Application of the liver category to assess the disease status of Eastern Baltic cod (*Gadus morhua*) infected with parasitic nematode larvae: assessing the robustness of the method and associations between parasite load and nutritional condition of infected fish

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Keywords: liver worm, Contracaecum osculatum, parasites, liver category, infection density, Fulton condition, disease monitoring, Baltic Sea, natural mortality

Abstract

Livers of Eastern Baltic cod (*Gadus morhua*) have become increasingly infected during the latest decade with the parasitic nematode (*Contracaecum osculatum*). Therefor Baltic International Fish Survey Working Group has from 2021 made it mandatory on BITS to assign a 'liver category' between 0 and 4 to individual livers, based on the number of nematodes visible on the surface. Although very useful as a proxy to follow the infections with a high spatiotemporal resolution, the liver category overlook nematodes hidden inside the liver. Because parasite load associates with impaired physiological condition and reduced condition of the infected fish, it is important to know also the total number of parasites. We present a tool for estimation of total number of nematodes in livers based on liver categorical data, and we test the area-specific robustness of the liver category. Data from 642 cod livers from ICES SD22, 24 and 25 were used in the estimation tool, and 594 cod livers from SD25 to examine associations between condition and parasite load. We found that the liver category is a good predictor of total number of nematodes in all three areas, and that the probability of cod having a critical condition increased with parasite load.

Introduction

Assessment of fish stocks is based on a series of input data such as numbers and/or biomass of fish, natural and fishing mortality, growth and recruitment. These data are classically derived from monitoring surveys and used for evaluation of the present status and prediction of the future development of exploited fish stock (Jennings *et al*., 2001). Fish health indicators such as parasite occurrence are usually not part of the routine sampling on monitoring surveys (Lloret *et al.*, 2012), mainly because collection of such data requires expert knowledge, is often time-consuming, cumbersome, difficult to conduct on board, and expensive. One way to overcome this discrepancy is to collect less ambitious but robust proxies of parasite loads on board of standard surveys.

The Eastern Baltic cod (*Gadus morhua*) stock, a key species in the Baltic Sea ecosystem and fisheries, represents an emerging case where information on infections with parasitic nematodes in the liver may assists in improving stock assessment, scientific advice and management. Fish in this stock are in a historically low nutritional condition, have reduced individual growth and low productivity, and natural mortality are in later years estimated more than three times higher than fishing mortality (Eero *et al.*, 2015; Sokolova *et al.*, 2018; Mion *et al.*, 2020). Several ecosystem changes such as deteriorating oxygen conditions and reduced quality and quantity of prey has been suggested to contribute to the poor state of the Eastern Baltic cod (Eero *et al.*, 2012, 2015; Plambech *et al.*, 2013; Casini *et al.*, 2016a; Neuenfeldt *et al.*, 2020). During the latest decade fish in this stock have also experienced a marked increase in infections with liver worm (*Contracaecum osculatum*), a nematode which parasitizes the liver of cod, and almost all larger cod in the central and eastern Baltic are now infected (Haarder *et al.*, 2014; Nadolna and Podolska, 2014; Horbowy *et al.*, 2016). Yet, so far infection levels remain low in the more Westerly stocks (Sokolova *et al.*, 2018). The parasite may negatively affect the condition of the fish as field observations reveal that cod with many parasitic nematodes in the liver have lower body condition than conspecifics with no or few of these parasites (Horbowy *et al*., 2016; Sokolova *et al*., 2018, Ryberg *et al*., in press). Low body condition can lead to increased natural mortality, hereby affecting stock productivity (Dutil and Lambert, 2000; Gislason *et al*., 2010; Casini *et al*., 2016b).

Poland made the first detailed determinations of number of liver worms in individual cod livers (National Marine Fisheries Research Institute) in 2010 (Nadolna and Podolska, 2014), followed by Denmark in 2012 (Haarder *et al*., 2014). However, such investigations where all individual nematodes are identified and counted are complexe, expensive and time-consuming. As a supplement, a more easily accessible way to follow the spatiotemporal development in infection levels in cod, is the so-called 'liver category' method (Figure 1) which has been implemented during recent years on monitoring surveys by some Baltic Sea countries, e.g. the German and Danish Baltic International Trawl Survey (BITS). Using a scale from 0 to 4, the liver category method assigns a category to each inspected liver, depending on the number of visible nematodes on the surface of the organ. This approach requires only basic training of staff 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 BITS protocol for participating countries to assign a liver category to all individual livers (WGBIFS, pers. comm.). Integration of the spatiotemporal development of liver worm infection loads in Baltic cod livers in assessment may thus improve the biological realism of explaining the causes of the deterioration of this stock, and hence improve management advice. Yet, to judge the usefulness of this initiative, the robustness of the liver category method needs to be evaluated.

Although some nematodes are visible on the surface of the liver, several specimens usually reside inside the liver parenchyma and are not visible (Nadolna and Podolska, 2014). Therefore, one obvious limitation of the liver category method is that it likely underestimates the true number of nematodes in the liver, the actual discrepancy being determined by the size and shape of the organ. A more precise estimate of the true number of nematodes in livers will enable us to evaluate potential health effect on the infected individual, as high infection density (i.e. number of nematodes per gram liver tissue) associates with impaired physiological condition of the fish (Ryberg *et al*., in press). It is therefore imperative to develop a tool that predicts the total number of nematodes based on the assigned liver category, and evaluate the area-specific strength of this tool. Using data from the main contemporary distribution areas of Baltic cod (ICES SD22, 24 and 25) (Figure 2), our main objectives were to 1) develop an estimation tool that converts already existing liver categorical data obtained from inspections of cod liver surfaces into estimates of total number of nematodes, 2) assess the area-specific robustness of the liver category method and 3) examine the probability of cod having a critical Fulton condition factor (Fulton's K) below 0.65 depending on infection density.

Material and method

Study areas and fish collection

Altogether 642 cod (*Gadus morhua)* from SD 22 (Kiel Bight and Mecklenburg Bay – western Baltic), SD24 (Arkona Basin) and SD25 (Bornholm Basin) were used in the analysis (Figure 2A, Table 1). 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 capture and transported on ice to the laboratory, and total length, wet weight of whole fish and of livers, gutted weight and gender recorded for each fish. To cover the length distribution commonly caught in monitoring cruises and to assure variability in infection load of fish, individuals ranging from 20-58 cm were included in the study (Table 1).

Analysis of livers for nematodes and parasite identification

To develop an estimation tool for prediction of the total number of nematodes based on liver categories, we established a database containing both liver category and counted total number of nematodes for each individual liver. All individual livers of the 642 sampled cod were assigned a liver category by using the so-called "liver category method" on fresh livers introduced by Thünen Institute in western Baltic areas. Today the liver category covers liver categories 0, 1, 2, 3 and 4 corresponding to counts of 0, 1-10, 11-20, 21-30 and >30 nematodes on the liver surface, respectively (Figure 1, in SD22 and SD24: scale 0-3, in SD25: scale 0-4).

After assignment of liver categories, individual livers were kept at -20 ^{0}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) 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).

Statistical analysis

From liver category to total number of nematodes

A full generalized linear model (GLM) including data from all three areas was defined to test for associations between liver categories and the counted total number of nematodes in livers and to provide estimates for further predictions of the total number of nematodes. In addition, to account for the area effect (i.e. SD22, SD24 and SD25), one model was defined for each area, resulting in another three separate models. In all four models, the counted number of nematodes was defined as the response variable (Eq. 1), which followed a negative binomial distribution where variance increases quadratically with the mean (Eq. 2). A log link function was defined by default in the model (Hardin *et al.*, 2007). The mean Y_i was independent with μ and over dispersion parameter θ > 0. This implied that the variance of the i'th observation becomes μ(1+μ/θ). A poisson distribution was tested, but this could not account for the variance in the data sets.

$$Y_i = nematodes \quad (1)$$

$$Y_i \sim NBin(\mu_i, \theta) \quad (2)$$

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 seasonal changes in the size of the livers, a hepatosomatic index (HSI) was calculated (Eq. 3) and also included as an explanatory variable in the model (Lambert and Dutil, 1997).

$$HSI = \frac{LW}{GW} * 100 \quad (3)$$

Where LW represents liver wet weight and GW represents gutted 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. Two-order interactions between TL and liver category as well as HSI and liver category were included in the full models to examine any differences of the effect of TL and HSI within each liver category (Eq. 4).

$$Log(Y_i) = \alpha(liver\ category_i) * HSI_i + \beta(liver\ category_i) * \mathrm{length_i} \quad (4)$$

However, due to data limitations for SD22 (dominance of category 0 livers), it was not possible to test any two-order interactions in the full model for this area. In this respect it is noteworthy 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, but 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 the statistical program 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 by using variance inflation factors (VIF) (Zuur *et al.*, 2009). No variables were excluded from the analysis due to collinearity (Table S1). Model selection was performed using a stepwise backward selection routine based on a likelihood ratio test for each of the variable included and excluded in the models. Extraction of residuals for model validation of each final model was done by using one-step predictions 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 (Figures S1-S4).

Fulton's K

To examine associations between Fulton's K and infections with liver worm in cod, two different analyses were performed on data from SD25. Calculation of Fulton's K was based on gutted weight (Eq. 5):

$$Fulton's\ K = \frac{GW}{(TL)^3} * 100 \qquad (5)$$

In the first case, we tested for significant difference of Fulton's K within the five levels of the liver category for each month represented in data. This was done by using Anova and a post hoc analysis (Tukey HSD). In the second case we included more own data (N=594) on total number of nematodes in livers from cod in SD25 (Table S2) in order to examine the association between Fulton's K and infection density and to estimate the probability of cod with critical Fulton's K (i.e. below 0.65) in relation to infection density. The critical Fulton's K reflects the level where cod are considered dying (Dutil and Lambert, 2000; Casini *et al.*, 2016b). The association between Fulton's K and infection density was examined by an exponential model (Eq. 6):

$$\log(\text{Fulton's } \text{K}_\text{i}) \sim N(\alpha * infection\ density_i, \sigma^2) \qquad (6)$$

The probability can be calculated as a tail probability directly from the model in equation 6 from each infection density. A parametric bootstrap was used to propagate the uncertainty from the estimates of α and σ to the probability estimates.

Results

Number and species of nematodes in analysed livers

A total of 11352 nematodes were recovered from the 642 livers examined, 32, 1487 and 9833 nematodes from SD22, SD24 and SD25, respectively. In SD22, 9 out of the 32 parasitic nematodes belonged to two other species of nematodes: herring or whale worm, *Anisakis simplex* (n=8) and seal worm or cod worm, *Pseudoterranova decipiens* (n=1). In SD24, 4 out of 789 parasitic nematodes were identified as *A. simplex* and the remaining as *C. osculatum*. In SD25, only *C. osculatum* was identified. Given the low contribution of species other than *C. osculatum*, the counted total number of nematodes used in the statistical analysis included all counted nematodes irrespectively of the species of nematodes.

Observed liver categories and counted number of nematodes

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 2B and 3, Table 2). Irrespectively of area, variance in counted number of nematodes within a liver category was highest in the highest liver category (Figure 3). When combining data from the three areas, the variance of counted number of nematodes within each liver category increased (Figure 3D). In area SD22 most livers were assigned liver category 0 and no livers were assigned to liver category 2 and 3 (Figure 2B, 3A and Table 2), while in area SD24 most livers were assigned to both liver category 0 and 1 and a few to liver category 2 and 3 (Figure 2B, 3B and Table 2). In area SD25, all five liver categories were present (Figure 2B, 3C and Table 2).

The estimation tool

Associations between liver categories and estimated number of nematodes, HSI and fish length

The model output of the final model for all data combined revealed that there was a significant increase in the estimated number of nematodes with increasing liver category (p=0.009, Table 3 and S3). Furthermore, the estimated 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 of the estimated number of nematodes ($p<0.001$, Table 3). There was no effect of HSI on the estimated number of nematodes (Table S3).

Area-specific patterns in estimated number of nematodes

In all three area models, the estimated number of nematodes increased significantly with increasing liver category ($p<0.001$, Table 5). In SD22, the estimated number of nematodes increased significantly with TL ($\beta=0.094$, $p=0.04$) and HSI ($\gamma=0.331$, $p=0.04$) (Table S3 and 5), whereas there was no effect of HSI and TL on estimation of nematodes in SD24 (Table S3). In SD25, estimation of the number of nematodes increased with TL ($\beta=0.049$, $p<0.001$) but decreased with increased HSI ($\gamma=-0.043$, $p=0.004$) (Table S3 and 5, Figure 4). 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 4). Model reduction of the three area models revealed that no interactions between liver category and HSI and liver category and TL were significant for the estimation of nematodes (Table S3).

Robustness of the liver category and estimation tool: liver categories and predicted number of nematodes

To evaluate the robustness of the three areas models, and to assess the strength of the estimation tool, predicted number of nematodes and confidence intervals (0.95) of the three area models were extracted (Table 6). Due to significance of TL and HSI in for SD22 and SD25, predictions were based on a cod of 40 cm length with the mean HSI of 2.7 for SD22 and 5.5 for SD25 (Table 6).

Predicted number of nematodes for area SD24 were calculated for all sizes and values of HSI, due to non-significance of the variables in this area (Table S2 and 5). Comparing predicted number of nematodes between the three areas revealed that predicted numbers within each liver category were highest in SD25 in all categories except for category 0, where the predicted number was highest in SD24 (Table 6). Accuracy of the three models decreased with increasing liver category as the confidence intervals became broader with each liver category level (Table 6).

Fulton's K

For all months, there was a tendency for Fulton's K to decrease (the decrease was only significant for June, $p=0.008$) with increasing liver category, except for liver category 4. However, Fulton's K decreased significantly with increase in infection density ($p<0.001$, $\alpha=\exp(-0.029)$, $sd=0.002$, $intercept=\exp(-0.262)$). The subsequent probability model revealed a sigmoid pattern between infection density and the probability of the fish having a critical Fulton's K, e.g. fish with an infection densify of 6 had a probability of having a critical Fulton's K of 50%, whereas fish with infection densities of 10 almost had 100% probability of having a critical Fulton's K (Figure 6).

Discussion

We here verify the robustness of the liver category method by showing that estimates of total number of nematodes increase significantly with increasing liver category. Thus, collection of liver categorical data during monitoring surveys provides an inexpensive and easy way to obtain pan-Baltic information on spatiotemporal changes in infection load with cods liver worm. However, we also show that the explanatory variables (i.e. TL and HSI) for estimation of nematodes differed between areas, implying the need to include a spatial component. In addition, we provide an estimation tool to predict total number of nematodes based on assigned liver category. From this, infection densities can be obtained, describing the severity of infections, which is known to relate to the health status of the infected individual. Finally, we illustrate how infection densities are related to the probability of cod having a critical Fulton's K.

The estimation tool: One size does not fit all

To simplify things, we initially set out to make one model for the estimation tool, covering SD22, 24 and 25. However, the effects of length and HSI on the estimations of the total number of nematodes differed substantially between the areas. Furthermore, the number of livers assigned to the different categories varied markedly from West to East, as also seen in a recent detailed investigation where a West-East gradient was found in prevalence and abundance of infection with *C. osculatum* in cod livers (Sokolova *et al.*, 2018). Therefore, the spatial component (i.e. a model for each of the three areas) had to be included to improve the strength of our estimations and predictions. Notably, the area-based models will enable identification of present and emerging areas with high infection densities, thus providing a spatial component to the description of future disease dynamics in cod.

How biological parameters affect estimations of total number of nematodes

Previous detailed investigations of cod in SD25 have shown that total number of nematodes in individual livers increase 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 SD22 and SD25 (Figure 5). The lack of significance of TL on estimated number of nematodes in SD24 may be a result of the narrow length range (35-50 cm) of the cod sampled for the analysis within this area, disenabling the model to capture a potential length effect, and/or be a result of stock mixing in this area (Hüssy *et al.*, 2016). Inter-individual differences in infection patterns in SD24 is 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 EBC cod and larger, less infected Western Baltic cod.

We chose to use HSI in the models to account for the seasonal difference in liver weight in relation to fish weight. In SD25, fish with low HSI had significantly higher predicted total number of nematodes for a given liver category than fish with high HSI (Figure 5). The opposite was the case in SD22, where increase in HSI resulted in higher predictions of total number of nematodes. The reason(s) for this difference between areas remains speculative, but maybe high numbers of nematodes (as seen in SD25) may cause destruction of liver structure and subsequent decrease in organ size. Previous detailed investigations of cod in SD25 have revealed that high liver parasite burdens cause reduced lipid content of the organ, resulting in reduced HSI (Petrushevsky and Shulman, 1955; Ryberg *et al.*, in press). On the contrary, livers in SD22 are only assigned to category 0 or 1 (i.e. no or low infection load) and a high HSI might leave more nematodes hidden inside the organ.

Cods liver worm, Fulton's K and natural mortality

Fish body condition is a key parameter in the dynamics of fish stocks. When cod reach a critically low Fulton's k of 0.65 it is expected to die, and natural mortality for use in stock assessment of Eastern Baltic cod has recently been adjusted for the observed low condition (Dutil and Lambert, 2000; Casini *et al.*, 2016b). Before a lethal low level is reached, low condition associates with reduced reproductive potential (Lambert *et al.*, 2000; Rätz and Lloret, 2003; Mion *et al.*, 2018)

316 and slow growth rates (Dutil *et al.*, 1999; Hüssy *et al.*, 2018). The combined effects of low condition has 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 and Dutil, 2000), thus stressing the importance of fish being in a good nutritional health for stock productivity. In the present study, there was a tendency for reduced Fulton's K with increase in liver category, except for cod with a liver category 4. The higher Fulton's K 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). Notably also, based on data from 594 cod, we found a significant decrease in Fulton's K and increase in the probability of cod with Fulton's K below 0.65 with increased infection density. Considering that the relative importance of the different ecosystem drivers on the poor state of the Eastern Baltic cod still remains uncertain, our results reveals the importance of including the link between infection density and Fulton's K when promoting the understanding for the main factors driving the cod stock status.

Limitations of the estimation tool

Although the categorization of cod livers now applied on board BITS surveys, in combination with the estimation tool provided here, undoubtedly will enable an essential step forward towards future assessments of cod liver parasite load, it also has a major shortcoming. While a liver contains a definite number of worms in its three-dimensional structure (unknown at the moment of liver categorization), the thoroughness and experience of the individual technicians looking at the liver surface to assign a category may well differ between persons. Furthermore, there may be an increasing uncertainty in assignment of liver category with increase in the number of nematodes on the surface of the liver. This call for careful intercalibration and training of technicians to minimize this error source.

Perspectives

Marine ecosystems are like dynamic landscapes where conditions and biological interactions change constantly over time (Barange *et al.*, 2011), and one way to ensure sustainable ecosystem-based management is to integrate solid time-series data from biological and ecological monitoring into stock assessment and management of exploited resources such as fish stocks. Fish disease monitoring is in general sparse, and mainly provides snapshot data in space and time. One previous effort is the establishment of the Fish Disease Index (FDI) (Lang and Wosniok, 2008), that e.g. has been used to monitor the health status of North Sea dab in relation to impacts of hazardous substances (Lang *et al.*, 2018). The liver worm has a complex life cycle, relying on several hosts (Valtonen *et al*., 1988; Zuo *et al.*, 2016; Nadolna *et al*., 2018), and their distribution and abundance – and hence interactions – will change through time. With this liver worm burdens may increase also in the more Westerly cod stocks, although it remains unclear if the parasite will thrive in these high salinity waters (Sokolova *et al*., 2018). The causality between infection load and the health status of the infected individual is still not fully understood (Ryberg et al*.,* press). Though, the assignment of liver categories, combined with the present estimation tool, will enable fishery scientist to follow the spatiotemporal development in both prevalence, abundance and infection density of this parasite. In addition, while there is still some way to go to actually estimate mortality rates based on infection density, we consider the probabilistic approach presented here has an important step ahead that hopefully also will inspire colleagues working with disease in other fish stocks.

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Author contributions

AN participated in the statistical analysis; BH participated in data collection and structuring and co-writing of manuscript; JD participated in data collection and input to the statistical analysis; JWB participated in structuring and co-writing of manuscript; KB supervised in species identification of the nematodes; MS participated in data collection and analysis of livers; MPR was responsible and carried out most analyses of livers, in charge of the liver data base and carried out the statistical analysis and led manuscript writing; UK participated in data collection and input to the statistical analysis; all authors edited the manuscript and approved of the final version of the manuscript.

Data availability statement:

The data that support our findings and the R code used for the analysis are available at https://github.com/mpla86/Liverworm.

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496 Figure 1. Photo of the five liver categories (0-4) of nematode infection levels of cod livers used in
497 the Baltic Sea. A liver category scale is assigned according the number of nematodes counted on
498 the surface of the liver and the categorical boundaries given by the scale. Photo by Bastian Huwer.

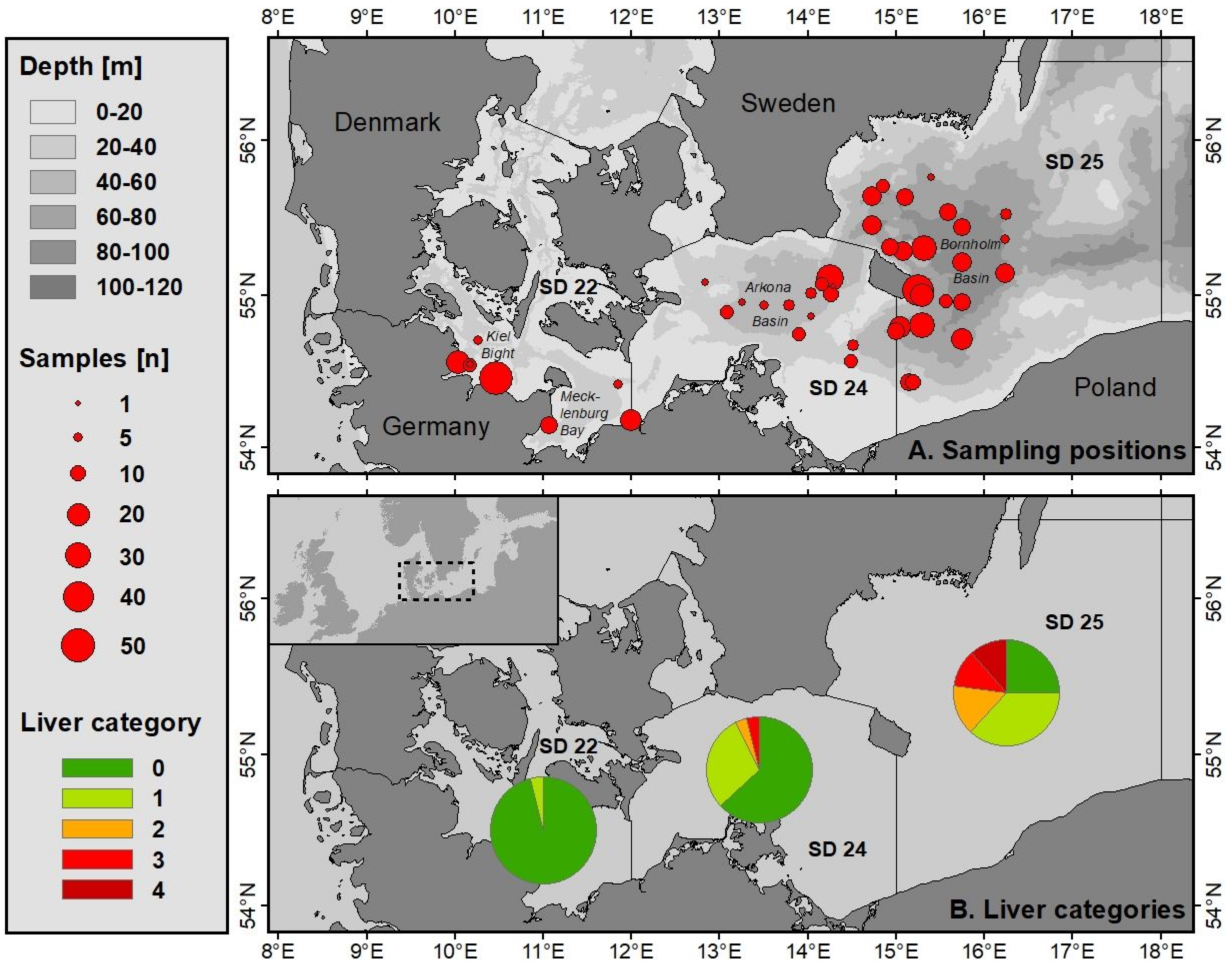


Figure 2: A) Study area and sampling positions within the three ICES subdivisions where cod *Gadus morhua* were collected for analysis of liver nematodes. Grey scale provides information of water depth and size of the bubbles represents number of samples examined from each position. B) Percentage of the total number of livers assigned to each liver category by subdivision.

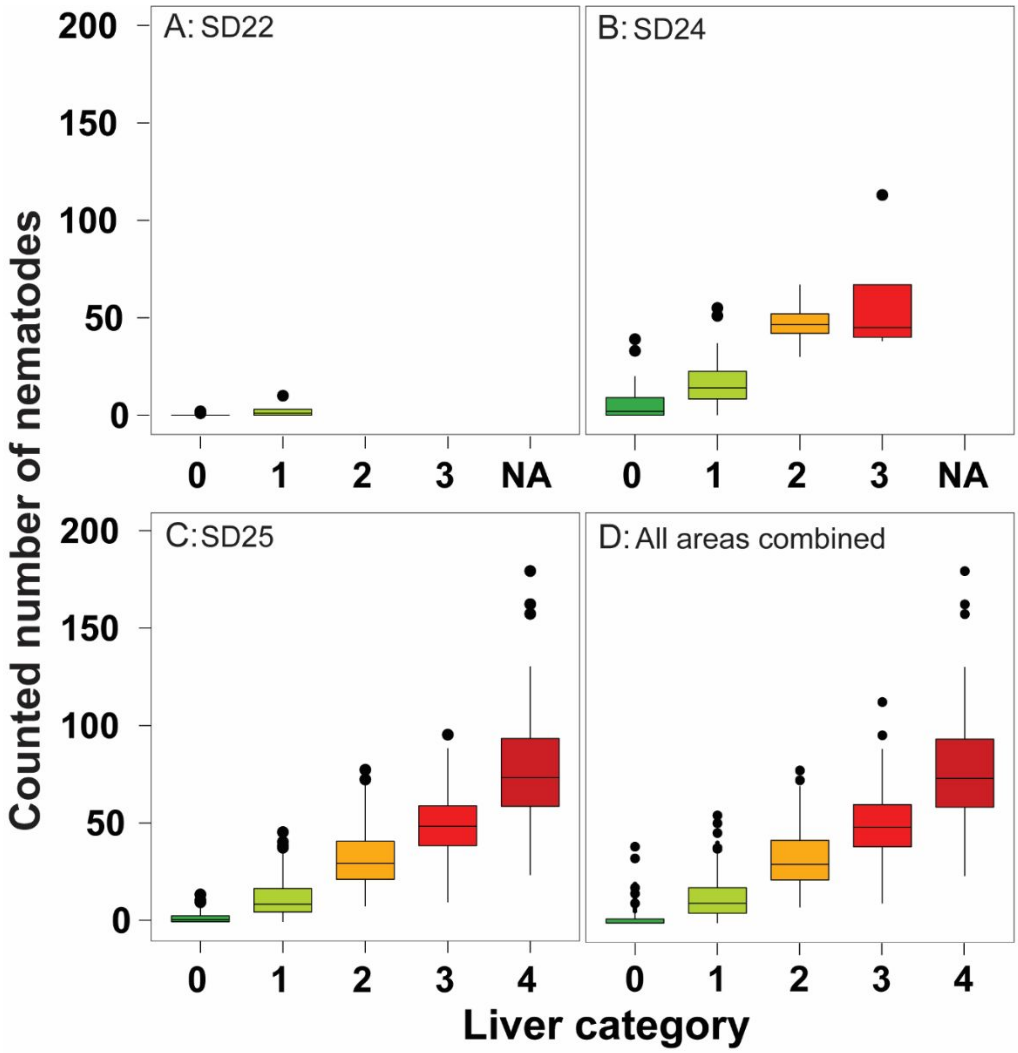


Figure 3. Relationship between the assigned liver categories and the counted total number of nematodes inside the livers of Baltic cod sampled in ICES SD22 (A), SD24 (B), SD25 (C) and all areas combined (D). See Figure 1 for visual appearance and a detailed description of the five liver categories. NA in panel A and B illustrates that to date this category has not been used in SD22 and SD24. For the box plots, the solid line is the median, 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. See table 2 for details on the total number of livers assigned to each category.

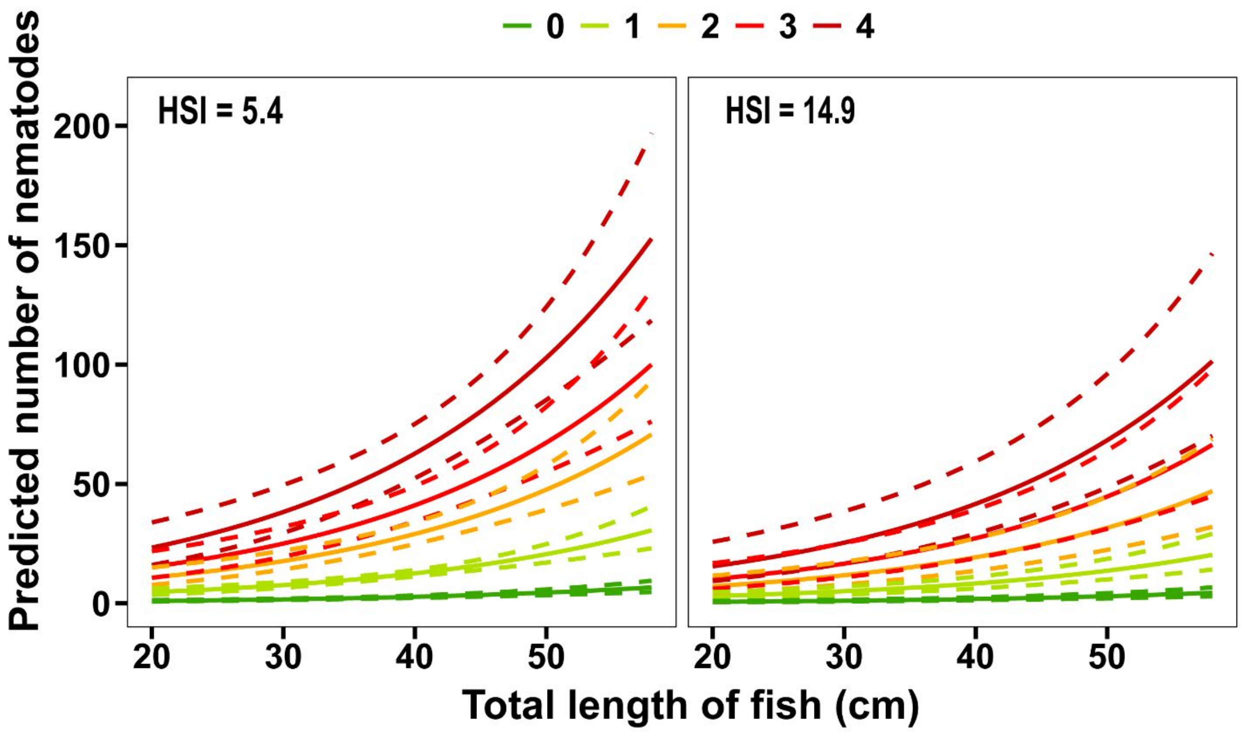
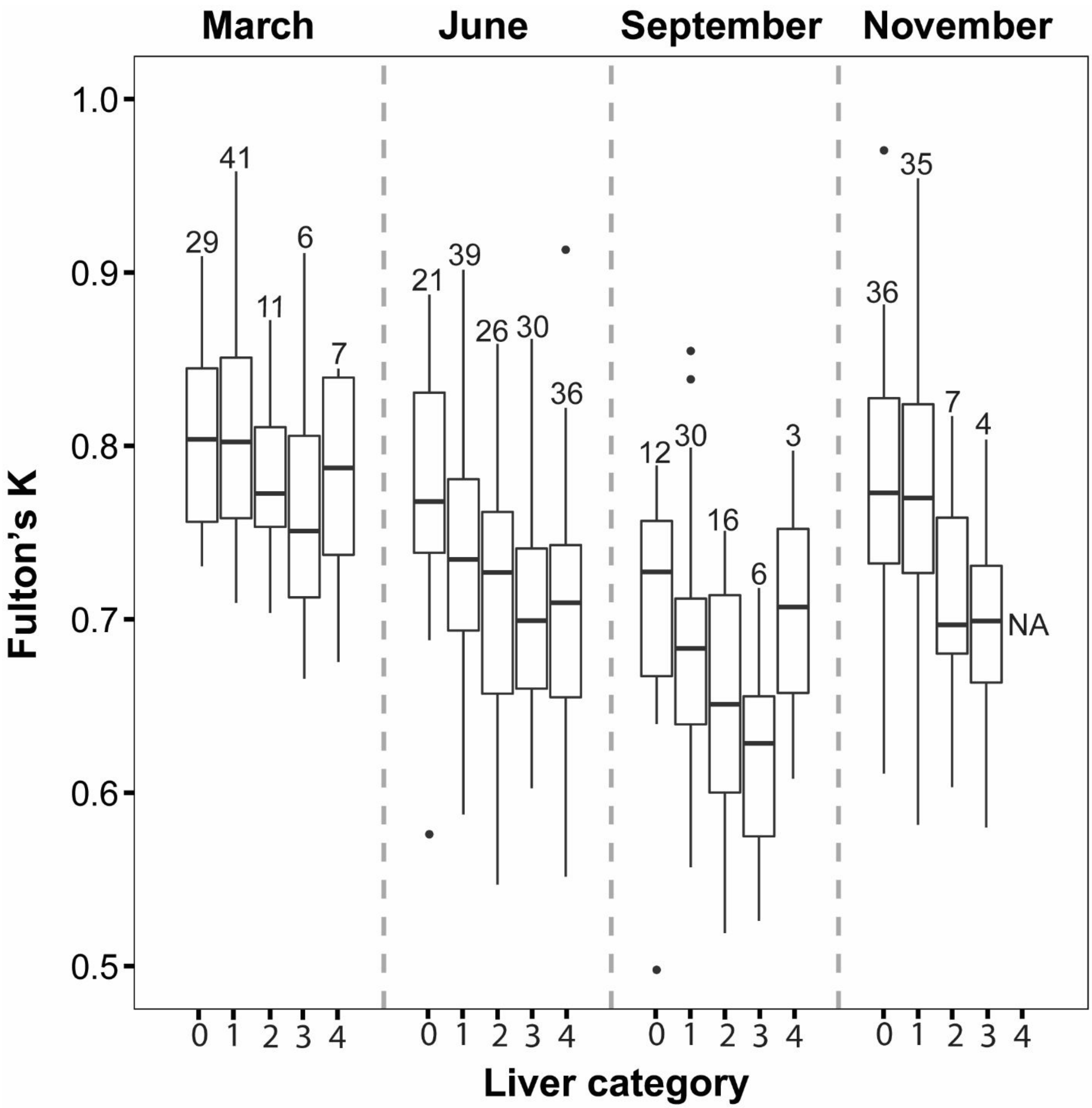
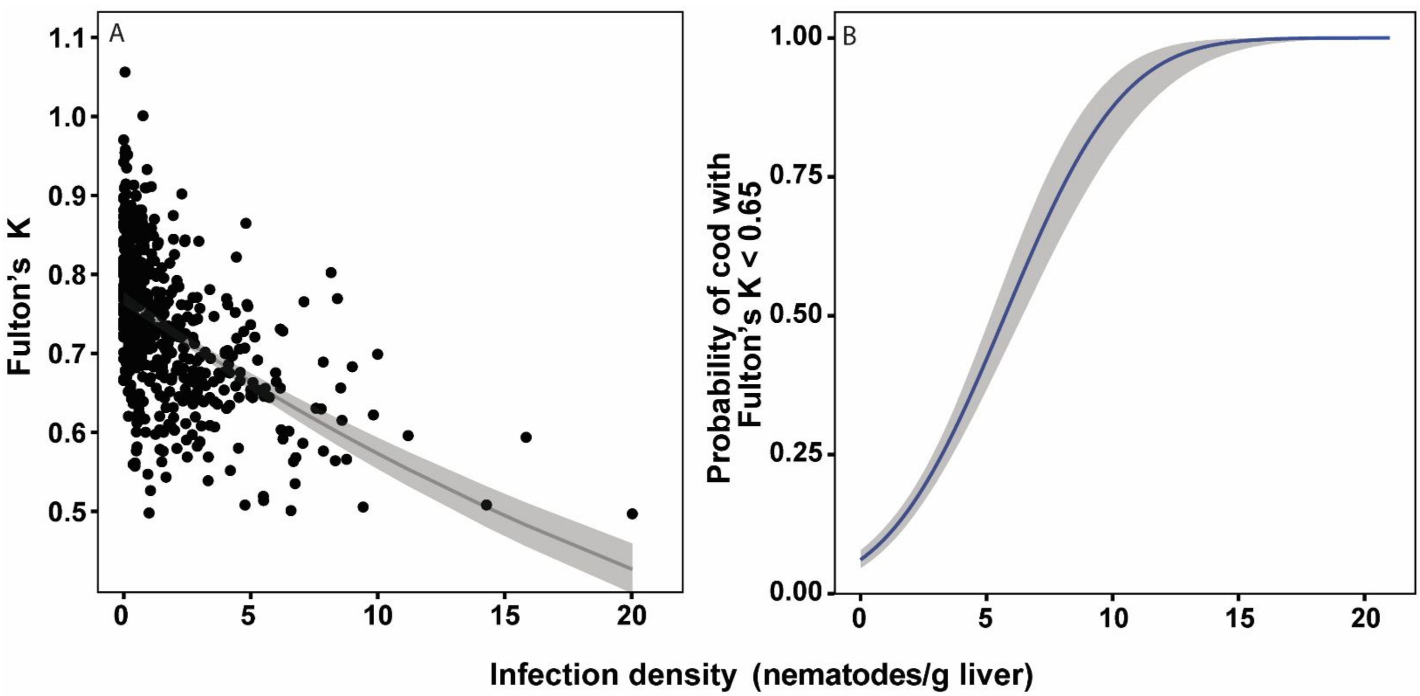


Figure 4. Predictions of the total number of nematodes in livers of Baltic cod from area SD25 derived from the final GLM model for different cod sizes with mean HSI index = 5.4 (A) and the highest observed HSI index = 14.9 (B). Colours represent the five liver categories 0-4. See Figure 1 for visual 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.



530 Figure 5 Fulton's K in relation to liver category of cod from SD25 (N=395) illustrated for four different months. Numbers above each box present number of cod within the given category. For the box plots, the solid line is the median, 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. See table 2 for details on the total number of livers assigned to each category.



536 6. A) Model fit (grey solid line) and uncertainty (grey area) of Fulton's K in relation to infection 537 density for the 594 Eastern Baltic cod sampled between 2016-2020 from SD25 included in the 538 present study. B) Mean (blue solid line) and uncertainty (grey area) of the probability for cod 539 having Fulton's K below 0.65 (i.e. critical Fulton's K) for different levels of infection density. The 540 probability model fit and the uncertainty are calculated based on the predictive output of the model 541 shown in panel A. The level of the critical Fulton's K where cod are considered dying is defined 542 in Casini *et al.*, 2016b based on findings of Dutil and Lambert, 2000.

Table 1. Overview of 642 cod sampled between 2017-2020 from ICES subdivisions SD22, SD24 and SD25 for the analysis of infections with liver nematodes (see also Fig. 2 for sampling positions and numbers). Values are mean ± SE and brackets represent range of data.

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**	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)

TL= total length; W= total wet weight; GW=gutted wet weight; LW= wet-weight of liver; HSI = hepatosomatic index; Prevalence: percentage of infected fish in the sample; Intensity of infection: mean number of counted parasites per fish, only including infected individuals. *GNS= gillnet set, OTB= otter trawl bottom, GTR= trammel net (gillnet consisting of three layers of net); ** scientific otter trawl, operated at the sea floor, similar to OTB

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

Table 3. Estimates of significant parameters and standard errors (SE) of the final model describing how the total number of nematodes changes within each category and with length. Wald Z provides the statistical result of each variable and factor level and p values below 0.05 are considered significant. Numbers are on log scale.

Parameter	Estimate	SE	Wald Z	p-value
Intercept	**α(category)**			**0.009**
0	3.137	0.546	5.750	
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 w.r.t length	**β(category)**			**<0.001**
0	-0.060	0.014	-4.365	
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	

Table 5. 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). Wald Z provides the statistical result of each variable and factor level and p values below 0.05 are considered significant. Numbers are on log scale.

Area	Parameter	Estimate	SE	Wald Z	p-value
SD22	**Intercept**	**α(category)**			**<0.001**
	0	-7.347	2.574	-2.854	
	1	-3.897	2.458	-1.585	
	Slope w.r.t. length	**β**			**0.04**
		0.094	0.054	1.726	
	Slope w.r.t. HSI	**γ**			**0.04**
		0.331	0.162	2.076	
SD24	**Intercept**	**α(category)**			**<0.001**
	0	1.756	0.152	11.559	
	1	2.831	0.213	13.267	
	2	3.861	0.615	6.282	
	3	4.104	0.549	7.478	
SD25	**Intercept**	**α(category)**			**<0.001**
	0	-0.731	0.264	-2.768	
	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 w.r.t. length	**β**			**<0.001**
		0.049	0.007	7.235	
	Slope w.r.t. HSI	**γ**			**0.004**
		-0.043	0.015	-2.880	

Table 6. Mean predicted numbers of nematodes based derived from the estimation tool for the three different areas. In 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 average HSI in area SD 22 (HSI = 2.7) and area SD25 (HSI=5.4). In SD24 predictions and confidence intervals (CI = 95%) of total number of nematodes are only based on the four liver category levels as length and HSI turned out to be non-significant in the model for this 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