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Published in: Conservation Physiology

Link to article, DOI: 10.1093/conphys/coaa093

Publication date: 2020

Document Version Peer reviewed version


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<th>Conservation Physiology</th>
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<td>Manuscript ID</td>
<td>CONPHYS-2020-087.R1</td>
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<tr>
<td>Manuscript Type:</td>
<td>Research Article</td>
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<td>Date Submitted by the Author:</td>
<td>06-Aug-2020</td>
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</table>
| Complete List of Authors: | Ryberg, Marie; Technical University of Denmark, National Institute of Aquatic Resources, DTU Aqua  
                             Skov, Peter; Technical University of Denmark, National Institute of Aquatic Resources, DTU Aqua  
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| Keywords:     | Compromised liver function, liver worm, parasites, energetic cost, nutritional condition, Eastern Baltic cod |
Title
Physiological condition of Eastern Baltic cod, Gadus morhua, infected with the parasitic nematode Contracaecum osculatum

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Lay summary
Nutritional condition, energy turnover, body, liver and plasma composition and digestive organ masses were evaluated in relation to varying infection densities with liver worm in Eastern Baltic cod. We show that fish with high infection load with this parasitic nematode have severely compromised physiological condition and poor health status.

Total word count
5571 words
Abstract

Establishing relationships between parasite infection and physiological condition of the host can be difficult and therefore are often neglected when describing factors causing population declines. Using the parasite-host system between the parasitic nematode *Contracaecum osculatum* and the Eastern Baltic cod *Gadus morhua* we here shed new light on how parasite load may relate to the physiological condition of a transport host. The Eastern Baltic cod is in distress, with declining nutritional conditions, disappearance of the larger fish, high natural mortality and no signs of recovery of the population. During the latest decade, high infection levels with *C. osculatum* have been observed in fish in the central and southern parts of the Baltic Sea. We investigated aerobic performance, nutritional condition, organ masses, and plasma and proximate body composition of wild naturally infected *G. Morhua* in relation to infection density with *C. osculatum*. Fish with high infection densities of *C. osculatum* had i) decreased nutritional condition, ii) depressed energy turnover as evidenced by reduced standard metabolic rate, iii) reduction in digestive organ masses, alongside iv) changes in the plasma, body and liver composition, and fish energy source. The significantly reduced albumin to globulin ratio in highly infected *G. morhua* suggests that the fish suffer from a chronic liver disease. Furthermore, fish with high infection loads had the lowest Fultons condition factor. Yet, it remains unknown whether our results stem from a direct effect of *C. osculatum*, or because *G. morhua* in an already compromised nutritional state are more susceptible towards the parasite. Nevertheless, impairment of the physiological condition can lead to reduced swimming performance, compromising foraging success while augmenting risk of predation, potentially leading to increase in natural mortality of the host. We hence argue that fish-parasite interactions must not be neglected when implementing and refining strategies to rebuild deteriorating populations.
Introduction

Parasitism is one of the most common animal lifestyles and can impact ecosystem functioning by affecting food-web stability, interaction strength and energy flow in both terrestrial and aquatic ecosystems (Marcogliese, 2004; Kuris et al., 2008; Lafferty et al., 2008; Hatcher et al., 2014). At the level of the individual, parasites can cause adverse effects on performance capacity of the host (McElroy and de Buron, 2014), e.g. by changing plasma protein and hormone levels (Akinyi et al., 2019; O’Dwyer et al., 2019), reducing aerobic and locomotor performances (Umberger et al., 2013; Hahn et al., 2018) and depleting energy reserves (Ferrer-Maza et al., 2016). Together, this shapes the physiological condition of an infected individual, and impairment may lead to reduced growth and increased mortality (Marcogliese, 2004; Khan, 2005; Behrens et al., 2014). For trophically transmitted parasites, such effects on transport hosts may make them more vulnerable to predators, increasing the probability of the parasite to reach its final host (Gabagambi et al., 2019).

Marine fish are hosts to a high diversity of parasitic organisms (Marcogliese, 2002; Rohde, 2002), and parasite-induced impairment of the physiological condition has been suggested to reduce fish stock productivity, leading to declining catches of both freshwater and marine fish populations (Lloret et al., 2012). However, establishing causality between parasite infection and physiological condition of the host can be difficult, and the mechanisms underlying parasite-altered host fitness remains largely unknown (Lloret et al., 2012; McElroy and de Buron, 2014).

Here, we use the host-parasite system between the third stage larvae liver worm Contracaecum osculatum (Zuo et al., 2018) and Eastern Baltic cod Gadus morhua as a case study to investigate the physiological performance of wild fish with high parasite load. The Eastern Baltic cod stock has exhibited a decline in nutritional condition during the past 20 years, an event that has occurred alongside deteriorating oxygen conditions and reduced prey
abundance, now leaving the fish historically malnourished and growth impaired (Eero et al., 2015; Casini et al., 2016b; Hüsy et al., 2018; Neuenfeldt et al., 2020). This has challenged management of the stock which at present show no signs of recovery, and with high natural mortality (ICES, 2019) and a fishing ban introduced in 2019. The grey seal Halichoerus grypus population has been severely reduced due to hunting and breeding problems between the 1960s and the 1990s where after recovery slowly began (Harding et al., 2007). Concurrent with the recovery of H. grypus, an increase in infections with the trophically transmitted C. osculatum that parasitizes the liver of cod has also been observed in G. morhua in the central and eastern Baltic Sea since the early 2010s, (Haarder et al., 2014; Nadolna and Podolska, 2014). This has coincided with even further deterioration of the health status and stock productivity of the fish (Eero et al., 2012, 2015). H. grypus is the main final host of this parasite while cod act as the last transport host in the life cycle (Koie and Fagerholm, 1995; Nadolna-Altyń et al., 2018; Zuo et al., 2018).

Field investigations have shown that infection intensity with C. osculatum in G. morhua coincides with poor nutritional status, and that more Westerly and Northwesterly cod stocks with little or no C. osculatum are in better nutritional condition (Horbowy et al., 2016; Sokolova et al., 2018). This parasite migrates to the liver of the cod following ingestion via smaller infected prey, e.g. sprat Sprattus sprattus (Zuo et al., 2016; Nadolna-Altyń et al., 2018), where it accumulates over time, resulting in a larger parasite burden in older fish (Horbowy et al., 2016; Zuo et al., 2016). The liver is responsible for nutrient assimilation, bile production, maintenance of metabolic homeostasis, protein synthesis, and also serves as an energy reserve and breeding capital for the fish (Hinton et al., 2017). It is thus intuitive to think that a high liver parasite burden leads to reduced function of the organ with negative effects on the nutritional condition of the infected individual. Yet, disentangling potential effects of parasites on their hosts from effects arising in the wake of unfavorable abiotic and food conditions
demands an interdisciplinary approach combining field and laboratory studies, and expert parasitologists, physiologists, and biologists (McElroy and de Buron, 2014).

To elucidate how high parasite load may relate to the physiological condition of wild fish, we here investigated aerobic performance, nutritional condition, mass of selected organs and plasma and proximate body composition of wild naturally infected *G. Morhua* in relation to infection density with *C. osculatum*.

**Material and methods**

**Pilot study**

The number of *C. osculatum* in cod livers increases with the length of the fish (Nadolna and Podolska, 2014; Horbowy et al., 2016). In 2017 we therefore conducted a pilot study to identify the length interval of *G. morhua* needed to obtain fish samples with sufficient variability in infection intensity to make a solid study design. More specifically, wild and naturally infected *G. morhua* (n=86) were captured by trawl East of Bornholm and used to assess the correlations between body mass (BM), total length (TL), liver mass (LM), gender and number of nematodes in the liver. A length range between 30-53 cm provided sufficient high variability in infection intensity of nematodes in the livers of the fish. Fish for experiments (see below) were consequently selected to cover this length range if possible.

**Experimental animals**

Wild naturally infected Eastern Baltic cod (n=152) were captured by trawl East of Bornholm between 2017 and 2019 (Table 1). Cod were either used live for respirometry or sampling was made directly on dead fish for investigations of body composition and organ sizes. For the latter investigations fish were killed and frozen at -20 °C immediately after trawling, and transported to DTU Aqua, Lyngby, for later analysis (Table 1). For fish used live in experiments (Table 1), trawling time never exceeded 20 minutes in order to minimize stress
and damage to the fish. Live cod were acclimated for 2 weeks in captivity at Bornholm’s 
Salmon Hatchery before being transferred to the fish holding facilities at DTU Aqua, Lyngby. 
Here they were held in two circular tanks (2000L each) with air-saturated recirculated water 
(10°C, 10‰ salinity, photoperiod of 8:16 light: dark, with a half hour period of sunrise and 
sunset), and allowed 3 weeks of acclimation before initiation of experiments. Fish were fed 
three times weekly with cooked blue mussels corresponding to ~2% of their BM. All 
experiments were carried out according to the animal welfare regulations of the Technical 
University of Denmark and EU directive 2010/63/EU for animal experiments. Ethical permit 
2017-15-0201-01282 from the Danish Animal Ethics Committee covered all experiments 
reported here.

Table 1

Recovery of nematodes from cod livers

All livers from fish used in the present study were dissected out and frozen separately (-20°C) 
before they were analysed for presence of nematodes. Individual livers, except those used for 
lipid and energy analysis, were placed in a plastic bag (200 × 400 × 0.07mm) and compressed 
between two glass plates (15 × 15 × 1cm) to a thickness of 1 mm by the addition of gentle 
pressure to the plates (Buchmann, 2007). Livers were subsequently examined under a Leica 
stereo microscope (6.3-40× magnification) (Leica Microsystems Germany) and detected 
nematodes were categorized as either small (<1cm body length) or large (>1cm body length). 
To minimize oxidation processes and tissue breakdown in livers dedicated to lipid analysis, 
individual defrosted livers (n=33) were placed on glass petri dishes kept on ice. Single 
nematodes were manually removed, using a tweezer, and the total number for each liver 
recorded and subsequently preserved in 70% ethanol. For all examined livers, nematode
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150 species identification was based on morphometric characteristics of the caudal and cephalic 
151 ends according to Fagerholm (Koie and Fagerholm, 1995). To compensate for differences in 
152 the number of nematodes related to liver size, infection density was calculated as number of 
153 nematodes per gram of liver tissue (i.e. liver tissue = wet weight of liver minus total weight of 
154 nematodes), rather than total number of nematodes per liver.

155

156 Nutritional condition

157 The association between infection density with nematodes and the nutritional condition of all 
158 cod included in this study (n=152) was analysed by calculating Fulton condition factor:

159 \[ \text{Fulton condition factor} = \left( \frac{BM}{TL^3} \right) \times 100 \] .

160

161 Aerobic performance

162 To investigate potential associations between infections and aerobic performance of the fish, 
163 we determined standard metabolic rate, maximum metabolic rate and aerobic metabolic scope 
164 (i.e. maximum metabolic rate minus standard metabolic rate) of cod (n=60) with varying 
165 infection densities of nematodes. Standard metabolic rate represents the energy requirements 
166 of the individual at a resting, non-digesting state, maximum metabolic rate represents the 
167 maximum aerobic performance (Chabot et al., 2016), while aerobic metabolic scope relates to 
168 the ability to perform aerobic work. Four static respirometers (6.6L or 8.2L, to accommodate 
169 for differences in TL of fish) were placed in a 250L black tank supplied with flow-through of 
170 aerated water from the same supply as the holding tanks (10°C, 10‰ salinity). To minimize 
171 disturbance from neighboring fish, non-transparent polyethylene plates were placed vertically 
172 between the respirometers, and to minimize disturbance from the outside, a curtain shielded 
173 the setup.
For standard metabolic rate determinations, oxygen consumption rates (mgO₂ kg⁻¹ h⁻¹) were measured over a period of >40 hours, using intermittent-flow respirometry (Steffensen, 1989). Oxygen consumption rate was measured in 12-minute loops consisting of a flush (420s), a wait (60s) and a measurement (240s) period. To obtain maximum metabolic rate, individual fish were exposed to a chase protocol (intense continuous chasing for 2 minutes in a circular 300L tank), and immediately thereafter (within maximally two minutes) placed in the respirometer, where the first oxygen consumption values were obtained rapidly, using a measurement periods of 90s, with 1 second of wait and without flushing. Measurements for maximum metabolic rate were terminated if oxygen saturation fell below 75% within the 90s measurements period. Hereafter, oxygen consumption measurements continued in 12 minutes loops (as described above) for a minimum of 40h (i.e. for standard metabolic rate determination).

The highest oxygen consumption measurement (i.e. maximum metabolic rate) occurred instantly after the chase protocol for 51 of the fish, whereas maximum metabolic rate occurred later (following spontaneous activity inside the respirometer) for the remaining 9 fish. For each fish, standard metabolic rate was determined as the average of the 10% lowest oxygen consumption (Chabot et al., 2016) values and aerobic metabolic scope was calculated as the difference between maximum and standard metabolic rate. Background respiration was found by linear regression of the start and the final background measurements, and subsequently subtracted from the corresponding oxygen consumption value. To eliminate potential effects of digestion on oxygen consumption, all cod were fasted 3-4 days prior to the experiment, the specific number of days being based on the cod gastric evacuation model made by Andersen, 2012. All cod were weighed upon initiation of the experiment. To assess for potential contributions from nematode oxygen consumption to the measured oxygen consumption of the cod, oxygen consumption (mgO₂ kg⁻¹ h⁻¹) was determined on 85 live nematodes (0.001-0.012g).
over a 24h period, using a 24-well glass microplate containing 5ml wells (Loligo Systems) with 10°C and 10‰ salinity water.

Organ size

To elucidate associations between infection density and size of selected organs, whole cod (n=59) were thawed and BM, TL, LM, gender and weight to nearest gram of the stomach (empty), intestine (empty), pyloric caeca and heart recorded.

Plasma composition

To reveal potential associations between the nematodes and the function of the liver and the disease status of the fish, hematological analysis were performed. Following respirometry, fish (n=60) were stunned by a sharp blow to the head, blood was immediately sampled by caudal puncture with a Lithium-heparinized 21-gauge hypodermic needle, and fish were euthanized by spinal transection. Blood samples were centrifuged at 1610G for 5min, and the plasma fraction stored at -18°C (Houston, 2002). Total blood protein content (gL⁻¹) was determined using an ADVIA 1800 Clinical Chemistry System (Siemens), while separation of plasma protein fractions into pre-albumin, albumin and the globulins (alpha-1, alpha-2, beta-1, beta-2 and gamma) was done using capillary electrophoresis (MINICAP PROTEIN 6, Sebia, Lisses). A/G ratios were calculated by dividing individual plasma albumin and globulins values.

Proximate composition and energy content

In order to examine changes in body composition and energy content of cod in relation to different infection densities, 33 whole cod (livers removed and with empty stomachs) were individually autoclaved and homogenized (Table 1). Crude protein (N * 6.25) content of the fish, and crude lipid content of fish and livers, were determined using the Kjeldahl (Foss Kjeltec...
224 2200, Hillerød, Denmark) and Bligh and Dyer methods (Bligh and Dyer, 1959), respectively.
225 To obtain dry matter and water content of the fish and liver, samples were dried for 24 h at
226 105˚C, and weight loss determined (Memmert UN110, Büchenbach, Germany). Ash content
227 determinations were based on weight loss after incineration of samples for 6h at 550˚C in a
228 muffle furnace (Hereaus Instruments K1252, Hanau, Germany) (Obirikorang et al., 2016), and
glycogen content of the fish was calculated as the difference between the initial dry weight and
229 the sum of the crude protein, fat and ash weights (Saint-Paul, 1984). Energy density of dry cod
230 tissue was determined from dried tissue samples combusted in a Parr 6300 bomb calorimeter,
231 and subsequently converted to energy density per gram of wet BM (kJg⁻¹) (Schloesser and
233
234 Data handling and analysis
235 Prior to infection density calculations, the total weight of nematodes retrieved from individual
236 livers was subtracted from the measured LM, small nematodes being assigned a weigh of
237 0.004g and large nematodes 0.009g, based on the weight range of nematodes used in
238 respirometry. Oxygen consumption measurements with R² < 0.95 (in total <0.8%) were
239 excluded from the analysis. Two of the 60 fish used in respirometry never entered into a resting
240 state (judged by observations of the raw oxygen consumption data), and were hence excluded
241 from the dataset.
242 Log linear Gaussian models were used to describe the associations between infection
243 density, TL and gender in all assessed variables except in the analysis of changes in organ size
244 where power function models were used instead. TL was included to account for accumulation
245 of nematodes in the liver over time (i.e. with increasing length of the fish) (Nadolna and
246 Podolska, 2014; Horbowy et al., 2016), and gender was included to test for any potential
247 differences between males and females. TL was not included in the analysis of standard
metabolic rate, maximum metabolic rate and aerobic metabolic scope as these were modelled
as the mass specific oxygen consumption because the scaling exponent for the relationship
between oxygen consumption and body mass of the fish was 1. Likewise, TL was not included
in the analysis of Fulton condition factor as TL is included in this parameter. For the analysis
of fish body composition, the effect of infection density on all the performed analyses where
carried out together with analysis of changes in whole body energy content, as well as the
redistribution between protein, lipid and glycogen and water within the fish. To test the
robustness of the results (due to high variation in infection density) we divided fish into 3
groups of infection densities, non-, medium- and high-infected to test for significant difference
between these 3 groups for selected parameters.

All statistical tests were conducted in R (R Core Team, 2016). 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 and S2). Model selection was performed using a stepwise backward selection routine
based on likelihood ratio test for each of the variables included and excluded in the models.
The model assumptions of normality and independence were subsequently validated by visual
inspection of model residuals (Figs S1-S12). ANOVA and post hoc (Tukey HSD) were used
for the test of robustness.
Results

A total of 4309 nematodes were recovered from the 152 livers examined, all belonging to the species *C. osculatum*. Mean and range of infection density were 2 (± s.e. 0.3) and 0-22 nematodes per gram liver, respectively. Upon retrieval of the livers from the fish for recovery of nematodes, it was noticed that for the 12 most heavily infected livers (all with infection density above 6 nematodes per gram liver; Fig. 1), the organ seemingly was losing its structure/integrity, and ‘melted’ upon removal from the body cavity of the fish.

Nutritional condition

The Fulton condition factor of cod decreased significantly (GLM: $n = 152$, SE = 0.003, $t = -8.1$, $P < 0.001$) with increasing infection density (Fig. 2, Table 2-3).

Aerobic performance

Standard metabolic rate decreased significantly (GLM: $n = 58$, SE = 0.003, $t = -3.2$, $P = 0.002$) with increasing infection density (Fig. 3, Table 2-3). In contrast, maximum metabolic rate was not associated with changes in infection density and aerobic metabolic scope and gender was not significant in any of the three cases. The oxygen consumption of *C. osculatum* was inconsiderable (mean ± s.e.; 0.0002 ± 2.2E-05, mgO$_2$ kg$^{-1}$hour$^{-1}$) and thus negligible for the measured oxygen consumption of the cod.
Organ size

The mass of intestine and pyloric caeca increased significantly (GLM: intestine, $n = 58$, $SE = 0.350$, $t = 6.3$, $P < 0.001$ & pyloric caeca, $n = 58$, $SE = 0.357$, $t = 7.3$, $P < 0.001$) with length of the fish, but decreased significantly with increasing infection density (GLM: intestine, $n = 58$, $SE = 0.012$, $t = -2.1$, $P = 0.03$ & pyloric caeca, $n = 58$, $SE = 0.011$, $t = -2.2$, $P = 0.03$).

Females had larger intestines compared to males but gender could not explain any variation found in weight of pyloric caeca (Table 2).

Plasma composition

Total protein in the plasma decreased significantly (GLM: $n = 60$, $SE = 0.005$, $t = -3.1$, $P = 0.01$, Fig. 4A) with increasing infection density, as did the albumin to globulin ratio (GLM: $n = 60$, $SE = 0.030$, $t = -5.4$, $P < 0.001$, Fig. 4B) (Table 2-3). Albumin decreased significantly while the sum of globulins and gamma-globulins alone increased significantly with increasing infection density (Table 2). These changes in protein fractions of the plasma were reflected by the highly significant decrease in albumin to globulin ratio. Pre-albumin, which is not a part of the albumin to globulin ratio, decreased significantly with increasing infection density (Table 2). On the contrary, there was no change in alpha-1, alpha-2 and beta-1-2 with increasing infection density. Gender and TL were only significant in the analysis of pre-albumin (Table 2).

Proximate composition and energy content

Overall, the body composition of the fish changed with increasing infection density. More specifically, total energy (GLM: $n = 33$, $SE = 0.009$, $t = -2.8$, $P = 0.006$, Fig. 5A) and protein content decreased significantly with increasing infection density (Table 2), while water
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(14)

323 (GLM: n = 33, SE = 0.001, t = 2.5, P = 0.01, Fig. 5B) and glycogen content increased significantly
324 (Table 2).

Figure 5 A-D

The decrease in total energy content of the fish was explained by a significant change in the
328 source of energy; a significant increase in protein (GLM: n = 33, SE = 0.025, t = 16.1, P < 0.001,
329 Fig. 6A) and a slightly increase in lipid energy, and a significant decrease in glycogen energy
330 (GLM: n = 33, SE = 0.057, t = -6.6, P < 0.001, Fig. 6C) with increase in total energy of the fish
331 (Fig 6, Table 2).

Figure 6 A-C

Total lipid energy of the fish did not change with changing infection density, and gender could
336 not be associated with the observed changes in the body composition of the fish. Liver lipid
337 content (GLM: n = 33, SE = 0.116, t = 2.1, P < 0.001, Fig. 5C) and dry matter decreased
338 significantly with increasing infection density, while ash and water content (GLM: n = 33,
339 SE = 0.074, t = -2.0, P = 0.001, Fig. 5D) significantly increased with increasing infection density.
340 Lipid, water and dry matter content of the liver differed significantly between males and
341 females, as livers from females contained more lipid and less water and less dry matter content
342 compared to livers from males (Table 2).

Test of robustness

346 Condition K-factor decreased significantly between the non-, medium and high-infected
347 groups with lowest value found in the high infected group (Table 3). The standard metabolic

14
rate, A/G ratio and lipid content of the liver decreased significantly in the high-infected groups compared to the non- and medium-infected groups (Table 3). Total energy and water content of the fish and the liver did not differ significantly between the high and medium groups but only between the non- and high-infected groups (Table 3). Total protein in the plasma did not differ significantly between non- and high-infected groups, only between the high- and the medium-infected groups (Table 3).

Table 3

Discussion

Using an integrative approach, we show that wild naturally infected G. morhua with heavy infections with the parasitic C. osculatum have impaired nutritional condition, reduced functionality of the digestive system, as indicated by the observed reduction in digestive organ masses (intestine and pyloric caeca), reduced baseline metabolism, and changes in body and plasma composition, and fish energy source. Notably, fish with high infection densities had decreased plasma albumin and increased globulin levels, resulting in reduced albumin to globulin ratio.

Synthesized in the liver, albumin is the main protein of the blood plasma. It has several functions, e.g. maintaining osmotic pressure, capillary permeability and transport and metabolism of an extraordinarily diverse range of molecules (McDonald and Milligan, 1992; Garcia-Martinez et al., 2013). In humans, reduced albumin to globulin ratios (driven by reduced albumin and increased globulins) are seen in individuals with chronic liver diseases associated with parenchymal damage, such as cirrhosis and liver cancer. Here the increased globulin levels are caused by alternations in the gamma fraction (synthesized in lymphatic tissues), with alpha and beta globulins remaining stable (Teloh, 1978; Suh et al., 2014).
Changes in plasma protein composition also occurs in diseased fish, where the albumin to globulin ratio has been used to reveal the physiological effects of specific pathogens (Aydin et al., 2001; Osmani et al., 2009). Notably, for some fish species gamma globulins are considered to represent the complex nature of parasitic nematode antigenicity (Meade and Harvey, 1969), which is in agreement with the present response of G. morhua to a parasitic nematode, where the gamma fraction of the globulins drive the observed increase in the plasma globulins. In support, in G. morhua, genes related to immune response are overall affected by infections with C. osculatum (Marnis et al., 2019). More specifically expression of immune related genes in G. morhua tend to be downregulated in the liver but upregulated in the spleen, suggestively due to local immune suppression in the liver caused by C. osculatum (Marnis et al., 2020).

Taken together, we argue that the significant decrease in the albumin to globulin ratio (caused by concomitant changes in plasma albumin and gamma globulin) that occurs with increasing C. osculatum infection, reveals that highly infected G. morhua suffer from a chronic pathological liver condition. This in turn probably impairs the organ functionality, likely due to extensive tissue damage by C. osculatum (Fig. 1) (Buchmann and Mehrdana, 2016).

Many parasites impose an energetic cost to their host (Lester, 1971; Östlund-Nilsson et al., 2005; Binning et al., 2013), yet we saw reduced standard metabolic rate in heavily infected fish. Because standard metabolic rate represents the summated energy expenditure in vivo, pinpointing the specific cause for this with certainty is not possible. However, as the site of infection of this particular parasite is the liver, and because the specific site of infection often determines how the parasite affect its hosts (Lafferty and Shaw, 2013), we suggest that a main cause of the reduced standard metabolic rate is an impaired functionality of this accessory digestive organ, leading to a compromised digestive system. This would result in reduced efficiency in protein turnover, reflected in a loss of body protein, low plasma total protein and albumin, and decrease in body energy content and a shift in the composition of the body energy
in fish as seen in the present study for *G. morhua* with high infection densities. Furthermore, as a large proportion of the maintenance costs are directed towards internal organs (Hulbert and Else, 2000) the observed decreased mass of intestinal tissue and pyloric caeca in highly infected fish may also partly explain the reduced standard metabolic rate. It is nevertheless important to note that the present results are derived from wild, naturally infected fish, with unknown feeding histories, potentially including periods of starvation. Starvation elicits a cascade of physiological responses, and many ectotherms (including fishes) and endotherms have been reported to reduce the mass of their gastrointestinal tissues to reduce energetic demands during starvation (McCue, 2010; Day *et al.*, 2014). Furthermore, during starvation, *G. morhua* initially exhausts its hepatic lipid and glycogen storage, and muscle glycogen, followed by mobilization of muscle protein (substituted by water) (Black and Love, 1986; Navarro and Gutiérrez, 1995). Thus, the reduced protein and lipid contents observed in the present study on *G. morhua* with high infection densities in some aspects resemble a starvation response. Yet one aspect differs markedly; fish that have starved long enough to deplete their hepatic lipid and muscle glycogen resources rely on muscle protein as the main source of energy (Navarro and Gutiérrez, 1995), whereas in the present study glycogen energy was found constitute the main proportion of the fish energy source for the fish with the highest infection intensities. Based on this, it seems plausible that high infection densities, and not starvation, is the main driver of the observed changes in body composition and preferred substrate utilization by the fish in the present study.

Although the present results reveal major associations between infections with *C. osculatum* and the physiological condition of *G. morhua*, the causality is unclear, and we are still, to some extent, dealing with ‘the chicken or the egg’ dilemma - especially in relation to the strong negative association between the Fulton condition factor and the increasing infection density. In various taxa including fish, nutritional state of an individual is recognized to impair
immune function and thereby increasing the risk of being infected by a pathogen (Gulland, 1992; Chandra, 1997; Johansen et al., 1997; Oliva-Teles, 2012). A gradual decrease in the nutritional condition of Eastern Baltic G. morhua has occurred since the 1990s, in all likelihood caused by reduced quantity and quality of prey in combination with deteriorating oxygen conditions (Plambech et al., 2013; Eero et al., 2015; Casini et al., 2016b; Neuenfeldt et al., 2020). In an already compromised nutritional state, G. morhua may be more susceptible to infection with C. osculatum. Notably, and irrespectively of potential causalties, history seems to repeat itself; in the late 1940s, the number of H. grypus (i.e. the main final host of C. osculatum) in the Baltic Sea was comparable to present days (Harding et al., 2007), and at that time G. morhua as now suffered high C. osculatum infection rates, with liver lipid content being highly comparable to our study when comparing parasite-free livers with livers infected with C. osculatum (Petrushevsky and Shulman, 1955). Furthermore, as in the present study, G. morhua infected with C. osculatum also had lower condition as compared with uninfected conspecifics.

Although admittedly speculative, we suggest infections to lead to augmented mortality for the most heavily infected fish (Fig. 7), as also suggested by Horbowy et al., 2016. Natural mortality increases for cod in critical condition (Dutil and Lambert, 2000), and the observed very low nutritional condition (Fulton condition factor) combined with low lipid levels in the liver in heavily infected G. morhua may thus have fatal consequences for the individual. One could speculate that heavily infected fish exhibit impaired swimming performance where predation on S. sprattus may become increasingly challenging, contributing further to the negative association between high infection load and low nutritional condition (Fig. 7). Likewise, cod in poor condition exhibit reduced swimming endurance, and cannot achieve as high swimming speeds as more well-conditioned conspecifics (Martínez et al., 2003).
Ultimately, this may lead to increased susceptibility towards predation, for example by the end host of the parasite (Fig. 7).

To fulfill its life cycle, *C. osculatum* depends on its main final host, the oceanic-living mammal *H. grypus*. As such, *H. grypus* not only has the role as a top predator in the Baltic Sea ecosystem, this marine mammal is also important in structuring part of the community and disease dynamics of *G. morhua* by introducing and maintaining the parasite burden of *C. osculatum*. Oceanic-living mammals are final hosts to all the major groups of parasites in the oceans, many possessing complex life cycles with several intermittent hosts, including invertebrates and fish (McClelland *et al.*, 1990). Although marine mammals during periods of time in history have suffered from intense hunting and humans have depleted their populations, in the 20th century, a shift from resource exploitation toward wildlife conservation resulted in recoveries of many of these populations (Magera *et al.*, 2013). The worldwide occurrence of these marine mammals thus stress the need of further investigations of potential influence of their parasite fauna on community dynamics, not least in relation to rebuilding of deteriorating fish populations.
Funding

This work was supported by the European Maritime and Fisheries Fund and The Danish Fisheries Agency (33113-B-16-070 and 33113-B-16-071) and by the European Union’s Horizon 2020 research and innovation programme PANDORA (773713).

Acknowledgements

We thank the technical staff onboard “Havfisken” for assistance in catching and processing fish, and express our gratitude to Sune Riis Sørensen for layout of Fig. 7. We are grateful to Bornholm’s Salmon Hatchery for acclimation of live cod. Finally, we want to thank Lise Nikolic Nielsen and Claus Stjernegaard from University of Copenhagen for their help with the analysis of the plasma samples.

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Figures

Figure 1 An example of a *G. morhua* with a liver having a high density of *C. osculatum* (19 nematodes per gram liver tissue), some visible on the surface of the organ, others hidden in the liver parenchyma.
Figure 2 Fulton condition factor of cod (n=152) with varying degrees of infection densities with *C. osculatum*. Thin grey line describes the model fit, significant negative association between infection density and Fulton condition factor. The 95% confidence interval is represented by the grey area.
Figure 3 The standard metabolic rate (MO$_2$, in mgO$_2$kg$^{-1}$hr$^{-1}$) of *G. morhua* (n=58) with varying degrees of infection densities with *C. osculatum*. The thin grey line describes the association between infection density and standard metabolic rate, revealing a significant negative correlation. The grey boxes are 95% confidence intervals.
Figure 4 A) Total blood protein (gL⁻¹) and B) albumin/globulin ratio in *G. morhua* (n=60) in relation to varying degrees of infection densities with *C. osculatum*. Both parameters decreased significantly with increasing infection density, as described by the thin grey lines. The grey boxes are 95% confidence intervals.
Figure 5 A) Total energy content (kJ g wet-weight\(^{-1}\)) of the whole fish excluding the liver, B) water content (%) of the whole fish, C) lipid content of the liver (g g liver\(^{-1}\)) and D) water content of the liver (%), all in relation to changes in infection density as described by the thin grey lines. Both total energy of the fish and lipid content of the liver decreased significantly with increasing infection density while water content increased significantly in both the fish and the liver with increasing infection density. In C and D, colors and symbols represent; blue and Δ = female, red and o = male. Grey, blue and red areas represent 95% confidence intervals.
Figure 6 Proportions of the fish energy source coming from A) protein energy (kJ g⁻¹), B) lipid energy (kJ g⁻¹) and C) glycogen energy (kJ g⁻¹), all in relation to the total energy of the fish (n=33). As described by the thin grey lines, the proportion of energy in the fish coming from protein increased significantly with increasing energy of the fish, and likewise for lipid energy (though not significantly), whereas the amount of energy coming from glycogen sources decreased with energy of the fish. The grey areas represent 95% confidence interval.
Figure 7 Schematic overview that summarizes possible mechanisms that suggestively can lead to increased natural mortality of *G. morhua* with high infection densities of *C. osculatum*, a parasitic nematode that infects the liver of the fish. Infections are associated with compromised function of the liver and digestive system that can lead to declining nutritional condition (red arrows) and potentially impaired swimming performance resulting in augmented susceptibility towards predation by seal (blue arrows) and changes in prey selection (black arrows). All mechanisms may lead to increase in natural mortality. The critical Fulton condition factor where cod are considered dying is defined in Casini *et al.*, 2016a based on findings of Dutil and Lambert, 2000.
Tables

Table 1: Overview of fish within all six assessments. n = number of fish, TL = total length, BW = body weight, LM = liver mass, prevalence: percentage of infected fish in the sample, and intensity of infection: mean number of parasites per liver, including only infected individuals. Number in brackets represents ranges of variables. All numbers are mean ± s.e.

<table>
<thead>
<tr>
<th>Assessment</th>
<th>n</th>
<th>TL (cm)</th>
<th>BW (g)</th>
<th>LM (g)</th>
<th>Prevalence (%)</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional condition</td>
<td>152</td>
<td>39 ± 0.4</td>
<td>510 ± 14.9</td>
<td>21 ± 1.0</td>
<td>89</td>
<td>32 ± 2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(29-53)</td>
<td>(209-1098)</td>
<td>(4-80)</td>
<td></td>
<td>(0-203)</td>
</tr>
<tr>
<td>Aerobic performance and plasma composition</td>
<td>60</td>
<td>42 ± 0.5</td>
<td>572 ± 22.2</td>
<td>19 ± 1.4</td>
<td>93</td>
<td>46 ± 5.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(34-53)</td>
<td>(260-1077)</td>
<td>(4-57)</td>
<td></td>
<td>(0-203)</td>
</tr>
<tr>
<td>Proximate composition of fish and liver</td>
<td>33</td>
<td>40 ± 1.0</td>
<td>532 ± 41.7</td>
<td>25 ± 3.3</td>
<td>85</td>
<td>23 ± 4.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(29-53)</td>
<td>(212-1098)</td>
<td>(6-80)</td>
<td></td>
<td>(0-104)</td>
</tr>
<tr>
<td>Organ size</td>
<td>59</td>
<td>36 ± 0.5</td>
<td>434 ± 16.7</td>
<td>20 ± 9.2</td>
<td>86</td>
<td>21 ± 2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(28-45)</td>
<td>(209-780)</td>
<td>(5-41)</td>
<td></td>
<td>(0-72)</td>
</tr>
</tbody>
</table>
Table 2 Symbols reflect the estimates of the final models (s.e. in brackets): $\alpha=$ infection density (INF), $\beta=$ intercept, $\gamma=$ gender (estimate for female), $\mu=$ length and $\lambda=$ total energy. – = variable not significant in the model and empty columns = variable was not included in the full model. Units of parameters: 1= mg O$_2$ kg$^{-1}$ h$^{-1}$, 2=g, 3= g L$^{-1}$, 4=%, 5= kJ g$^{-1}$ and 6=g g liver$^{-1}$. SMR = standard metabolic rate. Asterisks indicate the significance level of the estimated parameters (*p<0.05, **p<0.01, ***p<0.001). $R^2$ indicates how much of the variation of data each model explains. All reported estimated model parameters are on log scale except for organ size where estimates are on log10 scale.

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Parameter</th>
<th>$\alpha$ (INF)</th>
<th>$\beta$ (intercept)</th>
<th>$\gamma$ (gender)</th>
<th>$\mu$ (length)</th>
<th>$\lambda$ (total energy)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional condition</td>
<td>Fulton Condition</td>
<td>-0.02(0.003)**</td>
<td>-0.17(0.01)</td>
<td></td>
<td></td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td>Aerobic performance</td>
<td>SMR$^1$</td>
<td>-0.01(0.003)**</td>
<td>3.98(0.02)</td>
<td></td>
<td></td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td>Organ size</td>
<td>Pyloric caeca$^2$</td>
<td>-0.02(0.01)*</td>
<td>-3.44(0.55)</td>
<td>-2.60(0.36)**</td>
<td></td>
<td></td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>Intestine$^2$</td>
<td>-0.02(0.01)*</td>
<td>-2.94(0.54)</td>
<td>0.08(0.03)</td>
<td>2.22(0.35)**</td>
<td></td>
<td>0.43</td>
</tr>
<tr>
<td>Plasma composition</td>
<td>Total protein$^3$</td>
<td>-0.01(0.005)**</td>
<td>3.49(0.03)</td>
<td></td>
<td></td>
<td></td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Globulins$^4$</td>
<td>0.01(0.003)**</td>
<td>4.35(0.02)</td>
<td></td>
<td></td>
<td></td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>-0.16(0.03)**</td>
<td>-1.5(0.17)</td>
<td></td>
<td></td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Prealbumin$^4$</td>
<td>-0.08(0.02)**</td>
<td>2.35(1.42)</td>
<td>0.64(0.01)**</td>
<td>0.06(0.03)*</td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Gamma$^4$</td>
<td>0.02(0.008)*</td>
<td>2.16(0.04)</td>
<td></td>
<td></td>
<td></td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Albumin$^4$</td>
<td>-0.15(0.03)**</td>
<td>2.84(0.16)</td>
<td></td>
<td></td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td>Proximate composition of fish</td>
<td>Total energy$^5$</td>
<td>-0.03(0.01)**</td>
<td>1.28(0.01)</td>
<td></td>
<td></td>
<td></td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Water$^4$</td>
<td>0.003(0.001)*</td>
<td>4.39(0.002)</td>
<td></td>
<td></td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Protein$^5$</td>
<td>-0.04(0.01)**</td>
<td>1.03(0.02)</td>
<td></td>
<td></td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Glycogen$^5$</td>
<td>0.05(0.01)**</td>
<td>0.36(0.02)</td>
<td></td>
<td></td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Ash$^4$</td>
<td>0.04(0.01)</td>
<td>1.25(0.02)</td>
<td></td>
<td></td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Dry matter$^4$</td>
<td>-0.01(0.006)*</td>
<td>2.95(0.01)</td>
<td></td>
<td></td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Protein$^5$</td>
<td>-0.43(0.09)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.40(0.02)**</td>
</tr>
<tr>
<td></td>
<td>Glycogen$^5$</td>
<td>1.71(0.20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.38(0.06)**</td>
</tr>
<tr>
<td>Proximate composition of liver</td>
<td>Lipid$^6$</td>
<td>-0.11(0.03)**</td>
<td>3.59(0.24)</td>
<td>0.15(0.07)*</td>
<td></td>
<td></td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Water$^4$</td>
<td>0.10(0.03)**</td>
<td>3.76(0.06)</td>
<td>-0.15(0.07)*</td>
<td></td>
<td></td>
<td>0.37</td>
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<tr>
<td></td>
<td>Dry mat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.10(0.03)**</td>
<td>4.00(0.06)</td>
<td>0.15(0.07)*</td>
<td>-</td>
<td>0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ash</td>
<td>0.10(0.03)**</td>
<td>-0.49(0.06)</td>
<td>-</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Results of post hoc analysis Tukey HSD test for eight variables where infection density is divided into 3 groups. For the first 4 variables infection density (number of nematodes per gram liver tissue) in each group is: non=0, medium=4 and high>4, and for the 4 latter variables infection density in each group is: non=0, medium=2 and high>2 as a result of different range in infection densities between the two batches of fish. Units of parameters: 1= mg O$_2$ kg$^{-1}$ h$^{-1}$, 2= g L$^{-1}$, 3= kJ g$^{-1}$, 4=%, and 5= g g liver$^{-1}$. P-value = overall significance level between the groups, letters = groups that do not differ statistically from each other. * visualizes how group b or c differ significantly from group a where: *p<0.05, **p<0.01 and ***p<0.001. N represents number of fish within each infection groups (Non, medium, high). SMR = standard metabolic rate. All numbers are mean ± s.e.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Non</th>
<th>Medium</th>
<th>High</th>
<th>P value</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fulton Condition</td>
<td>0.88±0.2(a)</td>
<td>0.81±0.1(b**)</td>
<td>0.68±0.2(c***)</td>
<td>&lt;0.001</td>
<td>(17,115,20)</td>
</tr>
<tr>
<td>SMR$^1$</td>
<td>56.3±0.5(a)</td>
<td>54.6±0.2(a)</td>
<td>44.7±0.3(b**)</td>
<td>0.002</td>
<td>(4,39,15)</td>
</tr>
<tr>
<td>Total protein$^2$</td>
<td>32.1±0.6(ab)</td>
<td>32.4±0.2(a)</td>
<td>27.8±0.3(b*)</td>
<td>0.02</td>
<td>(4,39,15)</td>
</tr>
<tr>
<td>A/G</td>
<td>0.33±0.9(a)</td>
<td>0.18±0.4(a)</td>
<td>0.04±1.3(b*)</td>
<td>&lt;0.001</td>
<td>(4,39,15)</td>
</tr>
<tr>
<td>Total energy$^3$</td>
<td>3.67±0.5(a)</td>
<td>3.49±0.2(ab)</td>
<td>3.32±0.4(b*)</td>
<td>0.05</td>
<td>(5,21,7)</td>
</tr>
<tr>
<td>Water fish$^4$</td>
<td>80.2±0.4(a)</td>
<td>81.2±0.2(ab)</td>
<td>81.4±0.4(b*)</td>
<td>0.03</td>
<td>(5,21,7)</td>
</tr>
<tr>
<td>Lipid liver$^5$</td>
<td>0.58±0.5(a)</td>
<td>0.45±0.3(a)</td>
<td>0.30±0.6(b**)</td>
<td>0.03</td>
<td>(5,21,7)</td>
</tr>
<tr>
<td>Water liver$^4$</td>
<td>36.6±0.5(a)</td>
<td>44.2±0.3(ab)</td>
<td>54.0±0.5(b**)</td>
<td>0.02</td>
<td>(5,21,7)</td>
</tr>
</tbody>
</table>
Electronic Supplementary Material for:
Physiological condition of Eastern Baltic cod, *Gadus morhua*, infected with the parasitic nematode *Contracaecum osculatum*

Marie Plambech Ryberg, Peter V. Skov, Niccolò Vendramin, Kurt Buchmann, Anders Nielsen, Jane W. Behrens
1. Supplementary results

1.1 Test of collinearity: Variation Inflation Factor (table S1)

Table S1. Results from the test of collinearity of explanatory variables used in the different analyses. Test of variation inflation factor: \( VIF = \left( \frac{1}{1-R^2_i} \right) \) is used to assess the collinearity between the variables. The tests are only performed once for each batch of fish, and a VIF value is given for each variable. VIF is tested for the models including INF+length, length+total energy and in models including sum worm+ length+liver weight. The latter has been chosen to show that collinearity was low between liver weight and length of fish. Variables with VIF above 10 are defined as critical in relation to collinearity.

<table>
<thead>
<tr>
<th>Batch number of fish</th>
<th>Assessment</th>
<th>INF+length</th>
<th>Sum worm + Length + Liver weight</th>
<th>Length +Total energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1+2+3</td>
<td>Nutritional condition</td>
<td>1.05 &amp; 1.05</td>
<td>1.3 &amp; 1.8 &amp; 1.5</td>
<td>-</td>
</tr>
<tr>
<td>Batch 1</td>
<td>Aerobic performance + plasma composition</td>
<td>1.0 &amp; 1.0</td>
<td>1.2 &amp; 1.6 &amp; 1.4</td>
<td>-</td>
</tr>
<tr>
<td>Batch 2</td>
<td>Organ size</td>
<td>1.0 &amp; 1.0</td>
<td>1.5 &amp; 2.5 2.1</td>
<td>-</td>
</tr>
<tr>
<td>Batch 3</td>
<td>Prox com fish + liver</td>
<td>1.0 &amp; 1.0</td>
<td>1.2 &amp; 2.2 &amp; 2.0</td>
<td>1.0 &amp; 1.0</td>
</tr>
</tbody>
</table>
1.2 Effect of length on estimation of infection density (INF)

To show that the association between infection density and the different examined parameters is not a result of a length effect, we have tested all the models again, but this time length was kept in the models despite being non-significant in most cases. This analysis revealed that the effect of length did not influence estimates of infection density in any of the cases (Table S2).
Table S2. Additional analysis of the associations between infection density (INF) and the different parameters when length is included in the model, even though length is not significant in most models. In this analysis gender was included in the models where it was significant, however this estimate is not shown here.

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Parameter</th>
<th>$\alpha$ (INF)</th>
<th>$\mu$ (length)</th>
<th>$\lambda$ (total energy)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nutritional condition</strong></td>
<td>Fulton condition</td>
<td>-0.02(0.003)***</td>
<td>0.006(0.002)***</td>
<td></td>
</tr>
<tr>
<td><strong>Aerobic performance</strong></td>
<td>SMR(^1)</td>
<td>-0.01(0.003)**</td>
<td>-0.004(0.004)</td>
<td></td>
</tr>
<tr>
<td><strong>Organ size</strong></td>
<td>Pyloric caeca(^2)</td>
<td>-0.02(0.01)*</td>
<td>2.60(0.36)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intestine(^2)</td>
<td>2.22(0.35)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Plasma composition</strong></td>
<td>Total protein(^3)</td>
<td>-0.02(0.005)***</td>
<td>0.009(0.006)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Globulins(^4)</td>
<td>0.01(0.003)*****</td>
<td>0.002(0.004)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>-0.16(0.03)*****</td>
<td>0.003 (0.04)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prealbumin(^4)</td>
<td>-0.07(0.02)***</td>
<td>0.06(0.03)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gamma(^4)</td>
<td>0.02(0.008)*</td>
<td>0.007(0.009)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Albumin(^4)</td>
<td>-0.15(0.03)*****</td>
<td>0.005(0.034)</td>
<td></td>
</tr>
<tr>
<td><strong>Proximate composition of fish</strong></td>
<td>Total energy(^5)</td>
<td>-0.03(0.01)***</td>
<td>-0.001(0.002)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water(^4)</td>
<td>0.003(0.001)*</td>
<td>-3.1^-5(3.1^-4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protein(^5)</td>
<td>-0.04(0.01)***</td>
<td>-0.002(0.003)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glycogen(^5)</td>
<td>0.05(0.01)*****</td>
<td>0.000(0.003)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ash(^4)</td>
<td>0.04(0.01)*</td>
<td>0.003(0.003)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dry matter(^4)</td>
<td>-0.01(0.006)*</td>
<td>0.000(0.001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protein(^3)</td>
<td>0.000(0.001)</td>
<td>0.40(0.02)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glycogen(^5)</td>
<td>-0.002(0.002)</td>
<td>-0.38(0.06)****</td>
<td></td>
</tr>
<tr>
<td><strong>Proximate composition of liver</strong></td>
<td>Lipid(^6)</td>
<td>-0.18(0.04)*****</td>
<td>0.018(0.009)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water(^4)</td>
<td>0.10(0.03)*****</td>
<td>-0.009(0.006)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dry mat(^4)</td>
<td>-0.10(0.03)*****</td>
<td>0.011(0.006)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ash(^4)</td>
<td>0.10(0.03)***</td>
<td>-0.009(0.007)</td>
<td></td>
</tr>
</tbody>
</table>
1.3 Model validation plots

Fulton condition factor is calculated based on data compiled from all three batches of fish (i.e. all fish used in this study, n=152) (Fig S1). Examination of aerobic performance and plasma composition was performed on the same batch of fish (Figs. S2-S5). The study where changes in organ size was investigated involved fish from the second batch which was only used in this context (Fig S6). Analysis of composition of the fish and the liver was based on a third batch of fish and figure S7-S12 are therefore showing the same fish ID’s.

Figure S1. Model diagnostic plots for the final model of Fulton condition factor showing A) standardized residuals and fitted values and B) Q-Q plot for normal distribution of residuals.
Figure S2. Model diagnostic plots for the final model of standard metabolic rate (SMR) showing
A) standardized residuals and fitted values and B) Q-Q plot for normal distribution of residuals.
Figure S3. Model diagnostic plots for the final model of total protein (A+B) and globulins (C+D) in blood showing A) standardized residuals and fitted values and B) Q-Q plot for normal distribution of residuals.
Figure S4. Model diagnostic plots for the final model of A/G ratio (A+B) and prealbumin (C+D) showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution of residuals.
Figure S5. Model diagnostic plots for the final model of gamma (A+B) and albumin (C+D) showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution of residuals.
Figure S6. Model diagnostic plots for the final model of pyloric caeca (A+B) and intestine (C+D) showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution of residuals.
Figure S7. Model diagnostic plots for the final model of total energy (A+B) and water of fish (C+D) showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution of residuals.
Figure S8. Model diagnostic plots for the final model of protein (A+B) and glycogen in fish (C+D) showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution of residuals.
Figure S9. Model diagnostic plots for the final model of Ash (A+B) and dry matter of fish (C+D) showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution of residuals.
Figure S10. Model diagnostic plots for the final model of protein (A+B) and glycogen versus total energy (C+D) showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution of residuals.
Figure S11. Model diagnostic plots for the final model of lipid (A+B) and water of liver (C+D) showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution of residuals.
Figure S12. Model diagnostic plots for the final model of dry matter (A+B) and ash of liver (C+D) showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution of residuals.