Received: 12 February 2021 | Revised: 21 August 2021 | Accepted: 23 September 2021

DOI: 10.1111/fme.12516

ORIGINAL ARTICLE

Fisheries Management and Ecology WILEY

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

Marie Plambech Ryberg[1] | Bastian Huwer[1] | Anders Nielsen[1] | Jan Dierking[2] | Kurt Buchmann[3] | Maria Sokolova[4] | Uwe Krumme[5] | Jane W. Behrens[1]

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

[2]GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

[3]Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark

[4]National Institute of Aquatic Resources, Technical University of Denmark (DTU Aqua), Hirtshals, Denmark

[5]Thünen Institute of Baltic Sea Fisheries, Rostock, Germany

Correspondence
Jane W. Behrens, National Institute of Aquatic Resources, Technical University of Denmark (DTU Aqua), Kgs. Lyngby, Denmark.
Email: jabeh@aqua.dtu.dk

Funding information
The EU and the German BMBF, Grant/Award Number: 03F0682; The European Maritime and Fisheries Fund and The Danish Fisheries Agency, Grant/Award Number: 33113-B-16-070, 33113-B-17-110 and 33113-B-16-071; The European Union's Horizon 2020 research and innovation program, Grant/Award Number: 773713 (PANDORA); Director J.P.A. Espersen and Mrs. Dagny Espersen Foundation; The European Commission's Data Collection Framework

Abstract

During the 2010s, Atlantic cod *Gadus morhua* L. in the eastern Baltic Sea experienced increasing infection loads of the parasitic nematode *Contracaecum osculatum* (Rudolphi) in their livers. Starting in 2021, a mandatory part of the routine sampling protocol on Baltic monitoring surveys is to assign a liver category to individual cod livers, based on the number of nematodes visible on the liver surface, to follow spatiotemporal changes in nematode infection loads. The validity of the liver category method has never been evaluated. Based on data from 642 cod livers, the method was verified and found to be a good predictor of the total number of nematodes. Moreover, the probability of cod being in a critical condition increased with the parasite load. In addition to their direct applicability to Baltic cod, the present findings may inspire others working with disease in fish stocks to include parasite monitoring.

KEYWORDS
disease monitoring, eastern Baltic cod, Fulton condition factor, liver worm, natural mortality, stock assessment

1 | INTRODUCTION

Fish stock assessments require input data such as numbers and/or biomass of fish, natural and fishing mortality, growth and recruitment. These data, which are derived from the market sampling of commercial catches and from the monitoring surveys, are used to evaluate the present status of exploited fish stocks and project their future development (Jennings et al., 2001). Parasites can affect their hosts ranging from the level of the individual to the level of populations (Timi & Poulin, 2020). However, fish health indicators, such as parasite occurrence, are usually not part of the routine sampling on marine monitoring surveys (Lloret et al., 2012). This omission could be due to the collection of these data requiring expert knowledge, is often time-consuming, difficult to conduct, especially at sea, and, therefore, expensive.

The Atlantic cod *Gadus morhua* L. is a key species in the Baltic Sea ecosystem and commercial fisheries. The Baltic cod comprises two different stocks, the eastern and western stocks (Eero et al., 2012). The eastern Baltic cod stock is an emerging example for which information on infections with parasitic nematodes in the liver would improve scientific advice, stock assessments and management. This stock is at historically low levels of nutritional condition, individual growth and productivity. Natural mortality in eastern Baltic cod has consequently been estimated to be more than three times higher than fishing mortality in recent years (Eero et al., 2015; Mion et al., 2020; Sokolova et al., 2018). Several ecosystem changes, such as deteriorating oxygen conditions and reduced quality and quantity of prey, may have contributed to the poor state of the eastern Baltic cod stock (Casini et al., 2016b; Eero et al., 2015; Neuenfeldt et al., 2020; Plambech et al., 2013). During the latest decade, fish in this stock have also experienced a marked increase in infections with the nematode *Contracaecum osculatum* (Rudolphi), which parasitises the liver of cod (Haarder et al., 2014; Nadolna & Podolska, 2014). Most cod above 35 cm in the central and eastern Baltic are now infected, but so far infection levels remain low in the more westerly part of the Baltic region (Horbowy et al., 2016; Sokolova et al., 2018). Field studies show that cod with many parasitic nematodes in the liver have lower body conditions than conspecifics with no or few of these parasites (Horbowy et al., 2016; Ryberg et al., 2020; Sokolova et al., 2018). Low body condition can lead to increased natural mortality, reduced fecundity and skipped spawning, thus affecting the stock productivity (Casini et al., 2016a; Dutil & Lambert, 2000; Gislason et al., 2010; Mion et al., 2018).

Cod become infected with *C. osculatum* by ingesting smaller infected fish, such as sprat *Sprattus sprattus* (L.), herring *Clupea harengus* L. or greater sandeel *Hyperoplus lanceolatus* (Le Sauvage) (Nadolna-Ałtyn et al., 2017; Rodjuk, 2014; Valtonen et al., 1988; Zuo et al., 2016). Grey seal *Halichoerus grypus* (Fabricius) is the main final host of *C. osculatum* in the Baltic Sea, whereas cod is one of several transport hosts (Køie & Fagerholm, 1995). The abundance of the grey seals has increased markedly in recent years, now counting ≈ 38,000 animals (status 2019) compared with a few thousand in the 1980s (Harding et al., 2007; HELCOM, 2018; ICES, 2020). This trend has coincided with the marked increase in infection loads with *C. osculatum* in cod in the central and eastern Baltic in the International Council for Exploitation of the Sea (ICES) subdivision (SD) 25 and 26 (Sokolova et al., 2018).

In 2010, Poland's National Marine Fisheries Research Institute made the first detailed counts of *C. osculatum* numbers in individual cod livers (Nadolna & Podolska, 2014), followed by Denmark in 2012 (Haarder et al., 2014). However, detailed investigations in which all individual nematodes are identified and counted, are complex, expensive and time-consuming. Additionally, a few Baltic countries have recently included voluntary specimen collections during monitoring surveys (e.g. the German and Danish Baltic International Trawl Survey), using the so-called "liver category" method as a simpler protocol with which to follow the spatiotemporal development in infection levels in cod (Figure 1). This method categorises livers into five classes as a function of the number of visible nematodes on the surface of the entire organ including both lobes (i.e. 0, 1–10, 11–20, 21–30 and >30 nematodes). The method requires little training and is inexpensive, easy and fast to implement.

From 2021, the Baltic International Fish Survey Working Group (WGBIFS) has made it a mandatory part of the standard Baltic International Trawl Survey protocol for participating countries to assign a liver category to all individual livers (ICES, 2020). Including the spatiotemporal development of *C. osculatum* infection loads in

FIGURE 1 Photo of the five liver categories (0–4) of nematode infection levels of cod livers 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]

cod livers into assessments may lead to greater biological realism in explaining the causes of the deterioration of this stock, and hence improve management advice.

Until now, the liver categorisation method has not been evaluated to test whether the liver categories provide accurate estimates of differences in total numbers of nematodes and hence reliable information on the spatial and temporal development in prevalence and abundance of the infections. Since only a minority of nematodes are visible on the surface of the liver, whilst the majority are inside the liver parenchyma where they are not visible (Nadolna & Podolska, 2014), an obvious limitation of the liver category method is that it may underestimate the true number of nematodes in the liver, depending on the size and shape of the organ. Accurate and precise estimates of the true number of nematodes in livers will permit evaluation of the potential health effects on the infected fish because of high infection density (i.e. the number of nematodes per gram liver tissue) is associated with the impaired physiological condition of the fish (Ryberg et al., 2020).

The overarching aims of this study were to test the ability of liver categorisation to act as a surrogate for a true count of the total number of nematodes 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. The further aim was to estimate the total number of nematodes in livers, based on the liver category data, to determine infection load. To complement these aims, the association between infections and critical individual Fulton condition of cod was examined to test whether infections may result in increased natural mortality. The applicability of the liver category from a spatial perspective, including the estimation of the total number of nematodes, and how the infections may lead to changes at the population level are discussed.

2 | MATERIAL AND METHODS

2.1 | Study areas and fish collection

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 2a, Table 1). To account for the potential seasonal variation in condition and in the hepato-somatic index, sampling was performed in different months between 2017 and 2020 (Table 2). Cod were caught by trawling in SD24 and SD25 and by trawling, gillnets and trammel nets in SD22 (Table 1). All fish were processed fresh (without freezing or other conservation) either: (i) directly on board or (ii) within a few hours after the capture and transported with ice to the laboratory. Total length (TL, mm), wet body weight (entire fish g) and liver weight (g), eviscerated body weight (g) and sex was recorded for each fish. Individuals ranging from 20 to 58 cm TL were included to cover the length distribution and variability in the infection load of fish (Table 1).

2.2 | Analysis of livers for nematodes and parasite identification

All individual livers of the 642 sampled cod were assigned a "liver category" using the method introduced by Thünen Institute in western Baltic areas. Liver category (0 to 4), based on the number of nematodes on the liver surface (0, 1–10, 11–20, 21–30 and >30, respectively; Figure 1) and the total count of nematodes in each liver was recorded to test the ability of the liver category to act as a surrogate of the total number of nematodes. After the assignment of categories, individual livers were kept at −20°C for subsequent analysis of the total number of nematodes. This was done using the "compression method," where livers were placed in a plastic bag (200 × 400 × 0.07 mm) and compressed between two glass plates (15 × 15 × 1 cm) to a thickness of 1 mm by gentle pressure (Buchmann, 2007). Livers were subsequently examined under a Leica stereomicroscope (6.3–40× magnification, Leica Microsystems Germany). Nematode species identification was based on morphometric characteristics of the caudal and cephalic ends according to Fagerholm (1982) and carried out at the Laboratory of Aquatic Pathobiology, University of Copenhagen (Frederiksberg, Denmark). For further details on the methodologies, see Buchmann (2007) and Sokolova et al. (2018). The infection density (IFD), calculated as the number of nematodes per gram liver tissue (i.e. liver tissue = wet weight of the liver minus total weight of nematodes), was used to compensate for differences in the number of nematodes related to liver size. The total weight of nematodes was estimated using the mean weight (i.e. 0.0065 g) for small (<1 cm TL) and large (>1 cm TL) nematodes found in a previous study (Ryberg et al., 2020). The total weight of nematodes in livers was on average 0.59 ± 0.04% of the total weight of the liver.

2.3 | Statistical analysis

The data were unbalanced in terms of the number of observations within the five liver categories for the three examined areas (Figure 1; in SD22 and SD24: scale 0–3, in SD25: scale 0–4). This was due to spatial differences in parasite loads, with the lowest levels found in the western part and the highest levels in the eastern part of the Baltic region (Sokolova et al., 2018).

A full generalised linear model (GLM) including data from all three areas was defined to test the ability of the liver category to predict the total number of nematodes (Y). In addition, to focus on spatial differences, a separate model was applied for each area (i.e. SD22, SD24 and SD25). If the models predict no overlap in terms of predicted means and confidence intervals, then it can be stated that the liver category is a valid tool to monitor parasite load in the livers of Baltic cod. In all four models, the total number of nematodes was defined as the response variable, which followed a negative binomial distribution. The observations of the total number of nematodes $(Y_i)_{i=1,\dots,N}$ were assumed independent with mean μ_i and over dispersion parameter $\theta > 0$ for each of the N observations. This implied that the variance of the i^{th} observation becomes $\mu_i(1+\mu_i/\theta)$.

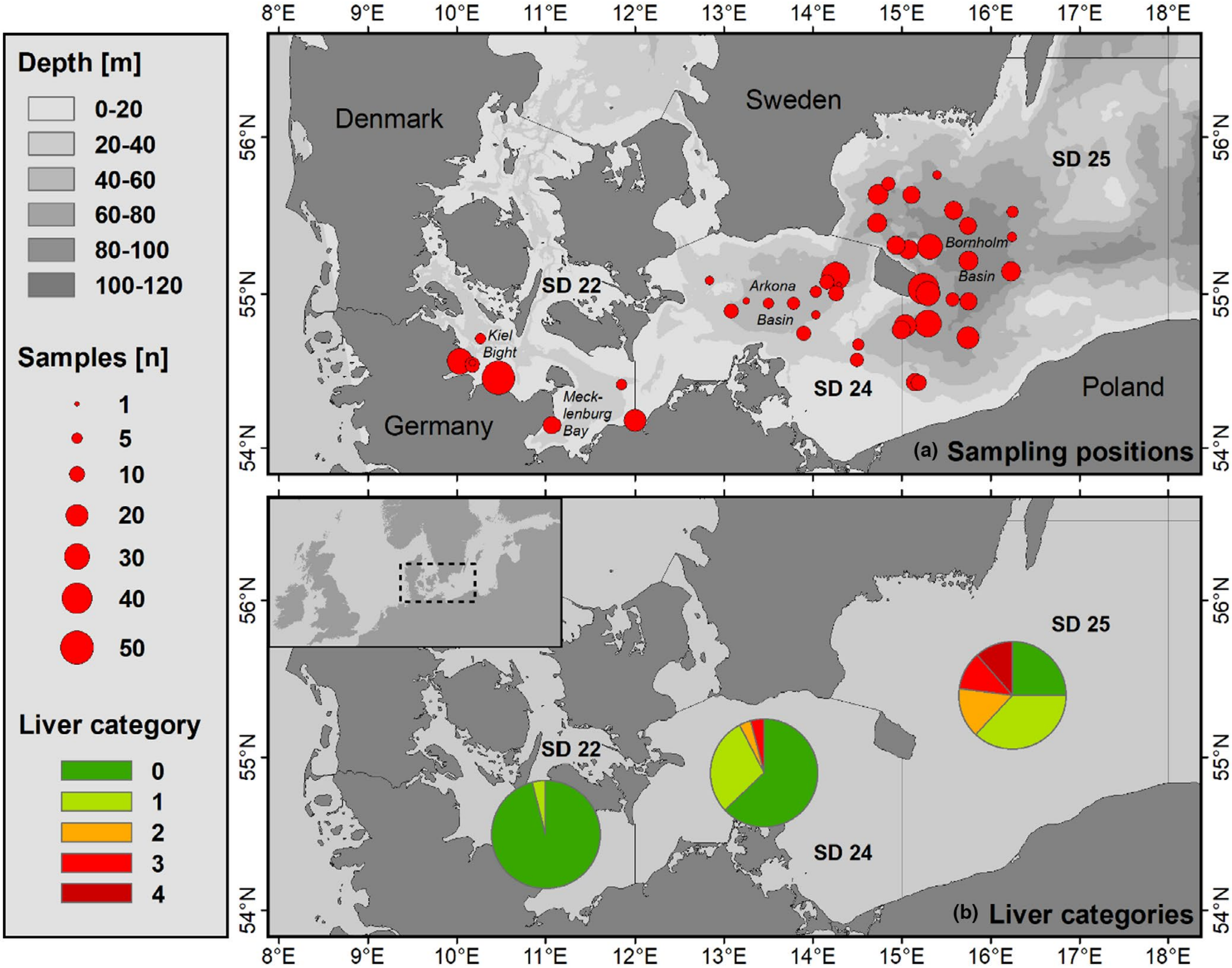

FIGURE 2 (a) The study area and the sampling positions within the three ICES subdivisions (SD22, SD24 and SD25) where cod were collected for analysis of liver nematodes: The grey scale indicates water depth of the Baltic and the size of the bubbles represents the number of samples examined from each position. (b) Percentage of the total number of livers assigned to each liver category by subdivision

A Poisson distribution was tested, but this could not account for the variance in the data sets. The logarithm of the mean μ_i is described by the linear model (Equation 1):

$$\log(\mu_i) = \alpha(livercategory_i) \cdot HSI_i + \beta(livercategory_i) \cdot TL_i + \delta(livercategory_i) \quad (1)$$

Total fish length (TL) was included as an explanatory variable to account for the accumulation of nematodes in the liver over time (Horbowy et al., 2016). To account for the seasonal changes in the size of the livers, a hepato-somatic index (HSI) based on eviscerated weight was calculated (Equation 2) and also included as an explanatory variable in the model (Lambert & Dutil, 1997):

$$HSI = \frac{LW}{GW} * 100 \quad (2)$$

where LW represents the liver wet weight and GW represents the eviscerated weight of the fish. GW of the fish was used in the calculation of HSI to eliminate potential bias related to gonad size and stomach fullness. A model including slopes with respect to HSI and TL for each liver category and an intercept for each liver category were defined to examine any differences in the effect of TL and HSI within each liver category (Equation 1).

Owing to the data limitations for SD22 (natural dominance of liver Category 0), it was not possible to include slopes with respect to HSI and TL for each liver category in the full model for this area. In this respect, note that the differences in the number of livers assigned to the different liver categories throughout the Baltic Sea do not reflect bias in sampling of livers – they simply reflect the spatial differences in infection load with nematodes in the livers of Baltic cod (Sokolova et al., 2018).

The statistical tests were carried out using R with Rstudio (version 3.4.1.) (R Core Team, 2016). The four GLM models were fitted with glmmTMB using the package "glmmTMB" (Brooks et al., 2017). Before model fitting, collinearity between explanatory variables was assessed using variance inflation factors (VIF) (Zuur et al., 2009). No variables were excluded from the analysis due to collinearity

TABLE 1 Overview of 642 cod sampled between 2017 and 2020 from ICES subdivisions SD22, SD24 and SD25 for the analysis of infections with liver nematodes (see also Figure 2 for sampling positions and numbers)

Area	Gear type	Number of livers	TL (cm)	W (g)	GW (g)	LW (g)	HSI	Prevalence (%)	Intensity of infection
SD22	GNS, OTB and GTR†	132	44 ± 0.5 (28–71)	910 ± 31 (190–2972)	827 ± 30 (178–2751)	23 ± 1.5 (2–87)	2.7 ± 0.1 (0.4–6.8)	13	2 ± 0.5 (0–10)
SD24	Bottom trawl‡	115	39 ± 0.4 (35–50)	562 ± 18 (300–1450)	481 ± 15 (270–1212)	23 ± 1.3 (3–72)	4.6 ± 0.2 (0.6–10.6)	74	17 ± 2.0 (0–113)
SD25	Bottom trawl[a]	395	39 ± 0.3 (20–58)	607 ± 16 (54–2280)	483 ± 11 (47–1648)	26 ± 0.9 (1–115)	5.4 ± 0.1 (1.0–14.9)	89	28 ± 1.6 (0–180)

Note: Values are mean ± SE and brackets represent the range of data

Abbreviations: GNS, gillnet set; GTR, trammel net (gillnet consisting of three layers of net); GW, eviscerated wet weight; HSI, hepato-somatic eviscerated index; LW, wet-weight of the liver; OTB, otter trawl bottom; Prevalence, percentage of infected fish in the sample; The intensity of infection, mean number of counted parasites per fish, only including infected individuals.TL, total length; W, total wet weight.

[a]Scientific otter trawl, operated at the sea floor, similar to OTB.

(Table S1). 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. Extraction of residuals for model validation of each final model was done using one-step prediction which is an implemented function in the R package "Template Model Builder" used to extract quantile residuals of models (Thygesen et al., 2017). The model assumptions of normality and independence were hereafter validated by visual inspection of model residuals (Figure S1–S4).

2.4 | Critical Fulton condition

To examine associations between the Fulton condition factor, which is a measure of "plumpness," and infections with *C. osculatum* in cod, two different analyses were performed on data from SD25. Calculation of Fulton condition factor was based on eviscerated weight and TL (Equation 3):

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

In the first case, the significant difference of the Fulton condition factor within the five levels of the liver category for each month represented in data was tested using ANOVA and a post hoc analysis (Tukey HSD). In the second case, more data were included ($n = 594$) on the total number of nematodes in livers from cod in SD25 (Table S2) to examine the association between Fulton condition factor and IFD, and to estimate the probability of cod being below the critical Fulton condition factor (0.65) in relation to IFD. The critical Fulton condition factor reflects the level where cod are considered dying (Casini et al., 2016a; Dutil & Lambert, 2000). The association between the Fulton condition factor and IFD was examined using:

$$\log\left(Fulton\ Condition\ factor_i\right) = \gamma * IFD_i + \varepsilon_i,\ where\ \varepsilon_i \sim N\left(0, \sigma^2\right) \quad (4)$$

The probability of having a critical Fulton condition factor at given IFD values can be calculated as a tail probability directly from the model in Equation 4. A parametric bootstrap (i.e. simulation of a large number of pairs of γ and σ) was used to propagate the uncertainty from the estimates of γ and σ to the probability estimates.

[Correction added on 14 October 2021, after first online publication: Equation 4 has been updated in this version.]

3 | RESULTS

3.1 | Number and species of nematodes and liver categories

A total of 11,352 nematodes were recovered from the 642 livers examined, 32, 1487 and 9833 nematodes from SD22, SD24 and SD25, respectively. Microscopic identification based on morphological

TABLE 2 The total number of livers assigned to each liver category for each area (SD22, SD24 and SD25) and month. For further details on liver categories, see Figure 1

	SD22			SD24		SD25				
	Nov	Jun	Aug	Nov	Apr	Sep	Jun	Nov	Mar	
Liver category	2017	2018	2018	2017	2018	2017	2018	2019	2020	Sum
0	48	14	65	37	35	12	21	36	29	297
1	3	0	2	4	30	30	39	35	41	184
2	0	0	0	2	2	16	26	7	11	64
3	0	0	0	4	1	6	30	4	6	51
4	NA	NA	NA	NA	NA	3	36	0	7	46
Sum	51	14	67	47	68	67	152	82	94	642
Sum: area		132		115			395			

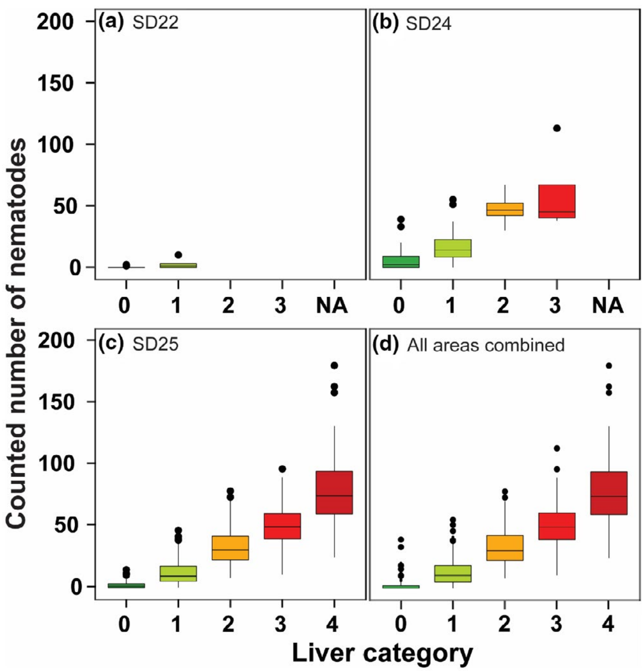

FIGURE 3 Relationship between the assigned liver categories and the counted total number of nematodes inside the livers of Baltic cod sampled in subdivisions (SD) defined by ICES (a = SD22; b = SD24; c = SD25) and all areas combined (d). See Figure 1 for visual appearance and a detailed description of 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 25^{th} and 75^{th} 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. See Table 2 for details on the total number of livers assigned to each category

characters showed that the majority of larvae belonged to the genus *Contracaecum*. In SD22, nine of the 32 parasitic nematodes belonged to two other species of nematodes: herring or whale worm *Anisakis simplex* (Rudolphi) ($n = 8$) and seal worm or cod worm, *Pseudoterranova decipiens* (Krabbe)($n = 1$). In SD24, four of the 789 parasitic nematodes were identified as *A. simplex* and the remainder as *C. osculatum*. In SD25, only *C. osculatum* was identified. Previous studies based on molecular analysis (sequencing of ITS, mtDNA cox 1 and 2; Sokolova et al., 2018; Zuo et al., 2018; Mohamed et al., 2020) showed that all recovered larvae were *C. osculatum*, *sensu stricto*, which suggests that the larvae in the present study are *C. osculatum*. Given the low contribution of species other than *C. osculatum*, the total number of nematodes counted that were used in the statistical analysis included all nematodes counted irrespective of the species of nematode.

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 number of nematodes counted within each liver category (Figures 2b and 3, Table 2). Irrespectively of area, variance in the number of nematodes counted within a liver category was highest in the highest liver category (Figure 3). When combining data from the three areas, the variance of the number of nematodes counted within each liver category increased (Figure 3d). In area SD22, most livers were assigned to liver Category 0, and no livers were assigned to liver categories 2 and 3 (Figures 2b and 3a, and Table 2), whereas in area SD24, most livers were assigned to either liver categories 0 or 1 and a few to liver categories 2 and 3 (Figures 2b and 3b, and Table 2). In area SD25, all five liver categories were present (Figures 2b and 3c, and Table 2).

3.2 | Variation in the estimated total number of nematodes

The final model for all data combined showed a significant increase in the total number of nematodes with increasing liver category (likelihood ratio test, $p = 0.009$, Table 3 and Table S3). The total number of nematodes increased significantly with TL for all liver categories, except for liver Category 0, where an increase in TL resulted in a decrease in the total number of nematodes (likelihood ratio test, $p < 0.001$, Table 3). There was no effect of HSI on the total number of nematodes (Table S3).

In all the three area models, the total number of nematodes increased significantly with increasing liver category (likelihood ratio test, $p < 0.001$, Table 4). In the area SD22, the total number of nematodes increased significantly with TL (likelihood ratio test, $\beta = 0.094$, $p = 0.04$) and HSI (likelihood ratio test, $\delta = 0.331$, $p = 0.04$) (Table S3 and S4), whereas there was no effect of HSI and TL on the total number of nematodes in area SD24 (Table S3). In SD25, the total number of nematodes increased with TL (likelihood ratio test, $\beta = 0.049$, $p < 0.001$) but decreased with increased HSI (likelihood ratio test, $\delta = -0.043$, $p = 0.004$) (Table S3 and S4, Figure 4). For a 40-cm cod with a liver Category 4 (i.e. highly infected) from SD25, the predicted total number of nematodes was e.g. 39% lower in fish with the highest observed HSI (14.9; $n = 42$ nematodes) than cod with a medium HSI (HSI = 5.4; $n = 61$ nematodes) (Figure 4). Model reduction of the three area models revealed that there were no interactions between the liver category and HSI, and the liver category and TL were significant for the total number of nematodes (Table S3). To evaluate the three areas models, and to assess the precision of the estimated total number of nematodes, the predicted number of nematodes and confidence intervals (0.95) of the three area models were extracted (Table 5). Owing to the significance of TL and HSI in areas SD22 and SD25, predictions were based on a cod of 40-cm TL with the mean HSI of 2.7 for the area SD22 and 5.5 for the area SD25 (Table 5). The predicted number of nematodes for the area SD24 were calculated for all sizes and the values of HSI, due to the non-significance of the variables in that area (Tables S2 and S4). The predicted numbers within each liver category were highest in the area SD25 in all categories except for the liver Category 0, where the predicted number was highest in the area SD24 (Table 5). The accuracy of the three models decreased with the increasing liver category as the confidence intervals became broader with each liver category level (Table 5).

TABLE 3 Estimates of significant parameters and standard errors (SE) of the final model, including all data combined, describing how the total number of nematodes changes within each category and with total length (TL)

Parameter	Estimate			
Intercept	**δ (category)**	**SE**	**Wald Z**	***p* value**
0	3.137	0.546	5.750	0.009
1	0.326	0.588	0.555	
2	1.745	1.172	1.488	
3	3.135	1.307	2.399	
4	2.920	1.535	1.903	
Slope	**β (category)**			
0	−0.060	0.014	−4.365	<0.001
1	0.056	0.015	3.740	
2	0.042	0.028	1.498	
3	0.018	0.030	0.602	
4	0.033	0.034	0.947	

Note: Wald Z provides the statistical result of each variable and factor level and *p* values below 0.05 are considered significant. The slope is given with respect to TL. The numbers are on a log scale.

Fulton condition factor tended to decrease in all months, though the decrease was significant for June only (ANOVA, $p = 0.008$), with increasing liver category, except for the liver Category 4 (Figure 5). However, Fulton condition factor decreased significantly with an increase in IFD (likelihood ratio test, $p < 0.001$, $\gamma = \exp(-0.029)$, SD = 0.002, intercept = exp(−0.262)). The subsequent calculation of the tail probability of equation 4 revealed a sigmoid pattern between IFD and the probability of the fish having a critical Fulton condition factor. For example, fish with an IFD factor of 6 had a probability of having a critical Fulton condition factor of 50%, whereas fish with an IFD of 10 almost had a 100% probability of having a critical condition factor (Figure 6).

4 | DISCUSSION

Assigning a liver category to individual cod livers has been a mandatory part of the routine sampling protocol on Baltic monitoring surveys since 2021 to monitor the parasite load in livers of Baltic cod (ICES, 2020). The present study verifies the ability of the liver category method to predict the total number of nematodes. The liver category is a good predictor of the total number of nematodes, and the GLMs developed provided good estimates of the total number of nematodes in livers. The total number of nematodes is needed to calculate IFD, which is known to relate to the health status of the infected fish (Ryberg et al., 2020). The present findings demonstrate that the explanatory variables (i.e. TL and HSI) for estimating a total number of nematodes differed between areas, implying the need to include a spatial component in the analysis, i.e. three different area models. Infection density is related to an increased probability of a cod having a critical Fulton condition factor, believed to lead to mortality. Altogether, this showed that the collection of liver category data during the monitoring surveys provides an inexpensive and easy way to obtain pan-Baltic information on spatiotemporal changes in infection load with *C. osculatum*. Arguably, fisheries scientists should not ignore the potential effects of parasites on fish stocks, as this may lead to flawed interpretations, e.g. of factors driving natural mortality or stock productivity (Timi & Poulin, 2020). In waters adjacent to Newfoundland and Labrador (eastern Canada) during the 1980s, high grey seal abundance coincided with the high infection loads in cod fillets with the seal-associated parasitic nematode *P. decipiens*, which impacted the commercial value of the fish in these stocks (Chandra & Khan, 1988; Malouf, 1986).

Previous investigations of cod in SD25 showed that the total number of nematodes in individual livers increases with TL, likely because the nematodes accumulate in the liver as cod consume infected prey (Horbowy et al., 2016; Zuo et al., 2016). The same pattern was found in the present study for fish in the areas SD22 and SD25. The lack of significance of TL on the estimated number of nematodes in the area SD24 might be a result of the narrow TL range (35–50 cm) of the cod sampled for the analysis within this

TABLE 4 Estimates of significant parameters and standard errors (SE) of the final models describing how the estimated total number of nematodes changes for each area within each liver category (intercept)

Area	Parameter	Estimate	SE	Wald Z	*p* value
SD22	Intercept	δ (category)			
	0	−7.347	2.574	−2.854	<0.001
	1	−3.897	2.458	−1.585	
	Slope (TL)	β			
		0.094	0.054	1.726	0.040
	Slope (HSI)	α			
		0.331	0.162	2.076	0.040
SD24	Intercept	δ (category)			
	0	1.756	0.152	11.559	<0.001
	1	2.831	0.213	13.267	
	2	3.861	0.615	6.282	
	3	4.104	0.549	7.478	
SD25	Intercept	δ (category)			
	0	−0.731	0.264	−2.768	<0.001
	1	0.787	0.271	2.908	
	2	1.625	0.289	5.629	
	3	1.971	0.299	6.602	
	4	2.395	0.310	7.734	
	Slope (TL)	β			
		0.049	0.007	7.235	<0.001
	Slope (HSI)	α			
		−0.043	0.015	−2.880	0.004

Note: Wald Z provides the statistical result of each variable and factor level and *p* values below 0.05 are considered significant. The slope is given with respect to total length (TL) and hepato-somatic index (HSI). The numbers are on a log scale.

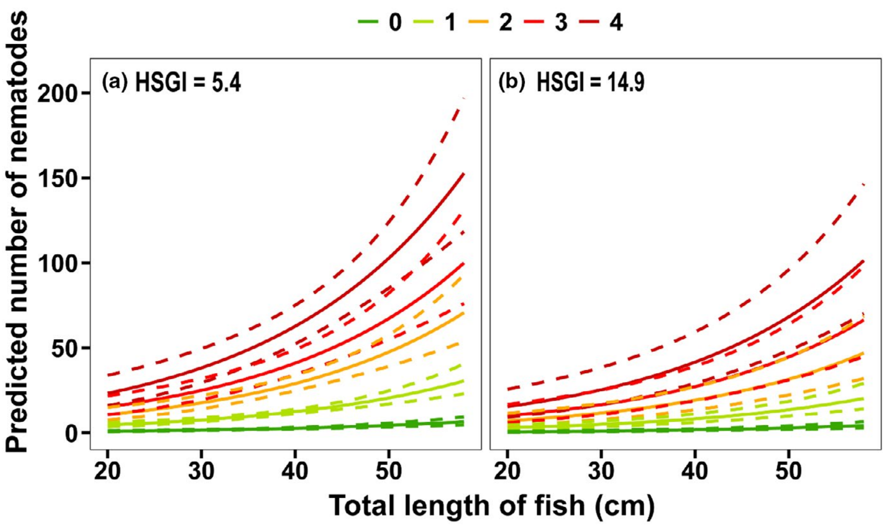

FIGURE 4 Predictions of the total number of nematodes in livers of Baltic cod from area SD25 derived from the final GLM model for different cod sizes with mean hepato-somatic eviscerated index (HSI) = 5.4 (a) and the highest observed HSI index = 14.9 (b). Colours represent the five liver categories 0–4. See Figure 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

TABLE 5 Mean predicted total numbers of nematodes derived from the three generalised linear models for the three different areas

Area	Liver category 0	1	2	3	4
SD22					
Mean	0.1	2.1	–	–	NA
CI (lower–upper)	0.0–0.2	0.5–9.6	–	–	NA
SD24					
Mean	5.8	17.0	47.5	60.6	NA
CI (lower–upper)	4.3–7.8	11.1–26.0	13.9–162.4	20.2–181.6	NA
SD25					
Mean	2.7	12.6	29.1	41.1	62.7
CI (lower–upper)	2.3–3.3	11.3–14.0	24.9–34.0	34.4–49.1	52.4–75.1

Note: In the areas SD22 and SD25, predictions and confidence intervals (CI = 95%) of the total number of nematodes within each liver category are represented for a 40-cm cod and the mean hepato-somatic eviscerated index (HSI) in area SD 22 (HSI = 2.7) and area SD25 (HSI = 5.4). In the area SD24, predictions and confidence intervals (CI = 95%) of the total number of nematodes are only based on the four liver category levels as the total length and HSI turned out to be non-significant in the model for this area.

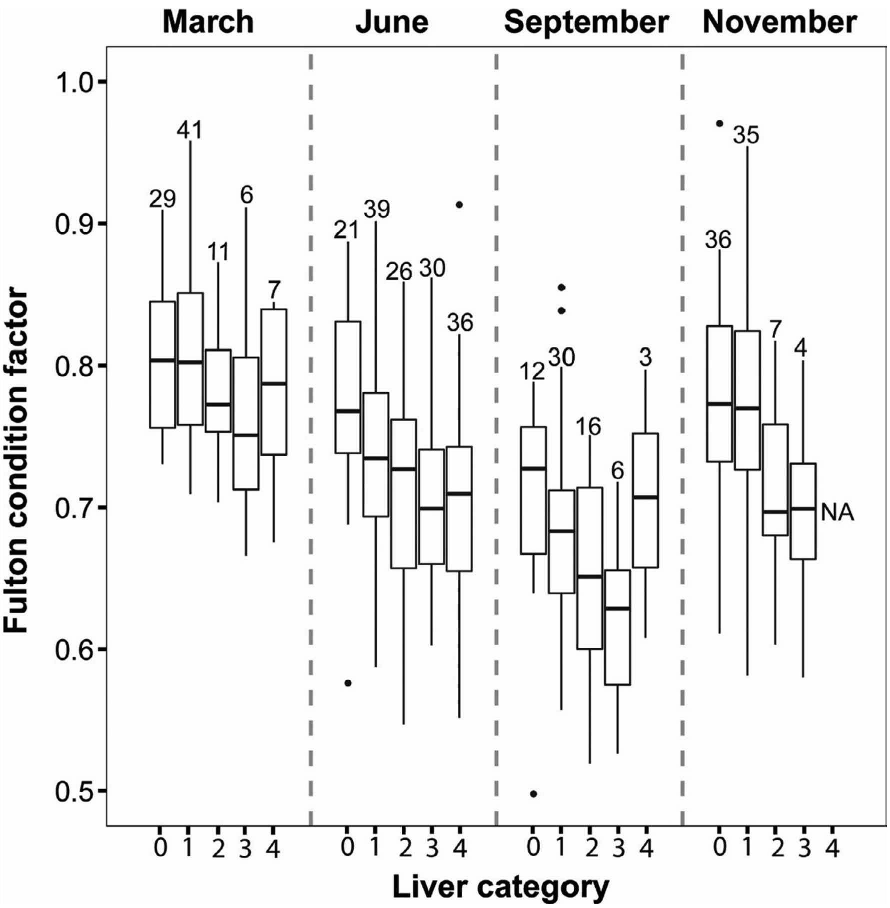

FIGURE 5 Fulton condition factor in relation to the liver category of cod from area SD25 (n = 395) illustrated for four different months. The numbers above each box present the number of cod within the given category. For the box plots, the solid line is the median and the box is the inter-quartile range (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 outliers in data. See Table 2 for details on the total number of livers assigned to each category

area, precluding the model from capturing a potential length effect. It could also be a result of stock mixing in this area (Hüssy et al., 2016). Inter-individual differences in infection patterns in SD24 are linked to the population of origin (Sokolova et al., 2018), and, although speculative, a potential length effect may be balanced out by the mixture of smaller infected eastern Baltic cod and larger, less-infected cod in the western Baltic.

In the present study, the HSI was used in the models to account for the seasonal difference in liver weight in relation to the fish weight. In SD25, fish with low HSI had significantly higher predicted total numbers of nematodes for a given liver category than the fish with high HSI. The opposite was the case in the area SD22, where an increase in HSI resulted in higher predictions of total numbers of nematodes. The reasons for this difference between

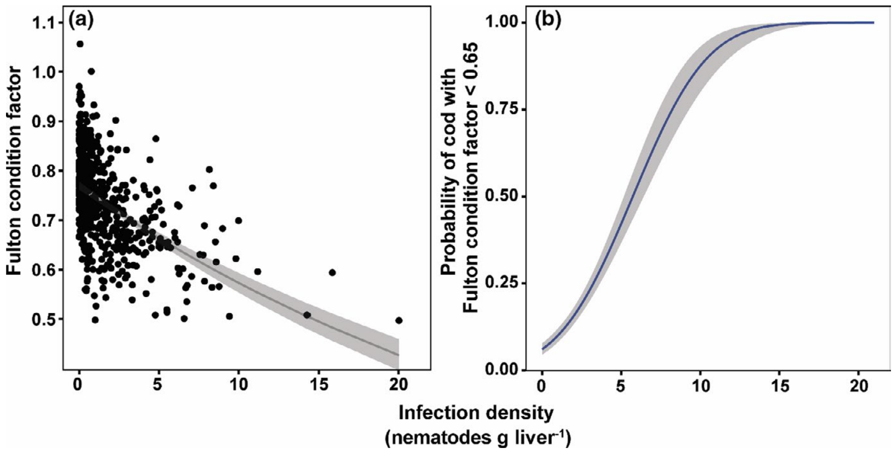

FIGURE 6 (a) Model fit (grey solid line) and uncertainty (grey area) of Fulton condition factor in relation to infection density (IFD) for the 594 eastern Baltic cod sampled between 2016 and 2020 from area SD25 and included in the present study. (b) Mean (blue solid line) and uncertainty (grey area) 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)

the areas remain speculative. High numbers of nematodes (as seen in area SD25) may cause destruction of liver structure and subsequent decrease in organ size. Previous investigations of cod in the area SD25 revealed that high liver parasite burdens cause reduced lipid content of the organ, resulting in reduced HSI (Petrushevsky & Shulman, 1955; Ryberg et al., 2020). Livers in area SD22 are all assigned to the liver category 0 or 1 (i.e. no or low infection load) and a high HSI might leave more nematodes hidden inside the organ.

Fish body condition is a widely used estimator of fish plumpness and, therefore, is a key parameter in fish stock assessments. When a cod reaches the critically low condition factor of 0.65, it is expected to die. Natural mortality for the use in stock assessment of cod has recently been adjusted to take account of the observed low condition (Casini et al., 2016a). Before a lethal low level is reached, the low condition is associated with reduced reproductive potential (Lambert et al., 2000; Mion et al., 2018; Rätz & Lloret, 2003) and slow growth rates (Dutil et al., 1999; Hüssy et al., 2018). The combined effects of the low condition have been suggested as one of the causes of the lack of recovery of the Gulf of St. Lawrence cod stock despite the fishery moratorium in the 1990s (Lambert & Dutil, 2000), thus stressing the importance of fish being in good nutritional health for stock productivity. In the present study, there was a tendency for reduced Fulton condition factor with an increase in the liver category, except for cod with a liver Category 4. The higher condition factor for fish with liver Category 4 may be a result of skipped spawning due to impaired health of fish with high infection loads, and/or reflect increased mortality of highly infected cod with very poor condition, as suggested by Horbowy et al. (2016). Increased IFD was associated with a significant decrease in the Fulton condition factor and an increase in the probability of a condition factor < 0.65, based on data from 594 cod. The relative effects of different ecosystem drivers on the poor state of cod remain uncertain, but the present results show the importance of including the link between IFD and Fulton condition factor in understanding the main factors driving cod stock status.

In conclusion, marine ecosystems are dynamic seascapes in which conditions and biological interactions change constantly over time (Barange et al., 2011). Sustainable ecosystem-based management requires reliable time-series from biological and ecological monitoring for stock assessment of exploited resources such as fish stocks. Fish disease monitoring is in general sparse and mainly provides snapshot data in space and time. A previous effort is the establishment of the Fish Disease Index (Lang & Wosniok, 2008), that has been used to monitor the health status of North Sea dab in relation to impacts of hazardous substances (Lang et al., 2017). *Contracaecum osculatum* has a complex life cycle, relying on several hosts (Køie & Fagerholm, 1995; Nadolna-Ałtyn et al., 2018; Valtonen et al., 1988; Zuo et al., 2018), and its distribution and abundance – and hence interactions – will change through time. *Contracaecum osculatum* burdens may increase in the more westerly cod stocks in future, although it remains unclear if the parasite will thrive in higher salinity water (Sokolova et al., 2018). The assignment of liver categories, combined with estimates of the total number of nematodes, will enable fishery scientists to follow the spatiotemporal development in both prevalence, abundance and IFD of this parasite. Whilst there is still some way to go to estimate actual mortality rates based on IFD, the probabilistic approach presented here may be an important step and may inspire colleagues working with parasites and disease in other fish stocks.

ACKNOWLEDGEMENT

This work was supported by the European Maritime and Fisheries Fund and The Danish Fisheries Agency (33113-B-16-070, 33113-B-16-071 and 33113-B-17-110), and by the European Union's Horizon 2020 research and innovation program under Grant Agreement No. 773713 (PANDORA) and by Director J.P.A. Espersen and Mrs.

Dagny Espersen Foundation. Collections on board RV ALKOR were conducted during the course of the BONUS BIO-C3 project and were supported by BONUS (Art 185), funded jointly by the EU and the German BMBF under grant No. 03F0682. The German sampling was partly co-funded by the European Commission's Data Collection Framework. We thank the scientific, technical and permanent staff of all research and commercial vessels that have contributed to the collection of samples for this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Marie Plambech Ryberg https://orcid.org/0000-0002-4485-9576
Jane W. Behrens https://orcid.org/0000-0002-0136-9681

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How to cite this article: Ryberg, M.P., Huwer, B., Nielsen, A., Dierking, J., Buchmann, K., Sokolova, M., et al. (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, 88–99. https://doi.org/10.1111/fme.12516