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Behrens, Jane W.; Ryberg, Marie Plambech; Chondromatidou, Virginia; Iburg, Tine Moesgaard

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## RESEARCH ARTICLE

# Comparative histopathology of livers from Baltic cod infected with the parasitic nematode *Contracaecum osculatum*

Jane W. Behrens  | Marie Plambech Ryberg  | Virginia Chondromatidou  |  
Tine Moesgaard Iburg 

National Institute of Aquatic Resources,  
Technical University of Denmark (DTU  
Aqua), Kgs. Lyngby, Denmark

## Correspondence

Jane W. Behrens, National Institute of  
Aquatic Resources, Technical University of  
Denmark (DTU Aqua), Kgs. Lyngby 2800,  
Denmark.  
Email: [jabeh@aqu.dtu.dk](mailto:jabeh@aqu.dtu.dk)

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## Abstract

Infection levels with the parasitic nematode *Contracaecum osculatum* in Eastern Baltic cod have increased in the last decades. Eastern Baltic cod is transport host for this parasite that has a high affinity for the liver of the fish. The liver is a highly vital organ and damage to the liver tissue can result in reduced functionality of the organ. Previous studies have revealed that cod with high infections loads reveal impaired physiological performance, reduced nutritional condition and show signs of having a liver disease. Yet, little is known about the pathological changes and inflammatory reactions of the cod liver related to the infections. In this study, we performed histological examinations on 30 Baltic cod livers caught in the eastern part of the Baltic Sea (length;  $38 \pm 0.9$  cm, weight;  $454 \pm 34.8$  gram) and three Sound cod livers (length;  $63 \pm 2.9$  cm, weight;  $3396 \pm 300.2$  gram) to categorize the degree of inflammation and its relation to pathological changes in infected cod livers. We further investigated how *C. osculatum* infection levels varied with intensity of inflammation and co-infections. We found that high infection loads with *C. osculatum* caused severe inflammation in the liver tissue of cod and reduced fat content of the hepatocytes. Conspicuous amounts of glycogen were found in the muscle and intestinal epithelial cells of the nematodes and parasitic co-infections occurred more frequently in the most heavily infected livers.

## KEYWORDS

*Gadus morhua*, hepatocytes, liver disease, liver worm, parasites

## 1 | INTRODUCTION

Fish collectively host several species of parasitic nematodes belonging to the Anisakidae family. Of these, especially *Anisakis simplex*, *Pseudoterranova decipiens* and *Contracaecum osculatum* are well-described. They all have quite similar life cycles, relying on a number of transport hosts (crustaceans, small fish) from different trophic levels

to reach their main final (definite) host, a marine mammal, which for *A. simplex* is the harbour porpoise (*Phocoena phocoena*), the harbour seal (*Phoca vitulina*) for *P. decipiens*, and the grey seal (*Halichoerus grypus*) for *C. osculatum* and *P. decipiens* (Aspholm et al., 1995; Hauksøn, 2011; Herreras et al., 2004; Køie & Fagerholm, 1995).

In the Baltic Sea, Eastern Baltic cod, *Gadus morhua*, is a known transport host for *C. osculatum* (Buchmann & Mehrdana, 2016;

Nadolna & Podolska, 2014), and concurrent with an increase in the abundance of grey seals (Harding et al., 2007; HELCOM, 2018; ICES, 2020), infection levels of *C. osculatum* have increased (Haarder et al., 2014; Nadolna & Podolska, 2014; Sokolova et al., 2018). As the common name—cods liver worm—imply, when cod eat infected prey, *C. osculatum* penetrates the stomach wall and migrates to the liver of the fish, where it either lays close to the surface of the liver or penetrates the liver parenchyma (Nadolna & Podolska, 2014). The nematodes accumulate during the life of fish, and older fish may have heavy parasite burdens, with up to several hundred *C. osculatum* in single livers (Horbowy et al., 2016; Ryberg et al., 2020; Zuo et al., 2016). The liver of fish, like livers in other taxa, is involved in multitude vital functions, such as the production of bile acids, egg yolk protein, vitellin and growth-related hormones, and it is a major site of leptin expression, which plays a role in regulation of feeding and metabolism (Roberts & Ellis, 2012). Damage to the liver tissue can result in reduced functionality of the organ, and this can lead to severe effects on the metabolism and reduce the production of vital elements of the individual (Roberts & Ellis, 2012). A recent study combining field and laboratory data has shown that high infection levels of *C. osculatum* in cod livers lead to impaired physiology of the fish, revealed by depressed energy turnover as evidenced by reduced standard metabolic rate, reduction in the digestive organ masses along with changes in the plasma, body and liver composition and fish energy source. Taken together, this suggests that cod with high infection levels suffers from a chronic pathological liver condition (Ryberg et al., 2020). In support, the expression of immune-related genes in the cod liver tends to be downregulated at high infection levels, suggestively due to local immune suppression in the organ caused by *C. osculatum* (Marnis et al., 2020), and several studies have furthermore revealed a negative association between infection levels and nutritional condition (Horbowy et al., 2016; Petrushevski & Shulman, 1955; Ryberg et al., 2020, 2022; Sokolova et al., 2018). The latter shows that cod infected with *C. osculatum* have a lower condition (i.e. are leaner) as compared with uninfected conspecifics. Studies have shown that *C. osculatum* becomes encapsulated in the liver of cod as part of an inflammatory reaction that is mainly caused by macrophages (Buchmann & Mehrdana, 2016; Zuo et al., 2017). However, the relation between the degree of inflammation and other changes in the liver tissue and infection level have not previously been described in detail. Our aim with this study was to use histological examinations to categorize the degree of inflammation associated with different levels of infection with *C. osculatum*

and to characterize the pathological changes in the liver of infected individuals. We therefore developed a descriptive scoring system. This investigation provides new knowledge on the pathological effects of this parasite in Eastern Baltic cod.

## 2 | MATERIALS AND METHODS

### 2.1 | Study area and fish collection

Eastern Baltic cod ( $n = 69$ ) were caught by trawl east in the Baltic Sea of Bornholm, close to the harbour of Nexø (latitude; 55.063073 and longitude; 15.12499) 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. This number of livers was chosen based on a previous pilot study examining the probability of finding fish with different infection loads in relation to their length based on number of visible nematodes on the surface of the liver (Ryberg, 2020), to ensure a high variation in infection load of the livers included in the study. Three healthy, well-fed and non-infected cod were used as baseline for histopathological comparison. These three cod had lived most of their life at the national aquarium 'The Blue Planet', Copenhagen, Denmark, but were originally caught in the Sound. These cod have mainly been fed boiled blue mussels throughout their life in captivity.

### 2.2 | Recovery and identification of parasites from cod livers

The total length (cm), TL, total wet weight (gram), TW, and liver weight (gram), LW, of the selected eastern Baltic cod and control cod were noted (Table 1). A total of three to four samples were taken from each of the 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  $-20^{\circ}\text{C}$  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). Detection and counting of nematodes in the livers was subsequently done with a stereo microscope, and the individual's nematodes were marked and divided

**TABLE 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*.

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

Note: Values are mean  $\pm$  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.

into two categories small (<1 cm) or large (>1 cm), as performed in a previous study by Ryberg et al. (2020). Hereafter, individual nematodes were removed, and species identification performed, based on visual inspection of the cephalic and caudal shape-ends characteristics (Fagerholm, 1982). These characteristics are visualized in the study by Zuo et al. (2018) who applied classical and molecular helminthological techniques to document *C. osculatum* in the liver of cod and stomach of seals.

## 2.3 | Sampling for histopathology

Liver tissue samples were fixed in 10% neutral-buffered formalin, processed conventionally and embedded in paraffin. Each paraffin block contained all liver samples from an individual fish (three–four pieces of liver; liver sections). Two slides, 3–4 µm thick, were made from each block. One slide was stained with haematoxylin and eosin (HE) for overall evaluation, and the other slide was stained with Picro-sirius Red (PR) for collagen. Two examiners evaluated the slides separately. Slide identities were blinded for the examiners during evaluation. If scoring differences were found, these were reconciled by consensus. After evaluation, two fish from each pattern of fatty change (see Section 2.4.2) were selected, and additional slides were stained with Periodic acid-Schiff (PAS) and PAS-diafase to evaluate the possible glycogen content.

## 2.4 | Histopathological evaluation

Haematoxylin and eosin slides were examined for overall inflammatory lesions, the presence of the nematode larvae and any other lesions in the liver tissues, as well as the presence of other parasites.

### 2.4.1 | Inflammatory response

A descriptive scoring system including five different categories was developed to examine the association between infection load and degree of inflammation in the cod livers. Each liver was given a category between 0 and 4, based on a semiquantitatively estimation of the overall degree of inflammation where lesions

related and unrelated to visible *C. osculatum* larvae in the lesions were included (Table 2).

The different inflammatory lesions used to categorize the livers were as follows: (1) small granulomas (Figure 1a, diameter below 280 µm), (2) large granulomas (Figure 1b,c, diameter above 280 µm), (3) immature granulomas, characterized by foci of an inorganized mixture of macrophages, lymphocytes, erythrocytes and few eosinophilic cells and plasma cells (Figure 1d) and (4) tract lesions, which were defined as focal areas with destruction of hepatocytes sometimes with bleeding or proteinaceous (fibrinous) exudation to the created space. Usually, no or minimal inflammatory reaction in the surrounding tissue was noted, but occasionally older lesions were lined with layers of macrophages and epithelioid cells and a thin layer of fibrous tissue mixed with lymphocytes forming a granuloma. Three types of small granulomas were seen; (i) small round to a little irregular aggregations of large macrophages (90–200 µm in diameter), well-demarcated and often surrounded with a thin capsule and scattered lymphocytes, (ii) small round granulomas (approximately 55 µm in diameter) with a necrotic centre often with erythrocytes, surrounded by moderate to thick fibrous capsule and (iii) small round granulomas of 110–280 µm in diameter, consisting of erythrocytes centrally bordered by one to three cell layers of epithelioid cells. Likewise, three types of larger granulomas could be seen: (I) large granuloma with a central parasite compatible with *C. osculatum* surrounded by a capsule of few to multiple layers of epithelioid cells, sometimes with scattered lymphocytes and a very thin fibrous layer. Usually, a space was seen between parasite and capsule, which often contained cellular debris and eosinophilic amorphous material; (II) large granulomas containing pale basophilic or pale eosinophilic, amorphous material centrally and bordered by few to multiple layers of epithelioid cells and demarcated by a capsule of mild-to-moderate fibrosis, sometimes intermixed with scattered lymphocytes; and (III) large granulomas with central bleeding, otherwise similar to large granuloma mentioned above.

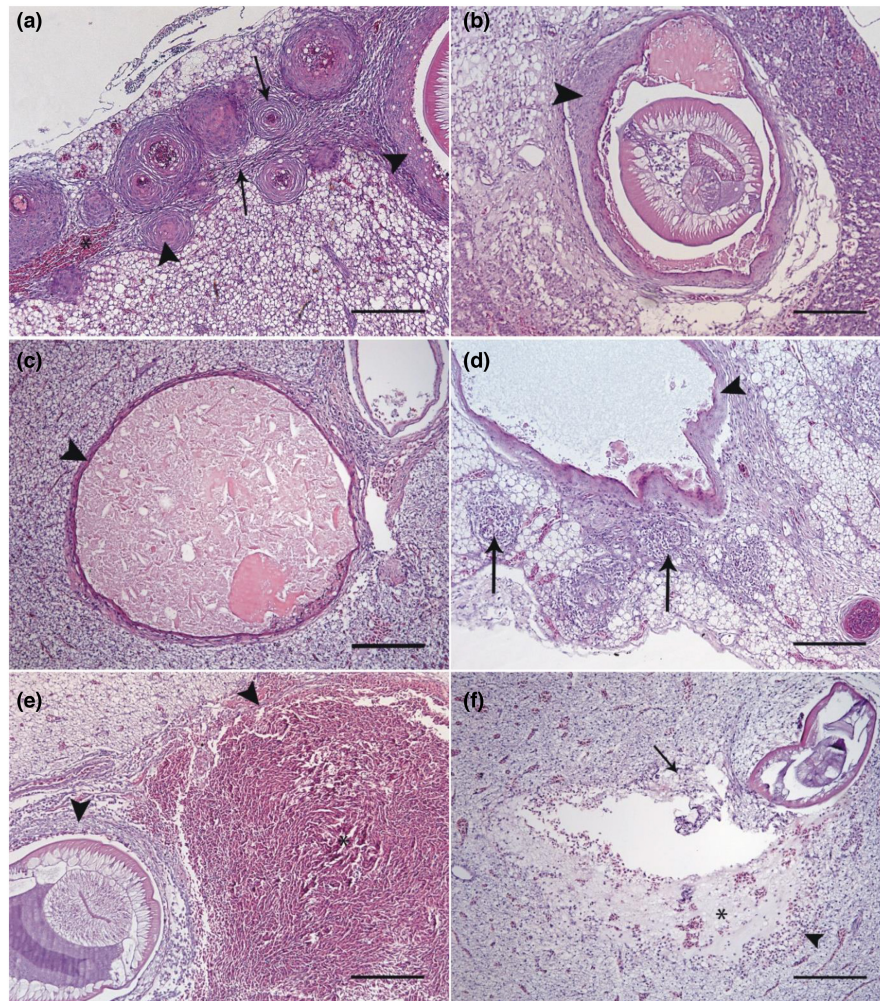
### 2.4.2 | Other changes and pathogens

If incidental findings of parasites other than *C. osculatum* were encountered during histology, these parasites were noted, identification was attempted based on a previous study (Bruno

TABLE 2 Criteria for categorization of the degree of inflammation in the cod liver.

Categories of the overall degree of inflammation	0	1	2	3	4
Immature and/or small granulomas, focal or multifocal	–	–/+	–/+	+	+
Large granulomas, focal or multifocal	–	–	–/+	+/++	++
Tract lesions, focal or multifocal	–	–/+	–/+	–/+	–/+
Presence of nematodes	–	–	+ in one liver section	+ in two liver sections	+ in all liver sections

Note: Each slide with 3–4 liver sections representing one fish were categorized based on the overall degree of inflammation and presence of liver nematodes or remnants of nematodes. – = no presence, + = few, ++ = many.



**FIGURE 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  $\mu$ m.

et al., 2006) and the number of parasites or the number of structures with parasites was estimated as few (1–10) or many (>10). No parasitological indices, such as prevalence, abundance or intensity was calculated. If other pathogens than parasites were seen, this was also noted. Furthermore, the dominating pattern of fat vacuoles and distribution and degree of fatty change in the hepatocytes were noted. The different patterns of fat vacuoles were either large, mixed, small or no vacuoles. PR slides were used to evaluate the degree of collagen in the livers in relation to granulomas, but also in general to see whether the parasite infection stimulated a general progressive fibrotic condition in the liver. PAS and PAS-diastase-stained slides were used to evaluate the presence of glycogen.

## 2.5 | Data handling and analysis

Infection density was calculated as number of nematodes per gram of liver tissue, as defined by Ryberg et al., 2020. Nematodes were either assigned a weight of 0.004 g (small nematodes) or 0.009 g (large nematodes) (Ryberg et al., 2020), and the summed weight of the nematodes in each liver was subtracted from the measured liver weight. The three healthy, well-fed and non-infected cod from the national aquarium the Blue Planet were not included in the statistical analysis as they only provided a baseline for the descriptive histological evaluation.

Two generalized linear models (GLM) with negative binomial distribution (Equation 1) 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 (see Table 2) and (2) the three categories each reflecting numbers of other parasites (none, few <10, and many >10). Weight of liver was inserted as an offset variable in the models to adjust the number of nematodes for size of the liver

$$Y_i \sim \text{NBin}(\mu_i, \theta) \quad (1)$$

The mean  $Y_i$  was independent with  $\mu$  and over dispersion parameter  $\theta > 0$ . This implied that the variance of the  $i$ 'th observation becomes  $\mu(1 + \mu/\theta)$ . Total number of nematodes was defined as the response variable (Equation 2)

$$Y_i = \text{nematodes} \quad (2)$$

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.

### 3 | RESULTS

A total of 960 nematodes were counted in the 30 Eastern Baltic cod livers. Microscopic identification based on morphological characters showed that all nematodes belonged to the genus *Contracaecum*. This is in accordance with a previous study showing that *C. osculatum* is the major nematode species present in cod in the Eastern part of the Baltic Sea as only 0.002% of the examined nematodes belonged to other species (Sokolova et al., 2018). In that study, the identification was based on molecular analysis (sequencing of ITS, mtDNA cox 1 and 2). Analysis of the control cod revealed no signs of parasites in their livers.

#### 3.1 | Histopathological evaluation

##### 3.1.1 | Inflammatory response

Of the 30 livers from the Eastern Baltic cod, five were inflammation category 0, two category 1, seven category 2, seven category 3 and 9 category 4 (Figures 2 and 3). One of the livers of the three well-fed control cod was categorized as inflammation category 0 and the other two livers as inflammation category 1. The two inflammation category 1 livers had disseminated small granulomas with no signs of pathogens. Track lesions were seen in categories 2–4, presumably created by the large nematodes moving around in the liver destroying or pushing hepatocytes aside, resulting in a rounded or elongated focus of bleeding or effusion of proteinaceous fluid and possibly necrotic hepatocytes (Figure 1e,f). Mainly, acute minimal inflammatory reactions were noted associated with track lesions, but occasionally older lesions were lined with layers of macrophages and epithelioid cells and a thin layer of fibrous tissue mixed with lymphocytes, similar to a granuloma.

Some periductular and or perivascular inflammation typically with lymphocytes and macrophages were seen in all inflammation categories of the Eastern Baltic cod livers. However, this reaction type was not seen in the control livers and the percentage of livers with periductular and/or perivascular inflammation was higher in the inflammation categories 2–4 (71%, 71% and 67% compared to 33% and 25%, respectively, in inflammation categories 0 and 1). However, this type of inflammatory reaction is considered a non-specific reaction that can be seen in connection with many inflammations in liver and intestine.

In terms of fibrosis in the livers, very little collagen was in general seen in both HE and PR, regardless of inflammatory category. Independent of the size of the granulomas, these were typically encapsulated by a thin-to-moderate thick rim of fibrotic tissue mixed with lymphocytes.

##### 3.1.2 | Other changes and pathogens

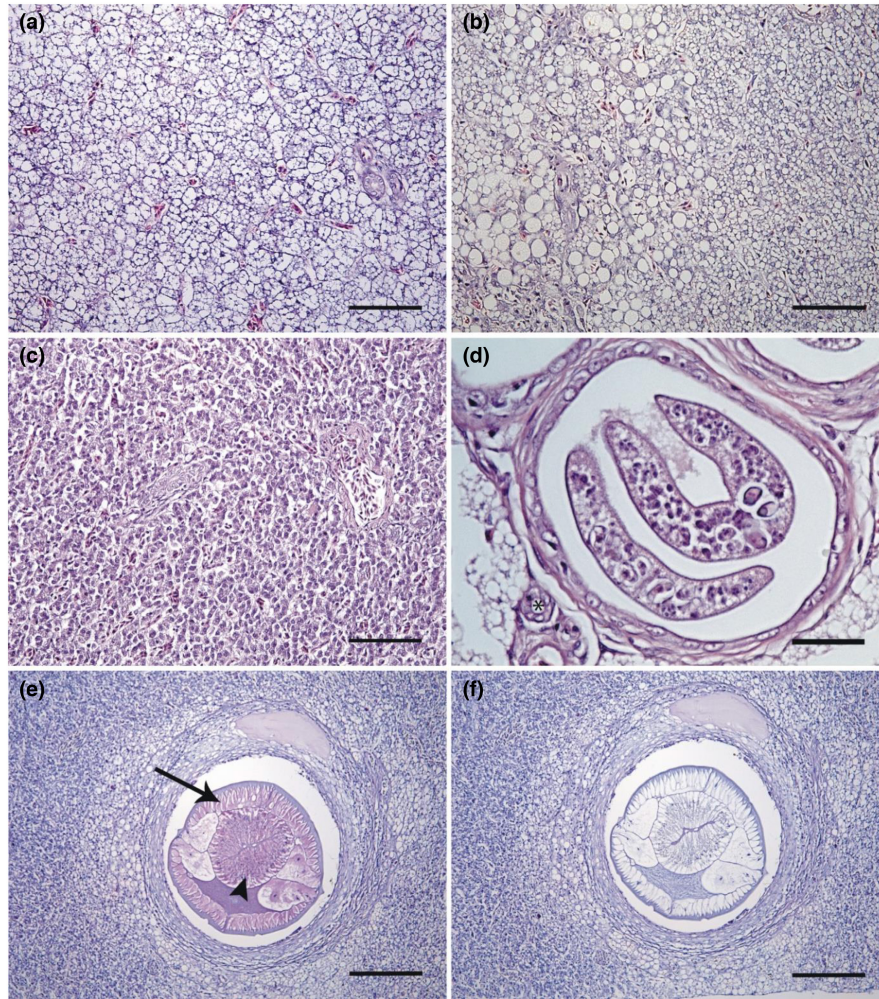
In most of the livers, a pattern of either large, small or a mixed sized fat vacuoles in the hepatocytes were diffusely distributed (Figure 2a–c). However, in five of the nine livers in inflammatory category 4, two of the seven livers in inflammatory category 3 and one of the six livers in category 2 very little fat was seen in the hepatocytes (Figure 2c). Livers from the in inflammatory categories 0 and 1 on the contrary showed a diffused pattern of small or mixed sized fat vacuoles. All the control cod livers ( $N = 3$ , two inflammatory category 0 and one inflammatory category 1) showed a diffused pattern of large fat vacuoles.

In terms of other pathogens, no visible bacteria or fungi were seen; however, in several livers, another parasite was discovered, which exclusively inhabited the bile ducts (Figure 2d). 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. No signs of cholestasis in connection to the infection were noted. In the selected slides that were stained with PAS and PAS-diastase, hepatocytes containing material staining as glycogen were located in a small rim under the liver capsule and in few hepatocytes scattered in the liver sections independent of the amount of fat in the livers. The nematodes present in the slides, however, stained very positive for glycogen in their cuticular and intestinal cells (Figure 2e,f).

#### 3.2 | Statistical analysis

The 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 categories 3 and 4 ( $df = 24$ ,  $p = .002$ , Figure 3a, Table 3).

Furthermore, when grouping intensity of infection of other parasites in the liver into none, few or many, the infection density with



**FIGURE 2** Patterns of liver fat vacuoles, co-infections and glycogen in *Contracaecum osculatum*. (a) Normal pattern of fatty content in a cod liver where hepatocytes are filled with large fat vacuoles in the cytoplasm. (b) Mixed pattern of fatty content, where some hepatocytes have large fat vacuoles in their cytoplasm and others have small vacuoles. (c) Cod liver with almost no fat vacuoles in the cytoplasm of the hepatocytes. (d) Dilated bile duct with flattened epithelium in a cod liver with Myxosporidian parasites. A normal bile duct (asterisk) is seen next to the dilated bile duct. (e) Granuloma with a cross section of a *Contracaecum osculatum*. Muscle cells (arrow), hypodermal lateral cords and intestinal epithelial cells (arrowhead) in the worm stain positive with PAS stain. (f) Same granuloma as in (e) but stained with PAS and diastase (an enzyme that digest glycogen). Notice the absence of staining in muscle cells, hypodermal lateral cords and intestinal epithelial cells in the worm, suggesting that the positive staining in (e) is caused by glycogen in the parasite cells. (a–c) Fatty content, HE, bar = 83  $\mu$ m and (e, f) examination for glycogen content, bar = 176  $\mu$ m.

*C. osculatum* was significant higher in the groups of livers designated many other parasites ( $df = 27$ ,  $p < .001$ , Table 3 and Figure 3b). No significant difference was found between the group with no other parasites (none) and the group with few other parasites (few) (Figure 3b).

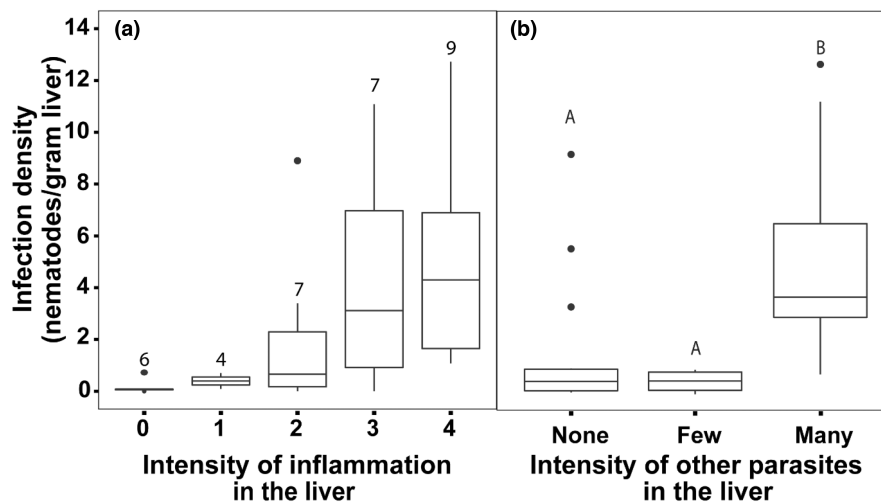
## 4 | DISCUSSION

*Contracaecum osculatum* infections at high infection loads caused severe inflammation in the liver tissue of cod, seen as granulomatous changes, large tracks of tunnels with tissue cellular debris and haemorrhages, as well as lesions to blood vessels. Furthermore, some of the severely infected livers revealed reduced fat content of the hepatocytes. Glycogen content in the hepatocytes were low in general, whereas conspicuous amounts were found in the muscle and intestinal

epithelial cells of the nematodes. Notably, parasitic co-infections occurred more frequently in the most heavily infected livers.

### 4.1 | Liver structure and functionality

Previous studies combining controlled experiments and field samplings have shown that cod with high *C. osculatum* infection loads have significantly reduced liver fat content, nutritional body condition and blood albumin to globulin ratio in addition to a pronounced immune response. Altogether, these parameters point to that these fish suffer from a chronic liver disease, with malfunctioning of the organ (Marnis et al., 2020; Ryberg et al., 2020). This is supported by the histopathological findings in the present study. We show that the degree of inflammation and the damage caused



**FIGURE 3** (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.

**TABLE 3** Estimates of significant parameters and standard errors (SE) of the two GLM models that describe how the infection density of nematodes changes with (1) intensity of inflammation and (2) with levels of other parasites.

Parameter	Estimate	SE	Z value	p-value
Inflammation category	$\alpha$			.002
1	-1.80	0.60	-2.97	
2	-0.96	0.89	-1.08	
3	0.66	0.46	1.45	
4	1.43	0.44	3.23	
5	1.85	0.39	4.75	
Other parasites				<.001
None	0.02	0.05	0.33	
Few	-0.02	0.11	-0.11	
Many	1.57	0.04	36.58	

Note: Z value provides the statistical result of each variable and factor level and p values below .05 are considered significant. Numbers are on log scale.

by the movements of *C. osculatum* in the liver lead to destruction of a high number of hepatocytes, likely influencing the functionality of the organ. Although little is known about this in fish, it is most likely that a gradual loss-of-functional hepatocytes will result in an emergent liver disease/development. Being a very lean fish with low fat content in the muscles, the liver has a vital function for the cod serving as an energy reserve in forms of lipids during feeding and availability of energy during periods of starvation and reproduction (Black & Love, 1986; Lambert & Dutil, 2000). Several studies have found a significant reduced fat content in cod livers highly infected with *C. osculatum* (Mohamed et al., 2020; Petrushevski & Shulman, 1955; Ryberg et al., 2020). In the current study, a pattern of reduced sizes of and/or the absence of fatty vacuoles was only seen in the livers with the most severe inflammations (inflammation categories 2–4), whereas a more normal pattern with both large, small and mixed sizes of fat vacuoles was seen in inflammation categories 0 and 1. This suggests that nutritional status of

highly infected cod is compromised. Eastern Baltic cod has been food deprived during the last decades (Neuenfeldt et al., 2020) and by comparing infected cod with well-fed control cod that has been held in captivity for the majority of their lives and have a normal pattern of mainly large fat vacuoles diffusely in their livers, we support several studies showing that Eastern Baltic cod is in distress with poor nutritional condition (Casini et al., 2016; Eero et al., 2015; Neuenfeldt et al., 2020).

## 4.2 | Inflammatory response

In humans, parasite-induced liver fibrosis can be very debilitating, and zebra fish models have been used to study the disease (Wang et al., 2021). In our study, the inflammatory response looked like an attempt to reduce migration of or destroy the nematodes inside the liver reflected by the encapsulation. The infected cod livers only

revealed fibrosis associated with the granulomas, with no signs of a progressive fibrotic process. In general, infections with parasitic nematodes in fish typically induce a host response that includes a granulomatous inflammation that encapsulates the parasite. The effectiveness of this host response in terms of either reducing the movement of the nematode or destroying the nematode will depend on the fish species (Feist & Longshaw, 2008). For example, *C. osculatum* have also been found in livers of Salmon (*Salmo salar*) in the Baltic Sea. However, in salmon, both the nematodes and the associated granulomas are typically smaller than the reaction found in cod livers (Mohamed et al., 2020; Setyawan et al., 2020). Cod and salmon species differ in their immune response, as cod lacks crucial components of the adaptive immune system (Star et al., 2011). In the host–parasite system between whiting, *Merlangius merlangus* (L.) and *Contracaecum* larvae, the infections lead to a response of the whiting similar to mammals reflected by a more complete encapsulation around the parasite compared with our study (Elarifi, 1982). In terms of functionality of livers infected with parasites, it is not always the case that the infections lead to reduced liver functionality. For example, high levels of nematode stages in livers of the fish species *Gymnotus inaequilabiatus* did not tend to affect the liver function, as degenerated hepatocytes and/or extensive granulomatous reactions were not observed and condition factor was not associated with the number of nematode stages in the liver (Galindo et al., 2017). In another host–parasite system between the third-larval stage of the nematode *Brevimulticaecum* sp. (*Heterocheilidae*) and the fish *Gymnotus inaequilabiatus* (*Gymnotidae*), a focal encapsulation of the parasites revealed that uninfected liver tissue maintained its functions and thus allowed the survival of the host despite the infections (Sayyaf Dezfui et al., 2016). The descriptive scoring system of inflammation developed in this study helped to reveal statistical correlation between inflammation and infection density where the most pronounced inflammation responses (categories 3 and 4) were found in livers with the highest infection densities of *C. osculatum*. The finding of track lesions supports that *C. osculatum* move within the liver, causing destruction of hepatocytes and hepatic structures with ensuing haemorrhages. In addition to movement by *C. osculatum*, these findings of glycogen in the muscle cells and intestinal epithelial of the nematodes indicate that the nematodes are metabolic active. This suggests that *C. osculatum* are growing inside the liver, which is supported by a pronounced size span (from a few mm to approximately 3 cm) of nematodes seen in cod livers (own observations; Mohamed et al., 2020; Ryberg et al., 2020). These findings combined with the knowledge that cod lacks crucial components of the adaptive immune system (Star et al., 2011) lead to the speculation that cod might not be able to prevent reinfections with *C. osculatum* and will be repeatedly be infected.

### 4.3 | Other pathogens

Although our results altogether strongly point to direct pathological effects of *C. osculatum* on the cod liver, the disease history of

the individual is not known when working with wild fish. Hence, we cannot exclude that some of the smaller granulomas may be caused by previous fungal or bacterial infections, for example francisellosis (Olsen et al., 2006) and atypical furunculosis (Cornick et al., 1984). We found myxosporidium parasites in the bile ducts where the bile ducts were often dilated, and occasionally very mild fibrosis surrounded the bile ducts. The presence of myxosporidium parasites in the bile ducts has also been found in cod from the southern Baltic Sea and other gadoid species from the North Atlantic Sea where pathological findings were found to be similar to our study (Borucinska et al., 2017; Hemmingsen & Mackenzie, 2001). Very interestingly, in the present study, the degree of co-infection was most pronounced in fish with heavy *C. osculatum* loads. This suggests that these severely infected cod may be more susceptible to other infections. This does not have to be a direct effect of the infections with *C. osculatum* but can be a result of the strong association between *C. osculatum* infection load and nutritional condition, as cod with high *C. osculatum* loads generally are in a poorer nutritional condition (Horbøw et al., 2016; Ryberg et al., 2020, 2022; Sokolova et al., 2018). Across taxa, including teleosts, individuals with reduced nutritional condition may experience increased susceptibility to pathogens, most likely because their immune response is compromised by their poor nutritional state (Chandra, 1997; Gulland, 1992; Johansen et al., 1997). Although not investigated in cod, malnutrition in some fish species is known to reduce the immune response (MacKinnon, 1998; Oliva-Teles, 2012; Salomoni et al., 1987), and Atlantic cod in poor condition have been found to be more susceptible to lethal disease outbreaks as compared to more well-fed conspecifics (Dutil et al., 2006).

### 4.4 | Conclusion

In conclusion, the present pathological changes and inflammatory reactions of the cod liver related to the infections of *C. osculatum*, together with indications that the parasite grow inside the liver, supports previous studies (Buchmann & Mehrdana, 2016; Mohamed et al., 2020; Ryberg et al., 2020, 2022) suggesting that infections with *C. osculatum* in cod can result in reduced liver functionality and thus reduced health status, as a result of damage to the liver tissue.

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### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

### DATA AVAILABILITY STATEMENT

Data are available by contacting last author.

## ORCID

Jane W. Behrens  <https://orcid.org/0000-0002-0136-9681>

Marie Plambeck Ryberg  <https://orcid.org/0000-0002-4485-9576>

Virginia Chondromatidou  <https://orcid.org/0009-0002-9160-0783>

Tine Moesgaard Iburg  <https://orcid.org/0000-0003-0246-5146>

## REFERENCES

- Aspholm, P. E., Ugland, K. I., Jødestøl, K. A., & Berland, B. (1995). Sealworm (*Pseudoterranova decipiens*) infection in common seals (*Phoca vitulina*) and potential intermediate fish hosts from the outer Oslofjord. *International Journal for Parasitology*, 25(3), 367–373.
- Black, D., & Love, R. M. (1986). The sequential mobilisation and restoration of energy reserves in tissues of Atlantic cod during starvation and refeeding. *Journal of Comparative Physiology B*, 156, 469–479.
- Borucinska, J. D., Morka, D., Grabowski, Z., & Smith, H. (2017). A follow-up study of selected biomarkers of health in cod *Gadus morhua* L. collected from the southern Baltic off the Polish coast. *Journal of Fish Diseases*, 40(12), 1883–1894.
- Bruno, D. W., Nowak, B., & Elliott, D. G. (2006). Guide to the identification of fish protozoan and metazoan parasites in stained tissue sections. *Diseases of Aquatic Organisms*, 70(1–2), 1–36.
- Buchmann, K. (2007). *An introduction to fish parasitological Methods: Classical and molecular techniques*. Biofolia.
- Buchmann, K., & Mehrdana, F. (2016). Effects of anisakid nematodes *Anisakis simplex* (s.l.), *Pseudoterranova decipiens* (s.l.) and *Contracaecum osculatum* (s.l.) on fish and consumer health. *Food and Waterborne Parasitology*, 4, 13–22. <https://doi.org/10.1016/j.fawpar.2016.07.003>
- Casini, M., Käll, F., Hansson, M., Plikshs, M., Baranova, T., Karlsson, O., Lundström, K., Neuenfeldt, S., Gårdmark, A., & Hjelm, J. (2016). Hypoxic areas, density-dependence and food limitation drive the body condition of a heavily exploited marine fish predator. *Royal Society Open Science*, 3(10), 160416. <https://doi.org/10.1098/rsos.160416>
- Chandra, R. K. (1997). Nutrition and the immune system: An introduction. *The American Journal of Clinical Nutrition*, 66, 460S–463S.
- Cornick, J. W., Morrison, C. M., Zwicker, B., & Shum, G. (1984). Atypical *Aeromonas salmonicida* infection in Atlantic cod, *Gadus morhua* L. *Journal of Fish Diseases*, 6(7), 495–499.
- Dutil, J. D., Godbout, G., Blier, P. U., & Groman, D. (2006). The effect of energetic condition on growth dynamics and health of Atlantic cod (*Gadus morhua*). *Journal of Applied Ichthyology*, 22(2), 138–144.
- Eero, M., Hjelm, J., Behrens, J., Buchmann, K., Cardinale, M., Casini, M., Gasyukov, P., Holmgren, N., Horbowy, J., Hussey, K., Kirkegaard, E., Kornilovs, G., Krumme, U., Koster, F. W., Oeberst, R., Plikshs, M., Radtke, K., Raid, T., Schmidt, J., ... Storr-Paulsen, M. (2015). Eastern Baltic cod in distress: Biological changes and challenges for stock assessment. *ICES Journal of Marine Science*, 72(8), 2180–2186. <https://doi.org/10.1093/icesjms/fsv109>
- Elarifi, A. E. (1982). The histopathology of larval anisakid nematode infections in the liver of whiting, *Merlangius merlangus* (L.), with some observations on blood leucocytes of the fish. *Journal of Fish Diseases*, 5(5), 411–419.
- Fagerholm, H. P. (1982). Parasites of fish in Finland. VI. Nematodes. *Acta Academiae Aboensis, B*, 40, 1–128.
- Feist, S. W., & Longshaw, M. (2008). Histopathology of fish parasite infections—importance for populations. *Journal of Fish Biology*, 73(9), 2143–2160.
- Galindo, G. M., Rodrigues, R. A., Marcondes, S. F., Soares, P., Tavares, L. E. R., & Fernandes, C. E. (2017). Morphological and morphometric features of nematode-cysts in *Gymnotus inaequilabiatus* liver in the Brazilian Pantanal. *Revista Brasileira de Parasitologia Veterinária*, 26, 285–291.
- Gulland, F. M. D. (1992). The role of nematode parasites in Soay sheep (*Ovis aries* L.) mortality during a population crash. *Parasitology*, 105(3), 493–503.
- Haarder, S., Kania, P. W., Galatius, A., & Buchmann, K. (2014). Increased *Contracaecum osculatum* infection in Baltic cod (*Gadus Morhua*) livers (1982–2012) associated with increasing grey seal (*Halichoerus Gryphus*) populations. *Journal of Wildlife Diseases*, 50(3), 537–543. <https://doi.org/10.7589/2012-12-320>
- Harding, K. C., Härkönen, T., Helander, B., & Karlsson, O. (2007). Status of Baltic grey seals: Population assessment and extinction risk. *NAMMCO Scientific Publications*, 6, 33. <https://doi.org/10.7557/3.2720>
- Hauksson, E. (2011). The prevalence, abundance, and density of *Pseudoterranova* sp. (p) Larvae in the flesh of cod (*Gadus morhua*) relative to proximity of grey seal (*Halichoerus grypus*) colonies on the coast off Drangar, Northwest Iceland. *Journal of Marine Biology*, 2011, 235832. <https://doi.org/10.1155/2011/235832>
- HELCOM. (2018). Population trends and abundance of seals (issue July).
- Hemmingsen, W., & Mackenzie, K. (2001). The parasite fauna of the Atlantic cod (*Gadus morhua* L.). *Advances in Marine Biology*, 40, 2–60.
- Herreras, M. V., Balbuena, J. A., Aznar, F. J., Kaarstad, S. E., Fernández, M., & Raga, J. A. (2004). Population structure of *Anisakis simplex* (Nematoda) in harbor porpoises *Phocoena phocoena* off Denmark. *Journal of Parasitology*, 90(5), 933–938.
- Horbowy, J., Podolska, M., & Nadolna-Ałtyn, K. (2016). Increasing occurrence of anisakid nematodes in the liver of cod (*Gadus morhua*) from the Baltic Sea: Does infection affect the condition and mortality of fish? *Fishery Research*, 179, 98–103.
- ICES. (2020). Working group on marine mammal ecology (WGMME). *ICES Scientific Reports*, 2, 39. <https://doi.org/10.17895/ices.pub.5975>
- Johansen, M. V., Bøgh, H. O., Giver, H., Eriksen, L., Nansen, P., Stephenson, L., & Knudsen, K. E. B. (1997). *Schistosoma japonicum* and *Trichuris suis* infections in pigs fed diets with high and low protein. *Parasitology*, 115(3), 257–264.
- Køie, M., & Fagerholm, H.-P. (1995). The life cycle of *Contracaecum osculatum* (Rudolphi, 1802) sensu stricto. *Parasitology Research*, 81, 481–489.
- Lambert, Y., & Dutil, J. D. (2000). Energetic consequences of reproduction in Atlantic cod (*Gadus morhua*) in relation to spawning level of somatic energy reserves. *Canadian Journal of Fisheries and Aquatic Sciences*, 57(4), 815–825.
- MacKinnon, B. M. (1998). Host factors important in sea lice infections. *ICES Journal of Marine Science*, 55(2), 188–192.
- Marnis, H., Kania, P. W., Syahputra, K., Zuo, S., & Buchmann, K. (2020). Local immune depression in Baltic cod (*Gadus morhua*) liver infected with *Contracaecum osculatum*. *Journal of Helminthology*, 94, e112. <https://doi.org/10.1017/S00222149X19001111>
- Mohamed, A., Zuo, S., Karami, A. M., Marnis, H., Setyawan, A., Mehrdana, F., Kirkeby, C., Kania, P., & Buchmann, K. (2020). *Contracaecum osculatum* (sensu lato) infection of *Gadus morhua* in the Baltic Sea: Inter- and intraspecific interactions. *International Journal for Parasitology*, 50(10–11), 891–898. <https://doi.org/10.1016/j.ijpara.2020.06.003>
- Nadolna, K., & Podolska, M. (2014). Anisakid larvae in the liver of cod (*Gadus morhua*) L. from the southern Baltic Sea. *Journal of Helminthology*, 88(2), 237–246. <https://doi.org/10.1017/s0022149x13000096>
- Neuenfeldt, S., Bartolino, V., Orio, A., Andersen, K. H., Ustups, D., Kulatska, N., Andersen, N. G., Niiranen, S., Bergstro, U., & Casini, M. (2020). Feeding and growth of Atlantic cod (*Gadus morhua* L.) in the eastern Baltic Sea under environmental change. *ICES Journal of Marine Science*, 77, 624–632. <https://doi.org/10.1093/icesjms/fsz224>
- Oliva-Teles, A. (2012). Nutrition and health of aquaculture fish. *Journal of Fish Diseases*, 35(2), 83–108.

- Olsen, A. B., Mikalsen, J., Rode, M., Alfjorden, A., Hoel, E., Straum-Lie, K., Haldorsen, R., & Colquhoun, D. J. (2006). A novel systemic granulomatous inflammatory disease in farmed Atlantic cod, *Gadus morhua* L., associated with a bacterium belonging to the genus *Francisella*. *Journal of Fish Diseases*, 29(5), 307–311.
- Petrushevski, G. K., & Shulman, G. G. (1955). Infection of Baltic cod liver with roundworms. *Trudy Akademii Nauk Litovskoi SSR. Seriya B*, 2, 119–125.
- R Core Team. (2016). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.
- Roberts, R. J., & Ellis, A. E. (2012). The anatomy and physiology of teleosts. In R. J. Roberts (Ed.), *Fish pathology* (4th ed., pp. 17–62). Wiley-Blackwell.
- Ryberg, M. P. (2020). *Eastern Baltic cod infected with Contracaecum osculatum: Physiological mechanisms and the importance of monitoring infection loads*. PhD thesis, DTU Aqua.
- Ryberg, M. P., Huwer, B., Nielsen, A., Dierking, J., Buchmann, K., Sokolova, M., Krumme, U., & Behrens, J. W. (2022). 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(1), 88–99.
- Ryberg, M. P., 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), coaa093. <https://doi.org/10.1093/conphys/coaa093>
- Salomoni, C., Fiorentino, M., & Palenzona, D. L. (1987). Effects of diet, sham-immunization and bleeding on the immune response kinetics for two strains of *Cyprinus carpio*. *Journal of Fish Biology*, 31, 93–99.
- Sayyaf Dezfuli, B., Fernandes, C. E., Galindo, G. M., Castaldelli, G., Manera, M., DePasquale, J. A., Lorenzoni, M., Bertin, S., & Giari, L. (2016). Nematode infection in liver of the fish *Gymnotus inaequaliatus* (Gymnotiformes: Gymnotidae) from the Pantanal Region in Brazil: pathobiology and inflammatory response. *Parasites and vectors*, 9(1), 473.
- Setyawan, A. C., Jensen, H. M., Kania, P. W., & Buchmann, K. (2020). Baltic cod endohelminths reflect recent ecological changes. *Journal of Helminthology*, 94, e155.
- Sokolova, M., Buchmann, K., Huwer, B., Kania, P. W., Krumme, U., Galatius, A., Hemmer-Hansen, J., & Behrens, J. W. (2018). Spatial patterns in infection of cod *Gadus morhua* with the seal-associated liver worm *Contracaecum osculatum* from the Skagerrak to the Central Baltic Sea. *Marine Ecology Progress Series*, 606, 105–118. <https://doi.org/10.3354/meps12773>
- Star, B., Nederbragt, A. J., Jentoft, S., Grimholt, U., Malmstrøm, M., Gregers, T. F., Rounge, T. B., Paulsen, J., Solbakken, M. H., Sharma, A., Wetten, O. F., Lanzén, A., Winer, R., Knight, J., Vogel, J.-H., Aken, B., Andersen, Ø., Lagesen, K., Tooming-Klunderud, A., ... Jakobsen, K. S. (2011). The genome sequence of Atlantic cod reveals a unique immune system. *Nature*, 477(7363), 207–210.
- Wang, X., Copmans, D., & de Witte, P. A. (2021). Using zebrafish as a disease model to study fibrotic disease. *International Journal of Molecular Sciences*, 22(12), 6404.
- Zuo, S., Barlaup, L., Mohammadkarami, A., Al-Jubury, A., Chen, D., Kania, P. W., & Buchmann, K. (2017). Extrusion of *Contracaecum osculatum* nematode larvae from the liver of cod (*Gadus morhua*). *Parasitology Research*, 116(10), 1–6. <https://doi.org/10.1007/s00436-017-5580-1>
- Zuo, S., Huwer, B., Bahloul, Q., Al-Jubury, A., Christensen, N. D., Korbut, R., Kania, P., & Buchmann, K. (2016). Host size-dependent anisakid infection in Baltic cod *Gadus morhua* associated with differential food preferences. *Diseases of Aquatic Organisms*, 120(1), 69–75.
- Zuo, S., Kania, P. W., Mehrdana, F., Marana, M. H., & Buchmann, K. (2018). *Contracaecum osculatum* and other anisakid nematodes in grey seals and cod in the Baltic Sea: Molecular and ecological links. *Journal of Helminthology*, 92(1), 81–89.

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