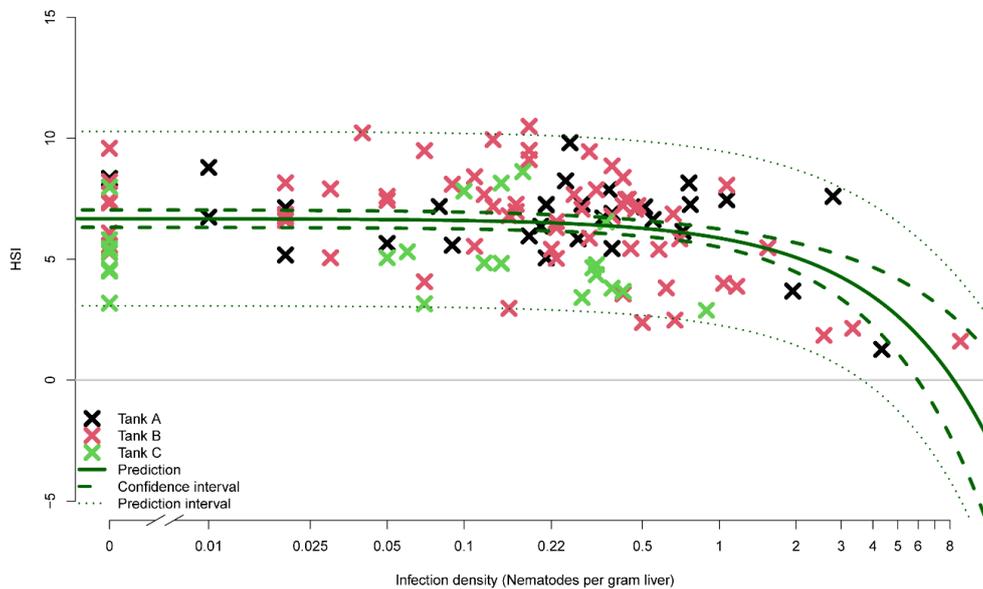


Figure 5.3. Model fit (grey solid line), uncertainty of the model fit (green dotted lines) and uncertainty related to observations (light green dotted lines) of the Hepatosomatic index (HSI) in relation to infection density (IFD) for the 119 cod included in the study. Colours represent the three groups of fish defined in the study; small cod (green), medium cod (red) and large cod (black).

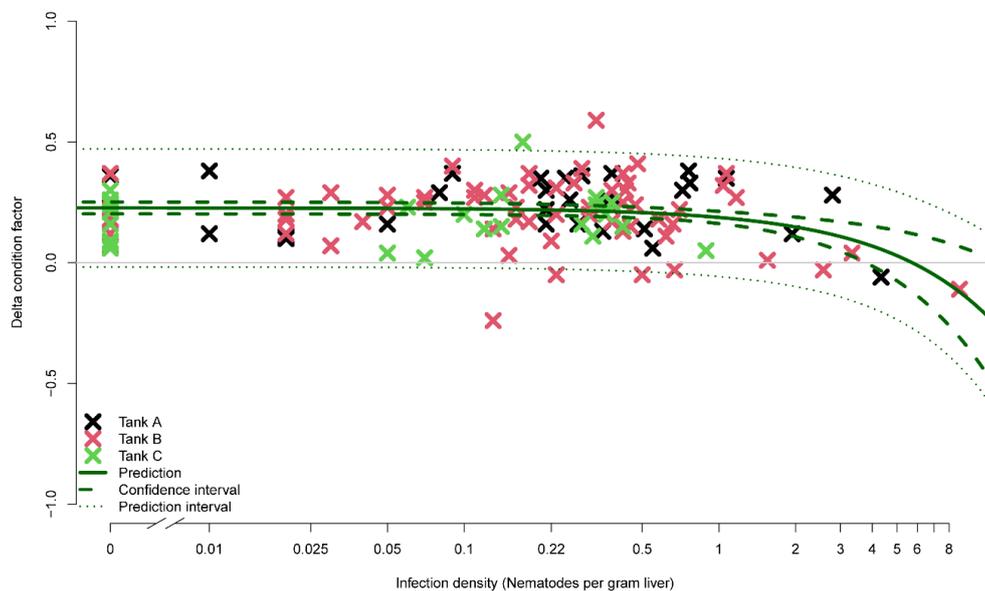


Figure 5.4. Model fit (grey solid line), uncertainty of the model fit (green dotted lines) and uncertainty related to observations (light green dotted lines) of Δ Fulton condition factor in relation to infection density (IFD) for the 119 cod included in the study. Δ Fulton condition factor reflects the changes in the Fulton condition factor over the experimental period calculated as the difference between the Fulton condition factor at the final day of the experiment minus the Fulton condition factor at the first day in the experiment.

Conclusions

We here for the first time show that the growth potential, liver index and Fulton condition factor are reduced in highly infected cod despite the fish being fed to saturation. The reason for these results may be that the liver function is so reduced due to the high infection loads that there is so little functional liver tissue at the start of the experiment that the fish cannot restore its normal liver function despite being offered food ad libitum. From these findings we support previous studies revealing that infections with liver worm lead to reduced health status of the cod (Behrens et al., 2023; Ryberg et al., 2020, 2022, 2023; section 1.1., 1.2. and 2 in the present report). Thus, we argue that the severity of these infections reflects that liver worm is a high-cost parasite to the cod. Thus, Eastern Baltic cod are not only food limited in nature, they also have to battle infections in the liver with this parasite, in an organ being one of the most vital for growth and nutrient turnover. A manuscript based on these results and intended to ICES Journal of Marine Science is being finalized for submission.

6. Spatial distribution of liver worm in sprat and energy content (TORVÆKST)

6.1 Spatio-temporal distribution of liver worm in sprat (TESLO)

Authors: Bastian Huwer, Katarzyna Nadolna-Ałtyn, Marie Storr-Paulsen, Uwe Krumme, Richard Klinger, Jan Dierking and Jane W. Behrens

Background

During the last decades, a shift from a cod-dominated (*Gadus morhua*) to a sprat-dominated (*Sprattus sprattus*) system was observed in the upper trophic levels of the Central Baltic Sea ecosystem (Köster et al., 2003). Sprat is a key fish species in the Baltic Sea ecosystem: it is the most important fish in the food webs of the open part of the sea (Ojaveer and Kalejs 2010), where it is a major prey of piscivorous consumers such as cod, salmon and marine mammals. Sprat is also economically important for the Baltic Sea Fisheries (Ojaveer and Kalejs 2010) and is targeted for both fish meal and fish oil as well as human consumption. Thus, due to the potential role of sprat in both the transmission of parasites in the Baltic Sea ecosystem and issues related to food safety, it is important to know the dynamics of sprat infection with zoonotic Anisakidae nematodes.

Three main species of parasitic nematode that use fish as transport host exist: Herring worm *Anisakis simplex*, cod/seal worm *Pseudoterranova decipiens* and liver worm *Contracaecum osculatum*. These species have quite similar life cycles, where marine mammals are the final hosts. In the final host the mature nematodes produce fertilized eggs, that are released to the water environment with the faeces of the marine mammals. In the water within the egg transformation to the larval stages L1, L2 or even L3 occurs (Køie and Fagerholm 1995). L2 or L3 larvae are eaten by crustaceans (intermediate host) where transformation from L2 to L3 can take place. Infected crustaceans are then eaten by pelagic fish (e.g. sprat, herring), which in turn are eaten by predatory fish (e.g. cod, salmon). These parasites are ingested by fish with food intake and can migrate from stomach and intestines to other organs like muscles or liver. Marine mammals feed on infected fish and thus become the definitive / final host and subsequently the life cycle is completed (Køie and Fagerholm 1995; Klimpel et al. 2004; Mouritsen et al. 2010).

Research conducted so far revealed the presence of liver worm in sprat in Estonian waters in the southern Baltic Sea (Turovsky et al. 1992), in the Russian economic zone (Rodjuk and Eliseev 2006, Rodjuk 2014), in the Gulf of Gdańsk (Skrzypczak and Rolbiecki 2015) and in central off-shore areas of Polish waters (Nadolna-Ałtyn et al. 2018) as well as the south-western area of the Baltic Sea (Zuo et al. 2016). Despite these reports on the infection of sprat with *C. osculatum* from various areas, a more comprehensive analysis of the spatial and seasonal occurrence and distribution of sprat infection in the Baltic is so far lacking. Furthermore, so far only liver worm was detected in Baltic Sea sprat while other parasitic nematode species were also reported in other fish species from that region.

Therefore, the aim of present study was to analyse the spatio-temporal dynamics of sprat infection with liver worm in the central and southern Baltic Sea. To achieve this goal sprat samples

were collected in different areas along a west-east transect from Kiel Bight to the Gulf of Gdańsk during surveys in four quarters of the year.

Materials and methods

Sampling

Samples of sprat were collected during various research cruises in 2021 and 2022 in collaboration with several of DTU Aqua's partner institutes. The following 5 institutes were involved in the sampling: National Institute of Aquatic Resources, Technical University of Denmark (DTU Aqua Denmark); Thünen Institute of Baltic Sea Fisheries (TI, Germany); Institute of Marine Ecosystem and Fishery Science, University of Hamburg (UH, Germany); GEOMAR Helmholtz Centre for Ocean Research Kiel (Geomar, Germany); National Marine Fisheries Research Institute (NMFRI, Poland).

The sprat were collected randomly from the catch (i.e. without any size selection), frozen on board and transported to the laboratory on land for further analysis. All collected samples were transported frozen to NMFRI, where the following analyses were conducted.

The aim was to collect samples (300 sprat each) in all four quarters of 2021 and from 6 different areas on a west-east transect in the southern Baltic Sea (see Figure 6.1.1): Kiel Bay (ICES SD 22), Mecklenburg Bay (ICES SD 22), Arkona Basin (ICES SD 24), Bornholm Basin (ICES SD 25), Midsjöbanke = Middle Bank (ICES SD 25) and Gulf of Gdańsk (ICES SD 26).

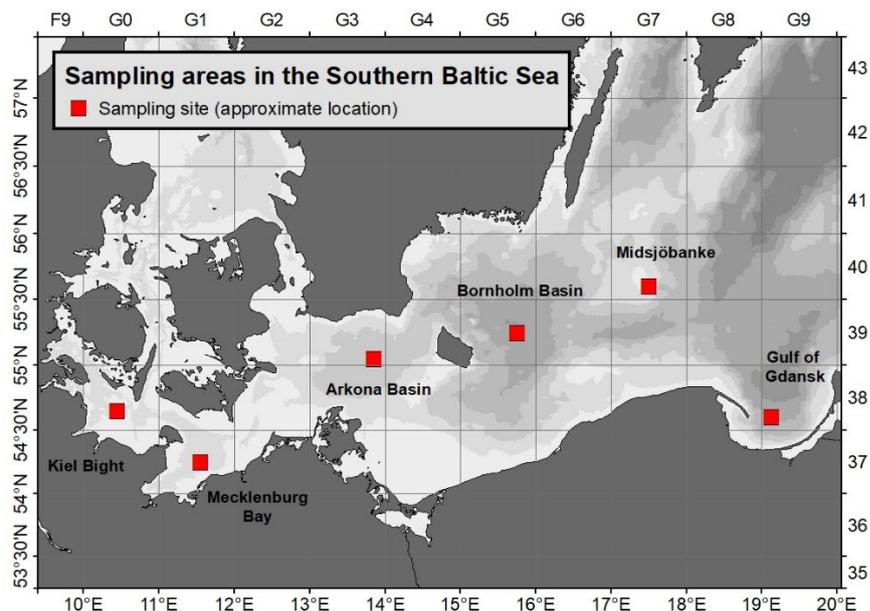


Figure 6.1.1. Map of the study area in the Southern Baltic Sea, indicating the 6 different areas for sample collection. Note that the symbols indicate the approximate locations of the sampling sites, while the exact sampling positions vary slightly in the different quarters of the year when samples were collected.

However, samples could unfortunately not be collected in all planned quarter/area combinations, due to severe weather conditions, technical/mechanical issues on the research vessels or simply the lack of sprat in the area. In addition, part of the samples from quarter 4 in 2021 were

lost by the shipping company during transport from Denmark to Poland. Therefore, sampling was repeated in quarter 4 in 2022. Table 6.1.1 presents a summary of all collected sprat samples by area and quarter of the year that were analyzed by NMFRI.

Table 6.1.1. Number of analysed sprat by sampling area and quarter of the year (q).

| AREA | q 1 - 2021 | q 2 - 2021 | q 3 - 2021 | q 4 - 2021 | q 4 - 2022 | Sum |
|-------------------|-------------|-------------|------------|------------|-------------|-------------|
| 1-Kiel Bight | 300 | | 277 | 90 | 300 | 967 |
| 2-Mecklenburg Bay | 300 | 174 | | 40 | 300 | 814 |
| 3-Arkona Basin | 600 | 300 | | 108 | 476 | 1484 |
| 4-Bornholm Basin | 300 | 300 | 250 | | 300 | 1150 |
| 5-Midsjöbanke | 300 | 300 | | | 300 | 900 |
| 6-Gulf of Gdansk | 300 | 300 | 300 | 300 | 300 | 1500 |
| Total | 2100 | 1374 | 827 | 538 | 1976 | 6815 |

Sample analyses

The following standard ichthyological analyses of thawed fish were conducted: length, total weight, sex, gonad developmental stage (Maier scale) and stomach fullness.

During the standard ichthyological analyses, a parasitological visual inspection focused on the presence of nematode parasites in each fish was conducted. Viscera were examined for the presence of parasites. All detected nematode parasites were collected and immediately identified to the lowest possible taxonomic level based on anatomical and morphological features with reference to descriptions given by Berland 1961 and Fagerholm 1982.

In addition, nematodes were fixed in ethanol for potential genetic analyses on selected sub-samples to corroborate the taxonomic identification. All analysed fish were subsequently digested in artificial gastric juice (water solution of pepsine and hydrochloric acid) at laboratory temperature. After 24 h digestion each sample was separately rinsed on a sieve under running tap water to reveal the presence of nematodes not detected during visual inspection (for example present in the muscle tissue). All collected nematodes were identified and fixed in the ethanol as described above.

Parasitological descriptors (prevalence, intensity and abundance of infection) were calculated for sprat infection with Anisakidae nematodes based on the definitions given by Bush et al. (1997):

- Prevalence of infection is the number of hosts infected divided by the number of fish examined.
- Intensity of infection is the number of individuals of a particular parasite species in a single infected host.
- Abundance is the number of individuals of a particular parasite in a single host regardless of whether or not the host is infected.

Results

The numbers of collected samples differs between areas and quarters of the year due to the challenges described above. The highest numbers of the samples were collected in quarter 1 and in the Gulf of Gdańsk (Table 6.1.1). As fish were collected randomly from the catch without size selection, the length distribution of collected sprat differs between areas and quarters of the year. The smallest individuals were caught in Gulf of Gdańsk, while bigger fish were collected west from that area (Table 6.1.2). The majority of collected sprat (about 80%) were in a size range between 10.5 - 13.5 cm.

Table 6.1.2. Mean length & length range (cm) of the analysed sprat by areas and quarters.

| AREA | q 1 - 2021 | | q 2 - 2021 | | q 3 - 2021 | | q 4 - 2021 | | q 4 - 2022 | |
|-------------------|-------------|-----------------|-------------|-----------------|-------------|-----------------|-------------|-----------------|-------------|-----------------|
| | MEAN | RANGE | MEAN | RANGE | MEAN | RANGE | MEAN | RANGE | MEAN | RANGE |
| 1-Kiel Bight | 12.5 | 7.5-14.5 | - | - | 12.5 | 8.5-15.0 | 11.5 | 9.0-14.0 | 10.4 | 6.5-15.0 |
| 2-Mecklenburg Bay | 11.9 | 7.0-14.5 | 12.6 | 8.5-15.5 | - | - | 12 | 10.0-15.0 | 11.8 | 7.5-15.5 |
| 3-Arkona Basin | 12.6 | 8.5-16.0 | 13.3 | 9.5-16.0 | - | - | 11.6 | 8.5-15.0 | 13.1 | 9.0-15.5 |
| 4-Bornholm Basin | 11.4 | 8.0-14.5 | 12 | 10.0-14.5 | 12.5 | 10.5-14.5 | - | - | 13 | 11.5-15.0 |
| 5-Midsjöbanke | 12.7 | 9.5-14.5 | 10.2 | 7.5-12.5 | - | - | - | - | 12.2 | 9.5-14.0 |
| 6-Gulf of Gdansk | 9.1 | 7.0-14.0 | 10.4 | 7.5-13.0 | 10.8 | 9.5-13.0 | 11.5 | 10.0-13.0 | 11.8 | 10.5-14.5 |
| Total | 11.8 | 7.0-16.0 | 11.6 | 7.5-16.0 | 11.9 | 8.5-15.0 | 11.5 | 8.5-15.0 | 12.1 | 6.5-15.5 |

The sex composition of collected sprat differed between areas and quarters of the year (Table 6.1.3). However, the proportions between males and females on average in each quarter of the year were similar.

Table 6.1.3. Sex composition of collected sprat by areas and quarters in % (M-male, F-female).

| AREA | q 1 - 2021 | | q 2 - 2021 | | q 3 - 2021 | | q 4 - 2021 | | q 4 - 2022 | |
|-------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| | M | F | M | F | M | F | M | F | M | F |
| 1-Kiel Bight | 36.7 | 63.3 | - | - | 76.9 | 23.1 | 60 | 40 | 47 | 53 |
| 2-Mecklenburg Bay | 57.7 | 42.3 | 70.7 | 29.3 | - | - | 57.5 | 42.5 | 56 | 44 |
| 3-Arkona Basin | 42.8 | 57.2 | 37.3 | 62.7 | - | - | 62 | 38 | 33.4 | 66.6 |
| 4-Bornholm Basin | 61.3 | 38.7 | 40.7 | 59.3 | 46.4 | 53.6 | - | - | 22 | 78 |
| 5-Midsjöbanke | 21.3 | 78.7 | 53.3 | 46.7 | - | - | - | - | 43.7 | 56.3 |
| 6-Gulf of Gdansk | 57 | 43 | 62.3 | 37.7 | 47 | 53 | 52.7 | 47.3 | 47.3 | 52.7 |
| Total | 45.7 | 54.3 | 51.2 | 48.8 | 56.8 | 43.2 | 56.1 | 43.9 | 40.8 | 59.2 |

In total 14 of 4839 analysed sprat sampled in 2021 have been infected with 19 parasites (prevalence of infection 0.29%; intensity of infection 1-3 parasites per fish; abundance 0.004). The smallest infected fish was 12 cm long. Most of infected individuals were females. The most heavily infected sprat was a 13 cm long male from the Arkona Basin. Among the 1976 sprat sampled in quarter 4 in 2022, a total of 10 were infected (prevalence of infection 0.51%, intensity of infection 1-8 parasites per fish, abundance 0.012), and a total of 23 parasites were found in these sprat (prevalence of infection 0.51%, intensity of infection 1-8 parasites per fish, abundance 0.012). Infected fish were of Arkona Basin, Mecklenburg Bay and Bornholm Basin origin. The smallest infected fish was 11.5 cm long and was caught in Mecklenburg Bay. Of the 42 parasites found in total, only three could not be confirmed to be liver worm (*C. osculatum*).

Both prevalence and intensity of infection differ between sampling areas and seasons (Table 6.1.4 and Figures 6.1.2 & 6.1.3 – note that the figures do not show results for Q4 in 2021, as the number of analyzed samples was rather low).

The highest prevalence and intensity of sprat infection with liver worm were observed in 2021 in the first quarter of the year, while no parasites were detected in samples collected during quarter 4 in 2021.

The majority of infected fish were caught in the Arkona Basin (5 fish) and Kiel Bight (4 fish) in quarter 1 in 2021. Overall, sprat sampled in quarter 4 in 2022 revealed the highest prevalence and intensity of infection. Here, the majority of infected sprat were from the Arkona Basin and Mecklenburg Bay, while the most heavily infected sprat were sampled in Arkona Basin (up to 8 nematodes per fish) and Bornholm Basin (6 nematodes in a single fish).

Table 6.1.4. Prevalence (P in %) and mean intensity (I) of sprat infection with parasitic nematodes by areas and quarters.

| AREA | q 1 - 2021 | | q 2 - 2021 | | q 3 - 2021 | | q 4 - 2021 | | q 4 - 2022 | |
|-------------------|-------------|------------|-------------|------------|-------------|------------|------------|----------|-------------|------------|
| | P | I | P | I | P | I | P | I | P | I |
| 1-Kiel Bight | 1.33 | 1 | - | - | 0 | 0 | 0 | 0 | - | - |
| 2-Mecklenburg Bay | 0 | 0 | 0 | 0 | - | - | 0 | 0 | 1.33 | 1.25 |
| 3-Arkona Basin | 0.83 | 1.6 | 0.33 | 1 | - | - | 0 | 0 | 1.05 | 2.4 |
| 4-Bornholm Basin | 0 | 0 | 0.33 | 2 | 0.8 | 1.5 | - | - | 0.33 | 6 |
| 5-Midsjöbanke | 0 | 0 | 0 | 0 | - | - | - | - | - | - |
| 6-Gulf of Gdansk | 0.33 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | - | - |
| Total | 0.48 | 1.3 | 0.15 | 1.5 | 0.24 | 1.5 | 0 | 0 | 0.51 | 2.3 |

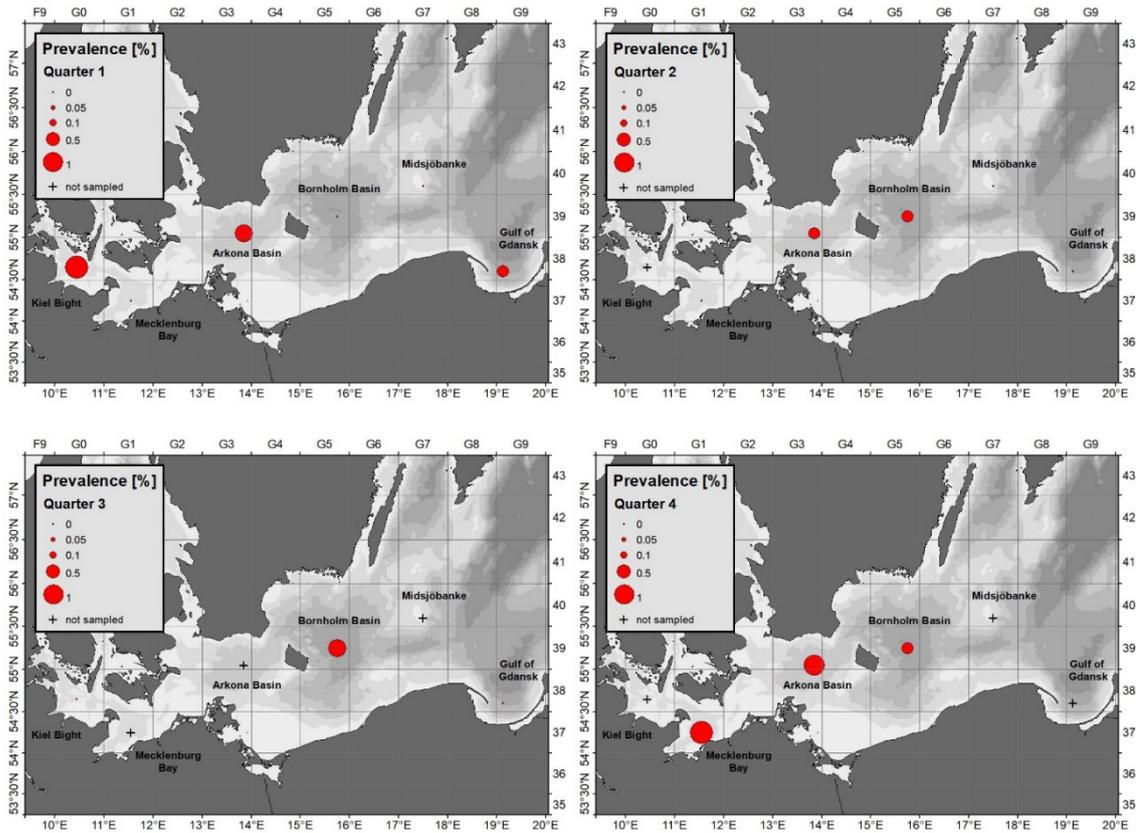


Figure 6.1.2. Prevalence of parasite infection (%) in the 6 different sampling areas in Quarter 1, Quarter 2 & Quarter 3 in 2021 and Quarter 4 in 2022. Note that the symbols indicate the approximate locations of the sampling sites, while the exact sampling positions vary slightly in the different quarters of the year when samples were collected.

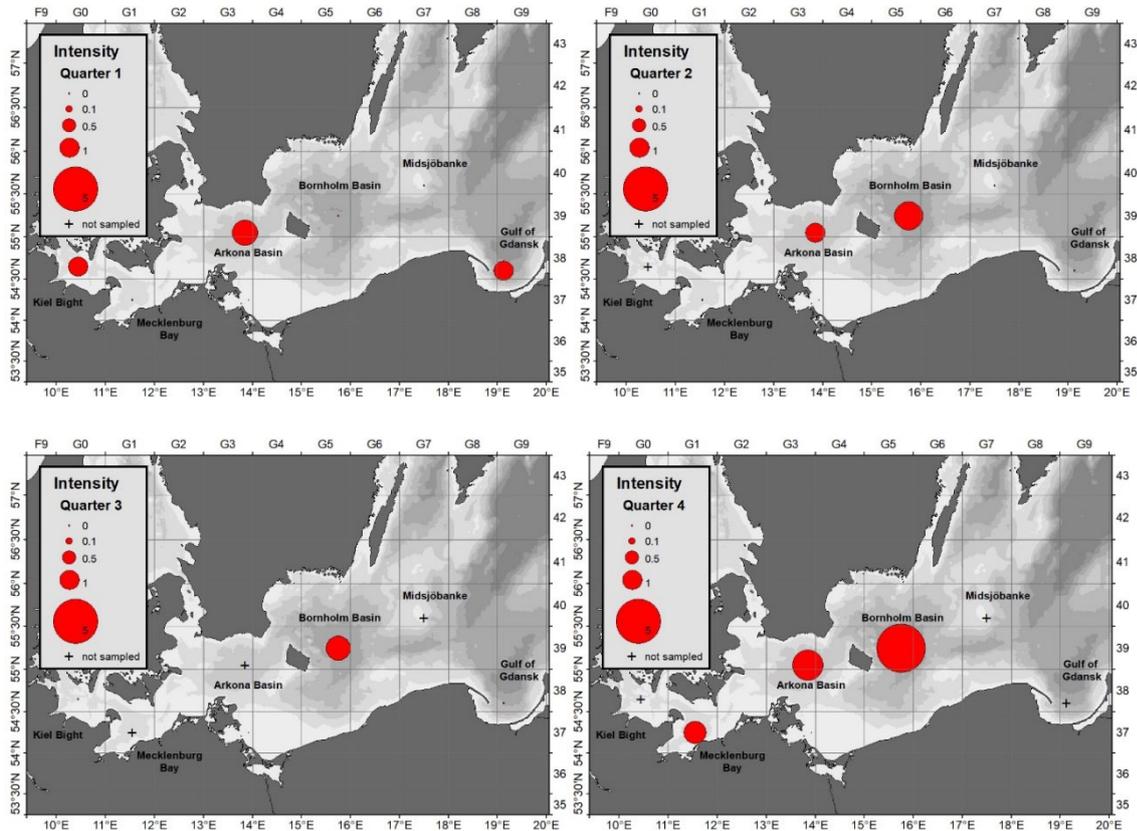


Figure 6.1.3. Intensity of parasite infection in the 6 different sampling areas in Quarter 1, Quarter 2 & Quarter 3 in 2021 and Quarter 4 in 2022. Note that the symbols indicate the approximate locations of the sampling sites, while the exact sampling positions vary slightly in the different quarters of the year when samples were collected.

Discussion and conclusions

Previous studies have shown the presence of parasitic nematodes – almost exclusively liver worm *C. osculatum* - in sprat from the southern Baltic Sea, but these were restricted to relatively few analyzed individuals and to local areas and short time periods. In contrast, the present study covered for the first time a large spatio-temporal range, extending over a broad area in central and Southern Baltic Sea and over 4 quarters of the year. Besides, an unprecedented large number of individual sprat (more than 6800) was investigated for that purpose. Thus, this study provides a comprehensive overview on the prevalence and intensity of sprat infection by parasitic nematodes (> 90% being liver worm, *C. osculatum*) in this region, covering the main distribution area of the Western and Eastern Baltic cod stocks.

Our results revealed that both prevalence and intensity of sprat infection with liver worm in the central and Southern Baltic Sea are generally low but show some differences between sampling areas and seasons. The areas showing the most regular prevalence and also the highest intensities are the Bornholm Basin and Arkona Basin. This may be due to the fact that several haul-out sites of grey seal are located in the Bornholm area and southern Sweden, i.e. in the vicinity of these sampling areas. As presence of the final host (marine mammals, in this case grey seals) is necessary to complete the life cycle of these parasites, areas where seal colonies are numerous likely pose a higher risk of getting infected with *Anisakidea* larvae (Hauksson 2011).

However, sprat can also undertake migrations, e.g. related to spawning (Ojaveer and Kalejs 2010) and feeding (Lindquist, 1971; van Khan et al., 1972), and may therefore transport parasites to areas distant from the place where they got infected. Notably however, our results should be interpreted with some caution, as not all sites were sampled in all seasons, and the number of analysed sprat in each area also varied between sites, which may bias the results and their interpretation. Yet, cod may become infected by alternative prey items than sprat. For example, various *Gammarus* species have also been shown to act as transport host of liver worm in the Baltic Sea (Pawlak et al., 2019), and these small crustaceans are like sprat a preferred prey item of cod.

Host organisms (invertebrates, fish, marine mammals) are unable to get rid of parasitic nematodes from the body cavity, and as a consequence they accumulate the parasites. However, invertebrates have a rather short life span and generally prey much less compared to fish and marine mammals, and therefore the risk of getting infected to accumulate the parasites is lower due to the shorter duration of exposure to parasites. This may explain why the level of infection with these nematodes in organisms on the lower levels of the Baltic Sea food web (invertebrates and sprat) is rather low compared to top predators that live longer and ingest more prey during their lifetime (cod, salmon, marine mammals).

An increasing number of individuals in the Baltic Sea grey seal population has been observed in recent years (Galatius et al. 2020) and a further increase may be expected in the future. This could also cause an increase in the availability of parasites that have grey seal as final host in the environment and, consequently, an increase in the infection levels of fish. Thus, our study may be used as “baseline” to follow the future development of parasite infection in sprat and other relevant species in the Baltic ecosystem.

In conclusion, our study showed that the level of parasite infection in Baltic sprat is relatively low but shows some variability in time and space. Although infection levels were still very low, sprat seemed to be most affected in areas located near grey seal colonies, where higher quantities of eggs and larvae of nematode parasites as well as infected invertebrate prey can be expected.

6.2 Energy content and condition of sprat (TORVÆKST)

Authors: Stefan Neuenfeldt, Jens Peter Hermann, Jane W. Behrens

Background

During the past four decades, the Baltic Sea ecosystem went through a major reorganization with two alternate states separated by a transition period in 1988–1993 (Moellmann et al., 2009). The first period is characterized by high predator (cod) and low prey (sprat) abundances, and the second characterized by decreased cod and increased sprat abundances (Alheit et al., 2005; Casini, 2013).

In the main part of the cod distribution area, the biomass of *Sprattus sprattus* L. (sprat) was low until the early 1990s, increased rapidly up to the mid-1990s and thereafter decreased again to levels comparable to the early 1990s (Casini et al., 2016b). Here, we investigated changes over one year in sprat energy density, i.e. the nutritional value sprat has as food item for cod measure in kJ/gram wet weight. We also calculated, how much sprat a cod of a given age is eating per

year during its life. The energy contained in sprat as well as feeding rates have been used in WP 3 as input for the bioenergetic modelling.

Materials and methods

From March 2002 until the end of 2003, GLOBEC Germany conducted 23 cruises that sampled along a standard 52 stations grid in the Bornholm Basin and adjacent areas. Both process-oriented and broad-scale surveys were performed to address the different research hypotheses of GLOBEC Germany. The sampling intensity (338 days at sea) allowed nearly complete seasonal coverage, encompassing the prolonged sprat spawning season. As part of this effort, sprat have been sampled monthly in 2002 to 2003. The sprat have been processed in a bomb calorimeter in order to determine their energy content.

The stochastic multispecies population models, SMS, is the currently applied tool to estimate predation mortality rates of cod on sprat. The predation mortalities are currently use in the Baltic sprat assessment, However, the model output was used here in order to estimate, how many sprat a cod on average consumes during each year of its life.

Results

Sprat energy content is between 4 and 8 kJ/g wet weight from March to September. Energy increase after September when the sprat start accumulating energy for spawning in spring (Fig. 6.2.1)

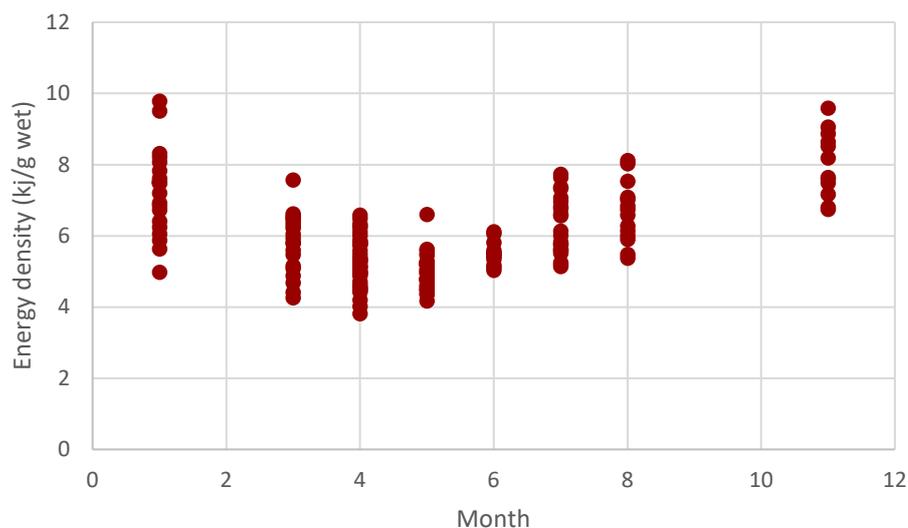


Fig. 6.2.1. Sprat energy density (data from 2002/3) over the yearly cycle. Here. All sprat length groups are pooled.

The pattern is the same, if length groups are considered separately, but variability in a given month is decreased. (Fig. 6.2.2).

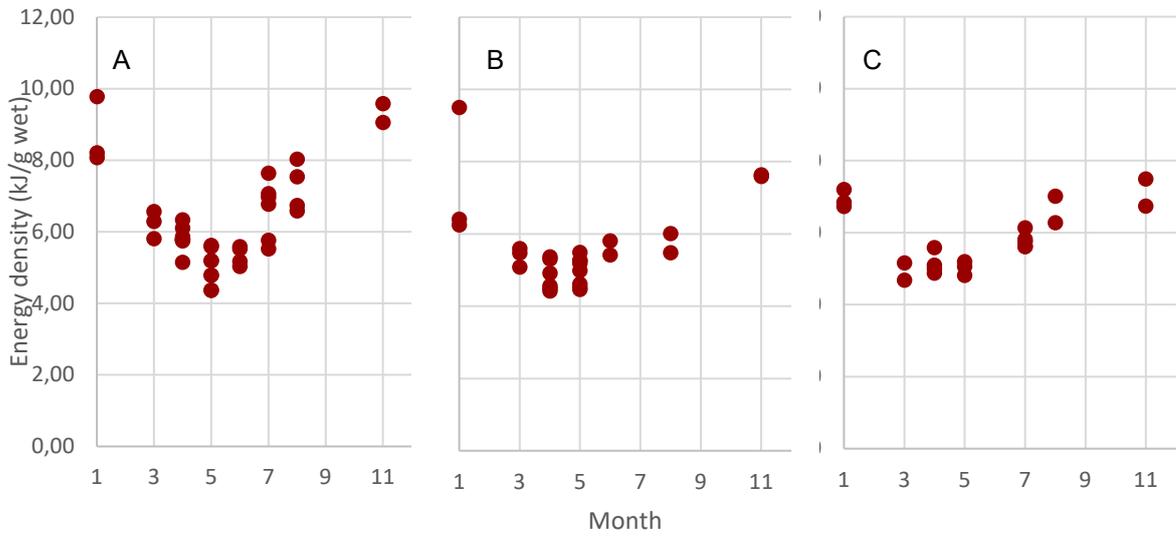


Figure 6.2.2. Sprat energy density over the yearly cycle for A) 8 cm sprat, B) 10 cm sprat and C) 12 cm sprat.

Cod start on average to forage on sprat at age 3 and consume around 18 sprat during this year of their life. During the next year, at age 4 they consumed 40 sprat, that is around 3 sprat per month. For large cod, sprat consumption is almost exponentially higher, for example at age 7 a cod consumed on average 170 cod, i.e. almost one every second day (Fig. 6.2.3).

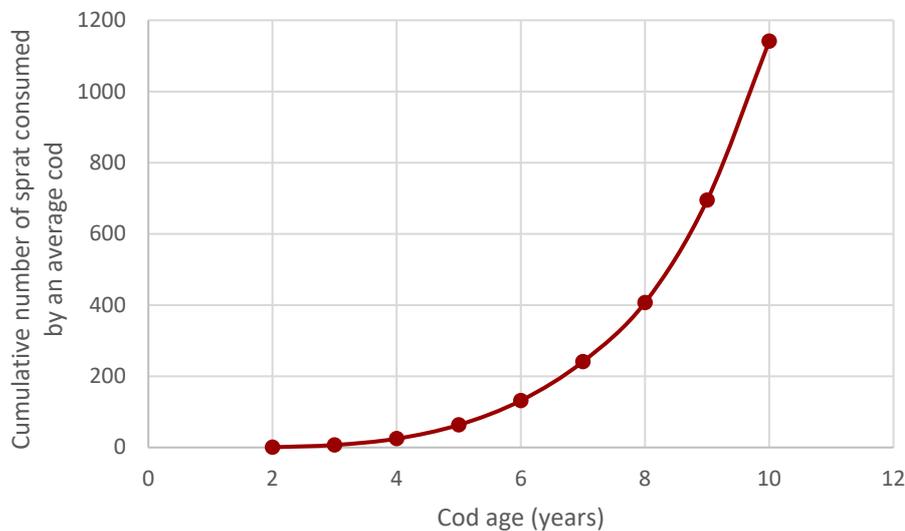


Fig. 6.2.3. Cumulative number of sprat eaten through the life span of a cod. The numbers given are an average for the whole cod population.

Conclusions

We measured sprat energy density over the annual cycle. The energy density is highest in winter when sprat are preparing for spawning. Cod consume sprat mostly before and after cod spawn-

ing. Hence, energy values between 5 and 7 kJ/g wet are appropriate for the bioenergetic modelling in Section 4. Until cod reach an age of around 4 years, the modelled consumption rate of sprat specimen is to be considered only moderate. First at ages >6 cod digest sprat almost daily.

7. Synthesis of knowledge

The Eastern Baltic cod stock is currently in an unprecedentedly poor state (Eero et al., 2023), and results from the present projects clearly suggests that high infection loads with the parasitic liver worm is one of several drivers causing this misery. Similar poor levels have occurred for the relationship between nutritional condition and liver worm load in the 1940s-1950s (where abundance of grey seal was comparable to present time) and subsequently improved, suggesting that recovery from such indicator states is possible (Eero et al., 2023). Yet, grey seal is currently recolonizing the southern and western Baltic Sea, including the Danish Straits and Kattegat (Galatius et al 2020), and infections load in cod will likely oscillate with grey seal abundance (Eero et al 2023). It however remains unclear to what extent the lower salinity and increasingly complex food-web in the more westerly parts of the Baltic, and the Danish Straits and Kattegat, may reduce the transmission of this parasite to cod (Sokolova et al., 2018). To this end, the recently implemented mandatory assignment of a liver category to individual cod livers in the routine sampling protocol on Baltic monitoring surveys from 2021 (ICES 2020) will reveal this.

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Appendix – scientific articles from the projects

This appendix provides an overview of the scientific papers that have stemmed from these projects.

Plambech Ryberg, M., Skov, P. V., Vendramin, N., Buchmann, K., Nielsen, A., & Behrens, J. W. (2020).

Physiological condition of Eastern Baltic cod, *Gadus morhua*, infected with the parasitic nematode *Contracaecum osculatum*.

Conservation Physiology, 8(1), Article coaa093.

<https://doi.org/10.1093/conphys/coaa093>

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Parasite load of Atlantic cod *Gadus morhua* in the Baltic Sea assessed by the liver category method, and associations with infection density and critical condition.

Fisheries Management and Ecology, 29, 88–99.

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Bioenergetics modelling of growth processes in parasitized Eastern Baltic cod (*Gadus morhua* L.).

Conservation Physiology, 11(1), Article coad007.

<https://doi.org/10.1093/conphys/coad007>

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