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**Physiological condition of Eastern Baltic cod, *Gadus morhua*,  
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osculatum***

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**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.

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## 26 Abstract

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

## 50 Introduction

51 Parasitism is one of the most common animal lifestyles and can impact ecosystem functioning  
52 by affecting food-web stability, interaction strength and energy flow in both terrestrial and  
53 aquatic ecosystems (Marcogliese, 2004; Kuris *et al.*, 2008; Lafferty *et al.*, 2008; Hatcher *et al.*,  
54 2014). At the level of the individual, parasites can cause adverse effects on performance  
55 capacity of the host (McElroy and de Buron, 2014), e.g. by changing plasma protein and  
56 hormone levels (Akinyi *et al.*, 2019; O'Dwyer *et al.*, 2019), reducing aerobic and locomotor  
57 performances (Umberger *et al.*, 2013; Hahn *et al.*, 2018) and depleting energy reserves (Ferrer-  
58 Maza *et al.*, 2016). Together, this shapes the physiological condition of an infected individual,  
59 and impairment may lead to reduced growth and increased mortality (Marcogliese, 2004; Khan,  
60 2005; Behrens *et al.*, 2014). For trophically transmitted parasites, such effects on transport  
61 hosts may make them more vulnerable to predators, increasing the probability of the parasite  
62 to reach its final host (Gabagambi *et al.*, 2019).

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

70 Here, we use the host-parasite system between the third stage larvae liver worm  
71 *Contracaecum osculatum* (Zuo *et al.*, 2018) and Eastern Baltic cod *Gadus morhua* as a case  
72 study to investigate the physiological performance of wild fish with high parasite load. The  
73 Eastern Baltic cod stock has exhibited a decline in nutritional condition during the past 20  
74 years, an event that has occurred alongside deteriorating oxygen conditions and reduced prey

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3 75 abundance, now leaving the fish historically malnourished and growth impaired (Eero *et al.*,  
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5 76 2015; Casini *et al.*, 2016b; Hüseyin *et al.*, 2018; Neuenfeldt *et al.*, 2020). This has challenged  
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7 77 management of the stock which at present show no signs of recovery, and with high natural  
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9 78 mortality (ICES, 2019) and a fishing ban introduced in 2019. The grey seal *Halichoerus grypus*  
10  
11 79 population has been severely reduced due to hunting and breeding problems between the 1960s  
12  
13 80 and the 1990s where after recovery slowly began (Harding *et al.*, 2007). Concurrent with the  
14  
15 81 recovery of *H. grypus*, an increase in infections with the tropically transmitted *C. osculatum*  
16  
17 82 that parasitizes the liver of cod has also been observed in *G. morhua* in the central and eastern  
18  
19 83 Baltic Sea since the early 2010s, (Haarder *et al.*, 2014; Nadolna and Podolska, 2014). This has  
20  
21 84 coincided with even further deterioration of the health status and stock productivity of the fish  
22  
23 85 (Eero *et al.*, 2012, 2015). *H. grypus* is the main final host of this parasite while cod act as the  
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25 86 last transport host in the life cycle (Koie and Fagerholm, 1995; Nadolna-Ałtyn *et al.*, 2018;  
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27 87 Zuo *et al.*, 2018).

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33 88 Field investigations have shown that infection intensity with *C. osculatum* in *G. morhua*  
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35 89 coincides with poor nutritional status, and that more Westerly and Northwesterly cod stocks  
36  
37 90 with little or no *C. osculatum* are in better nutritional condition (Horbowy *et al.*, 2016;  
38  
39 91 Sokolova *et al.*, 2018). This parasite migrates to the liver of the cod following ingestion via  
40  
41 92 smaller infected prey, e.g. sprat *Sprattus sprattus* (Zuo *et al.*, 2016; Nadolna-Ałtyn *et al.*, 2018),  
42  
43 93 where it accumulates over time, resulting in a larger parasite burden in older fish (Horbowy *et*  
44  
45 94 *al.*, 2016; Zuo *et al.*, 2016). The liver is responsible for nutrient assimilation, bile production,  
46  
47 95 maintenance of metabolic homeostasis, protein synthesis, and also serves as an energy reserve  
48  
49 96 and breeding capital for the fish (Hinton *et al.*, 2017) . It is thus intuitive to think that a high  
50  
51 97 liver parasite burden leads to reduced function of the organ with negative effects on the  
52  
53 98 nutritional condition of the infected individual. Yet, disentangling potential effects of parasites  
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55 99 on their hosts from effects arising in the wake of unfavorable abiotic and food conditions  
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3 100 demands an interdisciplinary approach combining field and laboratory studies, and expert  
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5 101 parasitologists, physiologists, and biologists (McElroy and de Buron, 2014).  
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8 102 To elucidate how high parasite load may relate to the physiological condition of wild  
9  
10 103 fish, we here investigated aerobic performance, nutritional condition, mass of selected organs  
11  
12 104 and plasma and proximate body composition of wild naturally infected *G. Morhua* in relation  
13  
14 105 to infection density with *C. osculatum*.  
15  
16

## 17 106 **Material and methods**

### 18 19 107 **Pilot study**

20  
21  
22 108 The number of *C. osculatum* in cod livers increases with the length of the fish (Nadolna and  
23  
24 109 Podolska, 2014; Horbowy *et al.*, 2016). In 2017 we therefore conducted a pilot study to identify  
25  
26 110 the length interval of *G. morhua* needed to obtain fish samples with sufficient variability in  
27  
28 111 infection intensity to make a solid study design. More specifically, wild and naturally infected  
29  
30 112 *G. morhua* (n=86) were captured by trawl East of Bornholm and used to assess the correlations  
31  
32 113 between body mass (BM), total length (TL), liver mass (LM), gender and number of nematodes  
33  
34 114 in the liver. A length range between 30-53 cm provided sufficient high variability in infection  
35  
36 115 intensity of nematodes in the livers of the fish. Fish for experiments (see below) were  
37  
38 116 consequently selected to cover this length range if possible.  
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### 43 117 44 118 **Experimental animals**

45  
46  
47 119 Wild naturally infected Eastern Baltic cod (n=152) were captured by trawl East of Bornholm  
48  
49 120 between 2017 and 2019 (Table 1). Cod were either used live for respirometry or sampling was  
50  
51 121 made directly on dead fish for investigations of body composition and organ sizes. For the  
52  
53 122 latter investigations fish were killed and frozen at -20 °C immediately after trawling, and  
54  
55 123 transported to DTU Aqua, Lyngby, for later analysis (Table 1). For fish used live in  
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57 124 experiments (Table 1), trawling time never exceeded 20 minutes in order to minimize stress  
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3 125 and damage to the fish. Live cod were acclimated for 2 weeks in captivity at Bornholm's  
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5 126 Salmon Hatchery before being transferred to the fish holding facilities at DTU Aqua, Lyngby.  
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7 127 Here they were held in two circular tanks (2000L each) with air-saturated recirculated water  
8  
9 128 (10°C, 10‰ salinity, photoperiod of 8:16 light: dark, with a half hour period of sunrise and  
10  
11 129 sunset), and allowed 3 weeks of acclimation before initiation of experiments. Fish were fed  
12  
13 130 three times weekly with cooked blue mussels corresponding to ~2% of their BM. All  
14  
15 131 experiments were carried out according to the animal welfare regulations of the Technical  
16  
17 132 University of Denmark and EU directive 2010/63/EU for animal experiments. Ethical permit  
18  
19 133 2017-15-0201-01282 from the Danish Animal Ethics Committee covered all experiments  
20  
21 134 reported here.  
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33 Table 1  
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### 138 **Recovery of nematodes from cod livers**

139 All livers from fish used in the present study were dissected out and frozen separately (-20°C)  
140 before they were analysed for presence of nematodes. Individual livers, except those used for  
141 lipid and energy analysis, were placed in a plastic bag (200 × 400 × 0.07mm) and compressed  
142 between two glass plates (15 × 15 × 1cm) to a thickness of 1 mm by the addition of gentle  
143 pressure to the plates (Buchmann, 2007). Livers were subsequently examined under a Leica  
144 stereo microscope (6.3-40× magnification) (Leica Microsystems Germany) and detected  
145 nematodes were categorized as either small (<1cm **body length**) or large (>1cm **body length**).  
146 To minimize oxidation processes and tissue breakdown in livers dedicated to lipid analysis,  
147 individual defrosted livers (n=33) were placed on glass petri dishes kept on ice. Single  
148 nematodes were manually removed, using a tweezer, and the total number for each liver  
149 recorded and subsequently preserved in 70% ethanol. For all examined livers, nematode



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3 150 species identification was based on morphometric characteristics of the caudal and cephalic  
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5 151 ends according to Fagerholm (Koie and Fagerholm, 1995). To compensate for differences in  
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8 152 the number of nematodes related to liver size, infection density was calculated as number of  
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10 153 nematodes per gram of liver tissue (i.e. liver tissue = wet weight of liver minus total weight of  
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12 154 nematodes), rather than total number of nematodes per liver.  
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### 16 156 **Nutritional condition**

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19 157 The association between infection density with nematodes and the nutritional condition of all  
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22 158 cod included in this study (n=152) was analysed by calculating Fulton condition factor:

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24 159 
$$\text{Fulton condition factor} = \left( \frac{BM}{TL^3} \right) * 100 .$$

### 25 26 27 160 28 29 161 **Aerobic performance**

30  
31 162 To investigate potential associations between infections and aerobic performance of the fish,  
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33  
34 163 we determined standard metabolic rate, maximum metabolic rate and aerobic metabolic scope  
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36 164 (i.e. maximum metabolic rate minus standard metabolic rate) of cod (n=60) with varying  
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38 165 infection densities of nematodes. Standard metabolic rate represents the energy requirements  
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40 166 of the individual at a resting, non-digesting state, maximum metabolic rate represents the  
41  
42 167 maximum aerobic performance (Chabot *et al.*, 2016), while aerobic metabolic scope relates to  
43  
44 168 the ability to perform aerobic work. Four static respirometers (6.6L or 8.2L, to accommodate  
45  
46 169 for differences in TL of fish) were placed in a 250L black tank supplied with flow-through of  
47  
48 170 aerated water from the same supply as the holding tanks (10°C, 10‰ salinity). To minimize  
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50 171 disturbance from neighboring fish, non-transparent polyethylene plates were placed vertically  
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52 172 between the respirometers, and to minimize disturbance from the outside, a curtain shielded  
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55 173 the setup.  
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3 174 For standard metabolic rate determinations, oxygen consumption rates ( $\text{mgO}_2\text{kg}^{-1}\text{h}^{-1}$ )  
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5 175 were measured over a period of >40 hours, using intermittent-flow respirometry (Steffensen,  
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7 176 1989). Oxygen consumption rate was measured in 12-minute loops consisting of a flush (420s),  
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9 177 a wait (60s) and a measurement (240s) period. To obtain maximum metabolic rate, individual  
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11 178 fish were exposed to a chase protocol (intense continuous chasing for 2 minutes in a circular  
12  
13 179 300L tank), and immediately thereafter (within maximally two minutes) placed in the  
14  
15 180 respirometer, where the first oxygen consumption values were obtained rapidly, using a  
16  
17 181 measurement periods of 90s, with 1 second of wait and without flushing. **Measurements for**  
18  
19 182 **maximum metabolic rate were terminated if oxygen saturation fell below 75% within the 90s**  
20  
21 183 **measurements period.** Hereafter, oxygen consumption measurements continued in 12 minutes  
22  
23 184 loops (as described above) for a minimum of 40h (i.e. for standard metabolic rate  
24  
25 185 determination).

26  
27 186 The highest oxygen consumption measurement (i.e. maximum metabolic rate) occurred  
28  
29 187 instantly after the chase protocol for 51 of the fish, whereas maximum metabolic rate occurred  
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31 188 later (following spontaneous activity inside the respirometer) for the remaining 9 fish. For each  
32  
33 189 fish, standard metabolic rate was determined as the average of the 10% lowest oxygen  
34  
35 190 consumption (Chabot *et al.*, 2016) values and aerobic metabolic scope was calculated as the  
36  
37 191 difference between maximum and standard metabolic rate. Background respiration was found  
38  
39 192 by linear regression of the start and the final background measurements, and subsequently  
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41 193 subtracted from the corresponding oxygen consumption value. To eliminate potential effects  
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43 194 of digestion on oxygen consumption, all cod were fasted 3-4 days prior to the experiment, the  
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45 195 specific number of days being based on the cod gastric evacuation model made by Andersen,  
46  
47 196 2012. All cod were weighed upon initiation of the experiment. To assess for potential  
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49 197 contributions from nematode oxygen consumption to the measured oxygen consumption of the  
50  
51 198 cod, oxygen consumption ( $\text{mgO}_2\text{kg}^{-1}\text{h}^{-1}$ ) was determined on 85 live nematodes (0.001-0.012g)  
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3 199 over a 24h period, using a 24-well glass microplate containing 5ml wells (Loligo Systems) with  
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5 200 10°C and 10‰ salinity water.  
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10 202 **Organ size**

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12 203 **To elucidate associations between infection density and size of selected organs**, whole cod  
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14 204 (n=59) were thawed and BM, TL, LM, gender and weight to nearest gram of the stomach  
15  
16 205 (empty), intestine (empty), pyloric caeca and heart recorded.  
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19 206

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21 207 **Plasma composition**

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23 208 **To reveal potential associations between the nematodes and the function of the liver and the**  
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25 209 **disease status of the fish, hematological analysis were performed.** Following respirometry, fish  
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27 210 (n=60) were stunned by a sharp blow to the head, blood was immediately sampled by caudal  
28  
29 211 puncture with a Lithium-heparinized 21-gauge hypodermic needle, and fish were euthanized  
30  
31 212 by spinal transection. Blood samples were centrifuged at 1610G for 5min, and the plasma  
32  
33 213 fraction stored at -18°C (Houston, 2002). Total blood protein content (gL<sup>-1</sup>) was determined  
34  
35 214 using an ADVIA 1800 Clinical Chemistry System (Siemens), while separation of plasma  
36  
37 215 protein fractions into pre-albumin, albumin and the globulins (alpha-1, alpha-2, beta-1, beta-2  
38  
39 216 and gamma) was done using capillary electrophoresis (MINICAP PROTEIN 6, Sebia, Lisses).  
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41 217 A/G ratios were calculated by dividing individual plasma albumin and globulins values.  
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49 219 **Proximate composition and energy content**

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51 220 **In order to examine changes in body composition and energy content of cod in relation to**  
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53 221 **different infection densities**, 33 whole cod (livers removed and with empty stomachs) were  
54  
55 222 individually autoclaved and homogenized (Table 1). Crude protein (N \* 6.25) content of the  
56  
57 223 fish, and crude lipid content of fish and livers, were determined using the Kjeldahl (Foss Kjeltec  
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59  
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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  
229 glycogen content of the fish was calculated as the difference between the initial dry weight and  
230 the sum of the crude protein, fat and ash weights (Saint-Paul, 1984). Energy density of dry cod  
231 tissue was determined from dried tissue samples combusted in a Parr 6300 bomb calorimeter,  
232 and subsequently converted to energy density per gram of wet BM (kJg<sup>-1</sup>) (Schloesser and  
233 Fabrizio, 2017).

### 235 **Data handling and analysis**

236 **Prior to infection density calculations**, the total weight of nematodes retrieved from individual  
237 livers was subtracted from the measured LM, small nematodes being assigned a weigh of  
238 0.004g and large nematodes 0.009g, based on the weight range of nematodes used in  
239 respirometry. Oxygen consumption measurements with  $R^2 < 0.95$  (in total <0.8%) were  
240 excluded from the analysis. Two of the 60 fish used in respirometry never entered into a resting  
241 state (judged by observations of the raw oxygen consumption data), and were hence excluded  
242 from the dataset.

243 **Log linear Gaussian models were used to describe the associations between infection**  
244 **density**, TL and gender in all assessed variables except in the analysis of changes in organ size  
245 where power function models were used instead. TL was included to account for accumulation  
246 of nematodes in the liver over time (i.e. with increasing length of the fish) (Nadolna and  
247 Podolska, 2014; Horbowy *et al.*, 2016), and gender was included to test for any potential  
248 differences between males and females. TL was not included in the analysis of standard

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3 249 metabolic rate, maximum metabolic rate and aerobic metabolic scope as these were modelled  
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5 250 as the mass specific oxygen consumption because the scaling exponent for the relationship  
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7  
8 251 between oxygen consumption and body mass of the fish was 1. Likewise, TL was not included  
9  
10 252 in the analysis of Fulton condition factor as TL is included in this parameter. For the analysis  
11  
12 253 of fish body composition, the effect of **infection density** on all the performed analyses where  
13  
14 254 carried out together with analysis of changes in whole body energy content, as well as the  
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16  
17 255 redistribution between protein, lipid and glycogen and water within the fish. To test the  
18  
19 256 robustness of the results (due to high variation in **infection density**) we divided fish into 3  
20  
21 257 groups of **infection densities**, non-, medium- and high-infected to test for significant difference  
22  
23  
24 258 between these 3 groups for selected parameters.

25  
26 259 All statistical tests were conducted in R (R Core Team, 2016). **Before model fitting,**  
27  
28 260 **collinearity between explanatory variables was assessed by using variance inflation factors**  
29  
30 261 **(VIF) (Zuur *et al.*, 2009). No variables were excluded from the analysis due to collinearity**  
31  
32 262 **(Table S1 and S2).** Model selection was performed using a stepwise backward selection routine  
33  
34 263 based on likelihood ratio test for each of the variables included and excluded in the models.  
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37 264 The model assumptions of normality and independence were subsequently validated by visual  
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39 265 inspection of model residuals (**Figs S1-S12**). ANOVA and post hoc (Tukey HSD) were used  
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42 266 for the test of robustness.

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## 273 **Results**

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

## 281 **Nutritional condition**

282 The Fulton condition factor of cod decreased significantly (GLM:  $n = 152$ ,  $SE = 0.003$ ,  $t = -$   
283  $8.1$ ,  $P < 0.001$ ) with increasing infection density (Fig. 2, Table 2-3).

285 Figure 2

## 287 **Aerobic performance**

288 Standard metabolic rate decreased significantly (GLM:  $n = 58$ ,  $SE = 0.003$ ,  $t = -3.2$ ,  $P = 0.002$ )  
289 with increasing infection density (Fig. 3, Table 2-3). In contrast, maximum metabolic rate was  
290 not associated with changes in infection density and aerobic metabolic scope and gender was  
291 not significant in any of the three cases. The oxygen consumption of *C. osculatum* was  
292 inconsiderable (mean  $\pm$  s.e.;  $0.0002 \pm 2.2E^{-05}$ ,  $mgO_2 kg^{-1}hour^{-1}$ ) and thus negligible for the  
293 measured oxygen consumption of the cod.

295 Table 2

297 Figure 3

### 298 **Organ size**

299 The mass of intestine and pyloric caeca increased significantly (GLM: intestine,  $n = 58$ ,  
300  $SE = 0.350$ ,  $t = 6.3$ ,  $P < 0.001$  & pyloric caeca,  $n = 58$ ,  $SE = 0.357$ ,  $t = 7.3$ ,  $P < 0.001$ ) with  
301 length of the fish, but decreased significantly with increasing infection density (GLM: intestine,  
302  $n = 58$ ,  $SE = 0.012$ ,  $t = -2.1$ ,  $P = 0.03$  & pyloric caeca,  $n = 58$ ,  $SE = 0.011$ ,  $t = -2.2$ ,  $P = 0.03$ ).  
303 Females had larger intestines compared to males but gender could not explain any variation  
304 found in weight of pyloric caeca (Table 2).

### 306 **Plasma composition**

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

317 Figure 4

### 319 **Proximate composition and energy content**

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

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

325

326 Figure 5 A-D

327

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

333

334 Figure 6 A-C

335

336 Total lipid energy of the fish did not change with changing infection density, and gender could  
337 not be associated with the observed changes in the body composition of the fish. Liver lipid  
338 content (GLM:  $n = 33$ ,  $SE = 0.116$ ,  $t = 2.1$ ,  $P < 0.001$ , Fig. 5C) and dry matter decreased  
339 significantly with increasing infection density, while ash and water content (GLM:  $n = 33$ ,  
340  $SE = 0.074$ ,  $t = -2.0$ ,  $P = 0.001$ , Fig. 5D) significantly increased with increasing infection density.

341 Lipid, water and dry matter content of the liver differed significantly between males and  
342 females, as livers from females contained more lipid and less water and less dry matter content  
343 compared to livers from males (Table 2).

344

### 345 **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



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3 348 rate, A/G ratio and lipid content of the liver decreased significantly in the high-infected groups  
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5 349 compared to the non- and medium-infected groups (Table 3). Total energy and water content  
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7 350 of the fish and the liver did not differ significantly between the high and medium groups but  
8  
9 351 only between the non- and high-infected groups (Table 3). Total protein in the plasma did not  
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11 352 differ significantly between non- and high-infected groups, only between the high- and the  
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13 353 medium-infected groups (Table 3).  
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19 355 Table 3  
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## 22 357 **Discussion**

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26 358 Using an integrative approach, we show that wild naturally infected *G. morhua* with heavy  
27  
28 359 infections with the parasitic *C. osculatum* have impaired nutritional condition, reduced  
29  
30 360 functionality of the digestive system, as indicated by the observed reduction in digestive organ  
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32 361 masses (intestine and pyloric caeca), reduced baseline metabolism, and changes in body and  
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34 362 plasma composition, and fish energy source. Notably, fish with high infection densities had  
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36 363 decreased plasma albumin and increased globulin levels, resulting in reduced albumin to  
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38 364 globulin ratio.  
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43 365 Synthesized in the liver, albumin is the main protein of the blood plasma. It has several  
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45 366 functions, e.g. maintaining osmotic pressure, capillary permeability and transport and  
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47 367 metabolism of an extraordinarily diverse range of molecules (McDonald and Milligan, 1992;  
48  
49 368 Garcia-Martinez *et al.*, 2013). In humans, reduced albumin to globulin ratios (driven by  
50  
51 369 reduced albumin and increased globulins) are seen in individuals with chronic liver diseases  
52  
53 370 associated with parenchymal damage, such as cirrhosis and liver cancer. Here the increased  
54  
55 371 globulin levels are caused by alternations in the gamma fraction (synthesized in lymphatic  
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57 372 tissues), with alpha and beta globulins remaining stable (Teloh, 1978; Suh *et al.*, 2014).  
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3 373 Changes in plasma protein composition also occurs in diseased fish, where the albumin to  
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5 374 globulin ratio has been used to reveal the physiological effects of specific pathogens (Aydin *et*  
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7 375 *al.*, 2001; Osmani *et al.*, 2009). Notably, for some fish species gamma globulins are considered  
8  
9 376 to represent the complex nature of parasitic nematode antigenicity (Meade and Harvey, 1969),  
10  
11 377 which is in agreement with the present response of *G. morhua* to a parasitic nematode, where  
12  
13 378 the gamma fraction of the globulins drive the observed increase in the plasma globulins. In  
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15 379 support, in *G. morhua*, genes related to immune response are overall affected by infections  
16  
17 380 with *C. osculatum* (Marnis *et al.*, 2019). More specifically expression of immune related genes  
18  
19 381 in *G. morhua* tend to be downregulated in the liver but upregulated in the spleen, suggestively  
20  
21 382 due to local immune suppression in the liver caused by *C. osculatum* (Marnis *et al.*, 2020).  
22  
23 383 Taken together, we argue that the significant decrease in the albumin to globulin ratio (caused  
24  
25 384 by concomitant changes in plasma albumin and gamma globulin) that occurs with increasing  
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27 385 *C. osculatum* infection, reveals that highly infected *G. morhua* suffer from a chronic  
28  
29 386 pathological liver condition. This in turn probably impairs the organ functionality, likely due  
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31 387 to extensive tissue damage by *C. osculatum* (Fig. 1) (Buchmann and Mehrdana, 2016).  
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38 388 Many parasites impose an energetic cost to their host (Lester, 1971; Östlund-Nilsson *et*  
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40 389 *al.*, 2005; Binning *et al.*, 2013), yet we saw reduced standard metabolic rate in heavily infected  
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42 390 fish. Because standard metabolic rate represents the summated energy expenditure *in vivo*,  
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44 391 pinpointing the specific cause for this with certainty is not possible. However, as the site of  
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46 392 infection of this particular parasite is the liver, and because the specific site of infection often  
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48 393 determines how the parasite affect its hosts (Lafferty and Shaw, 2013), we suggest that a main  
49  
50 394 cause of the reduced standard metabolic rate is an impaired functionality of this accessory  
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52 395 digestive organ, leading to a compromised digestive system. This would result in reduced  
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54 396 efficiency in protein turnover, reflected in a loss of body protein, low plasma total protein and  
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56 397 albumin, and decrease in body energy content and a shift in the composition of the body energy  
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3 398 in fish as seen in the present study for *G. morhua* with high infection densities. Furthermore,  
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5 399 as a large proportion of the maintenance costs are directed towards internal organs (Hulbert  
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7 400 and Else, 2000) the observed decreased mass of intestinal tissue and pyloric caeca in highly  
8  
9 401 infected fish may also partly explain the reduced standard metabolic rate. It is nevertheless  
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11 402 important to note that the present results are derived from wild, naturally infected fish, with  
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13 403 unknown feeding histories, potentially including periods of starvation. Starvation elicits a  
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15 404 cascade of physiological responses, and many ectotherms (including fishes) and endotherms  
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17 405 have been reported to reduce the mass of their gastrointestinal tissues to reduce energetic  
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19 406 demands during starvation (McCue, 2010; Day *et al.*, 2014). Furthermore, during starvation,  
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21 407 *G. morhua* initially exhausts its hepatic lipid and glycogen storage, and muscle glycogen,  
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23 408 followed by mobilization of muscle protein (substituted by water) (Black and Love, 1986;  
24  
25 409 Navarro and Gutiérrez, 1995). Thus, the reduced protein and lipid contents observed in the  
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27 410 present study on *G. morhua* with high infection densities in some aspects resemble a starvation  
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29 411 response. Yet one aspect differs markedly; fish that have starved long enough to deplete their  
30  
31 412 hepatic lipid and muscle glycogen resources rely on muscle protein as the main source of  
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33 413 energy (Navarro and Gutiérrez, 1995), whereas in the present study glycogen energy was found  
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35 414 constitute the main proportion of the fish energy source for the fish with the highest infection  
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37 415 intensities. Based on this, it seems plausible that high infection densities, and not starvation, is  
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39 416 the main driver of the observed changes in body composition and preferred substrate utilization  
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41 417 by the fish in the present study.

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43 418 Although the present results reveal major associations between infections with *C.*  
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45 419 *osculatum* and the physiological condition of *G. morhua*, the causality is unclear, and we are  
46  
47 420 still, to some extent, dealing with 'the chicken or the egg' dilemma - especially in relation to  
48  
49 421 the strong negative association between the Fulton condition factor and the increasing infection  
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51 422 density. In various taxa including fish, nutritional state of an individual is recognized to impair  
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3 423 immune function and thereby increasing the risk of being infected by a pathogen (Gulland,  
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5 424 1992; Chandra, 1997; Johansen *et al.*, 1997; Oliva-Teles, 2012). A gradual decrease in the  
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7 425 nutritional condition of Eastern Baltic *G. morhua* has occurred since the 1990s, in all likelihood  
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9 426 caused by reduced quantity and quality of prey in combination with deteriorating oxygen  
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11 427 conditions (Plambech *et al.*, 2013; Eero *et al.*, 2015; Casini *et al.*, 2016b; Neuenfeldt *et al.*,  
12  
13 428 2020). In an already compromised nutritional state, *G. morhua* may be more susceptible to  
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15 429 infection with *C. osculatum*. Notably, and irrespectively of potential causalities, history seems  
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17 430 to repeat itself; in the late 1940s, the number of *H. grypus* (i.e. the main final host of *C.*  
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19 431 *osculatum*) in the Baltic Sea was comparable to present days (Harding *et al.*, 2007), and at that  
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21 432 time *G. morhua* as now suffered high *C. osculatum* infection rates, with liver lipid content  
22  
23 433 being highly comparable to our study when comparing parasite-free livers with livers infected  
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25 434 with *C. osculatum* (Petrushevsky and Shulman, 1955). Furthermore, as in the present study, *G.*  
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27 435 *morhua* infected with *C. osculatum* also had lower condition as compared with uninfected  
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29 436 conspecifics.

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35 437 Although admittedly speculative, we suggest infections to lead to augmented mortality  
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37 438 for the most heavily infected fish (Fig. 7), as also suggested by Horbowy *et al.*, 2016. Natural  
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39 439 mortality increases for cod in critical condition (Dutil and Lambert, 2000), and the observed  
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41 440 very low nutritional condition (Fulton condition factor) combined with low lipid levels in the  
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43 441 liver in heavily infected *G. morhua* may thus have fatal consequences for the individual. One  
44  
45 442 could speculate that heavily infected fish exhibit impaired swimming performance where  
46  
47 443 predation on *S. sprattus* may become increasingly challenging, contributing further to the  
48  
49 444 negative association between high infection load and low nutritional condition (Fig. 7).  
50  
51 445 Likewise, cod in poor condition exhibit reduced swimming endurance, and cannot achieve as  
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53 446 high swimming speeds as more well-conditioned conspecifics (Martínez *et al.*, 2003).  
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3 447 Ultimately, this may lead to increased susceptibility towards predation, for example by the end  
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5 448 host of the parasite (Fig. 7).

6  
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8 449 To fulfill its life cycle, *C. osculatum* depends on its main final host, the oceanic-living  
9  
10 450 mammal *H. grypus*. As such, *H. grypus* not only has the role as a top predator in the Baltic Sea  
11  
12 451 ecosystem, this marine mammal is also important in structuring part of the community and  
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14 452 disease dynamics of *G. morhua* by introducing and maintaining the parasite burden of *C.*  
15  
16 453 *osculatum*. Oceanic-living mammals are final hosts to all the major groups of parasites in the  
17  
18 454 oceans, many possessing complex life cycles with several intermittent hosts, including  
19  
20 455 invertebrates and fish (McClelland *et al.*, 1990). Although marine mammals during periods of  
21  
22 456 time in history have suffered from intense hunting and humans have depleted their populations,  
23  
24 457 in the 20<sup>th</sup> century, a shift from resource exploitation toward wildlife conservation resulted in  
25  
26 458 recoveries of many of these populations (Magera *et al.*, 2013). The worldwide occurrence of  
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28 459 these marine mammals thus stress the need of further investigations of potential influence of  
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30 460 their parasite fauna on community dynamics, not least in relation to rebuilding of deteriorating  
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32 461 fish populations.  
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673 **Figures**

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675 **Figure 1** An example of a *G. morhua* with a liver having a high density of *C. osculatum* (19  
676 nematodes per gram liver tissue), some visible on the surface of the organ, others hidden in the  
677 liver parenchyma.

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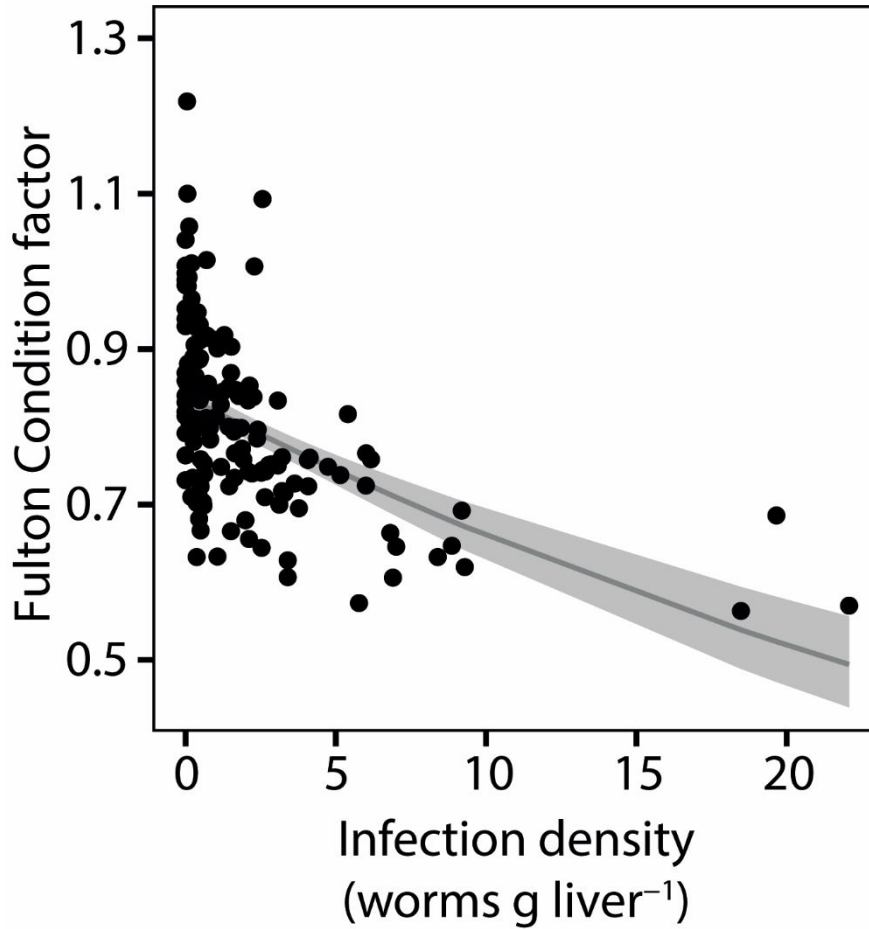
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687 Figure 2 Fulton condition factor of cod (n=152) with varying degrees of infection densities  
688 with *C. osculatum*. Thin grey line describes the model fit, significant negative association  
689 between infection density and Fulton condition factor. The 95% confidence interval is  
690 represented by the grey area.

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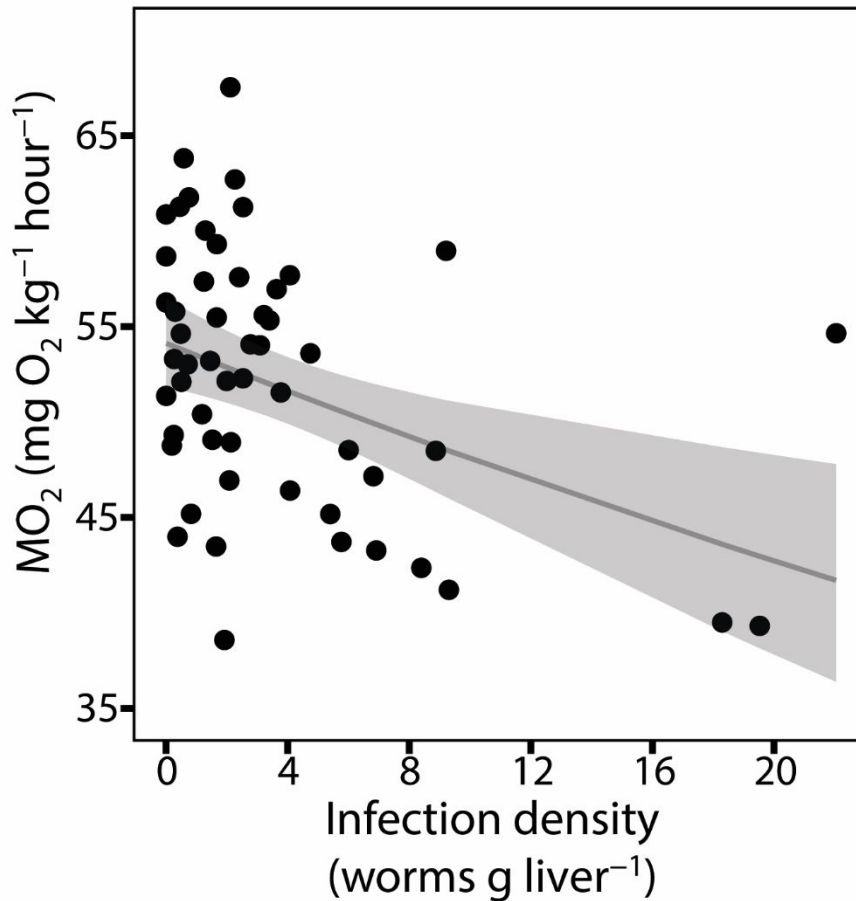
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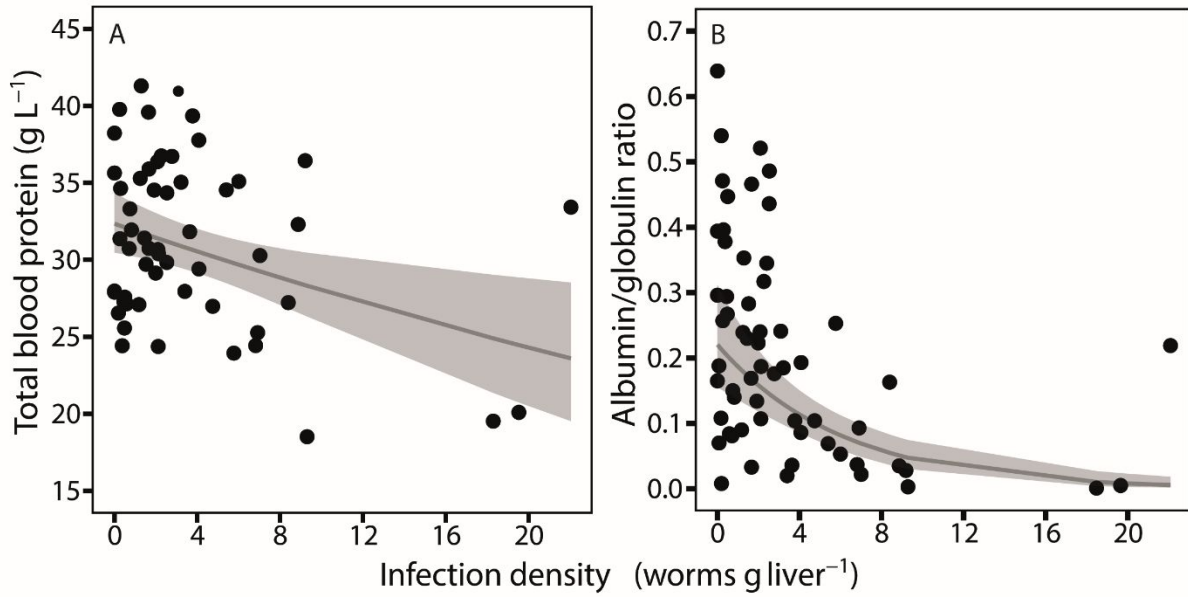
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699 Figure 3 The standard metabolic rate ( $\text{MO}_2$ , in  $\text{mgO}_2\text{kg}^{-1}\text{hr}^{-1}$ ) of *G. morhua* ( $n=58$ ) with varying  
700 degrees of infection densities with *C. osculatum*. The thin grey line describes the association  
701 between infection density and standard metabolic rate, revealing a significant negative  
702 correlation. The grey boxes are 95% confidence intervals.

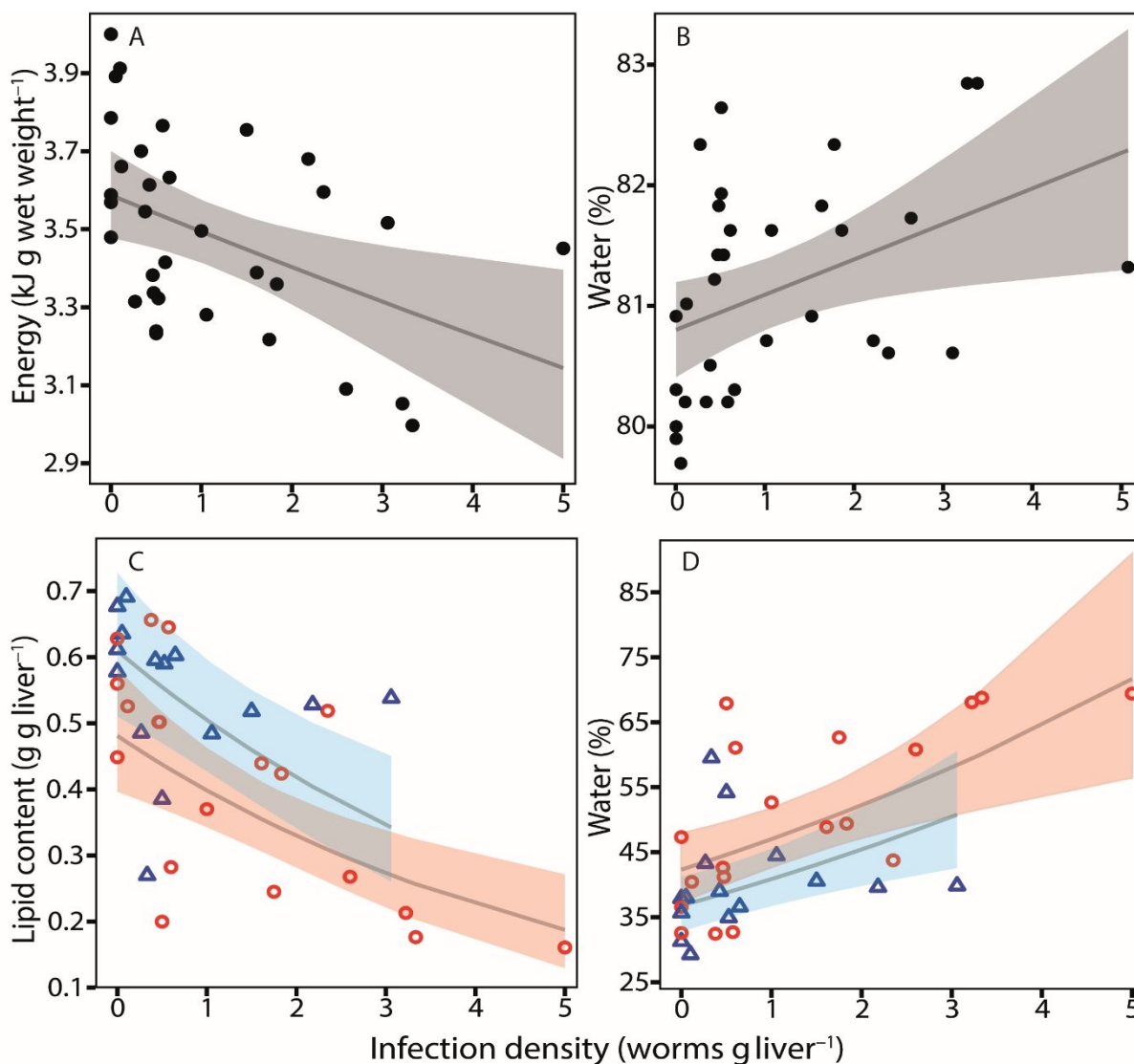




703 Figure 4 A) Total blood protein (g L<sup>-1</sup>) and B) albumin/globulin ratio in *G. morhua* (n=60) in  
704 relation to varying degrees of infection densities with *C. osculatum*. Both parameters decreased  
705 significantly with increasing infection density, as described by the thin grey lines. The grey  
706 boxes are 95% confidence intervals.

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710 Figure 5 A) Total energy content (kJ g wet-weight<sup>-1</sup>) of the whole fish excluding the liver, B)711 water content (%) of the whole fish, C) lipid content of the liver (g g liver<sup>-1</sup>) and D) water

712 content of the liver (%), all in relation to changes in infection density as described by the thin

713 grey lines. Both total energy of the fish and lipid content of the liver decreased significantly

714 with increasing infection density while water content increased significantly in both the fish

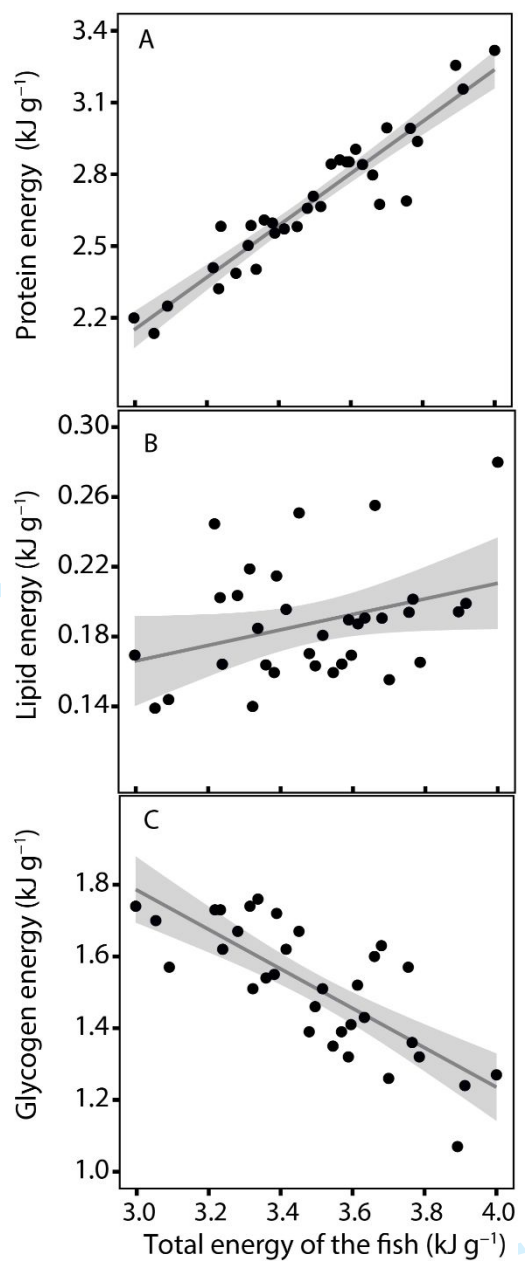
715 and the liver with increasing infection density. In C and D, colors and symbols represent; blue

716 and  $\Delta$  = female, red and o = male. Grey, blue and red areas represent 95% confidence intervals.

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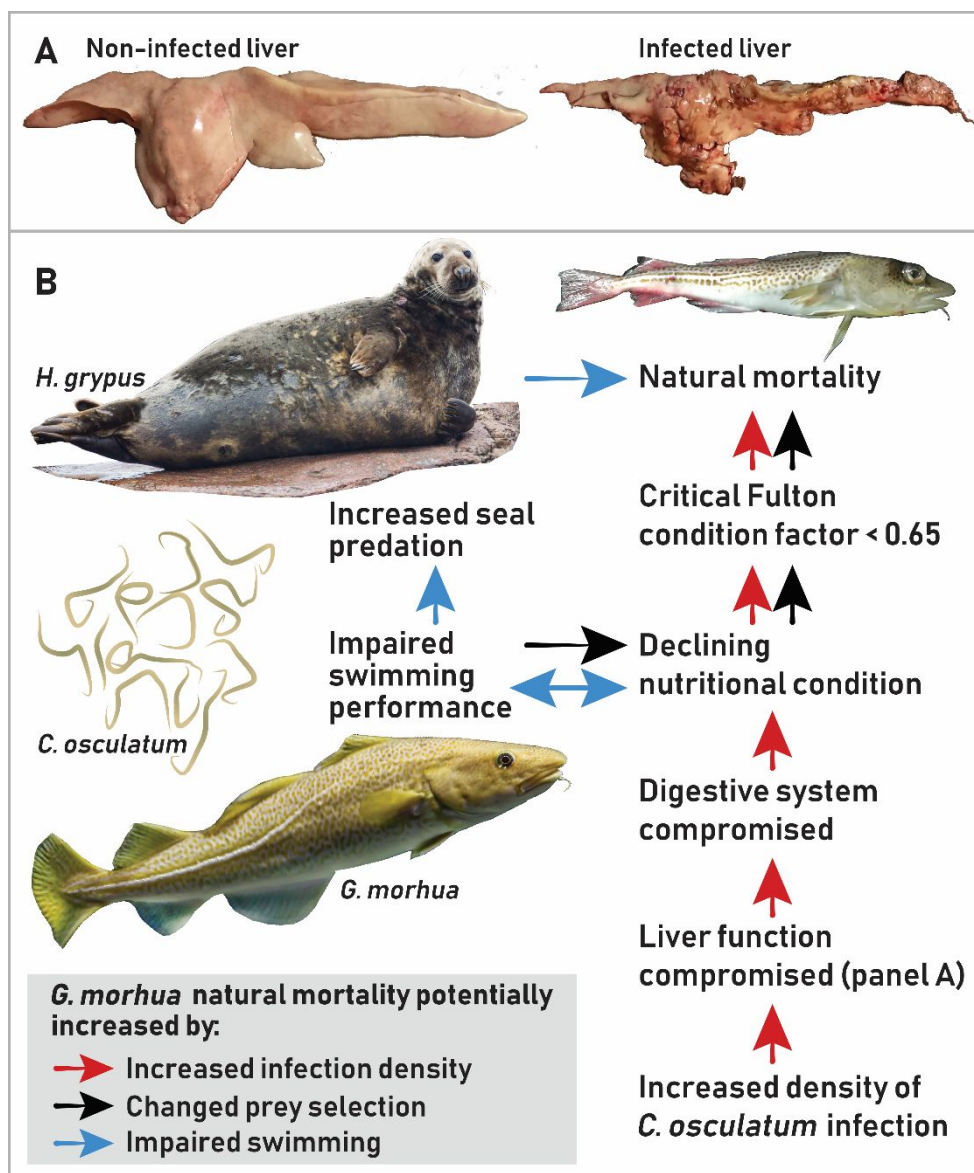




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720 Figure 6 Proportions of the fish energy source coming from A) protein energy (kJg<sup>-1</sup>), B) lipid  
 721 energy (kJg<sup>-1</sup>) and C) glycogen energy (kJg<sup>-1</sup>), all in relation to the total energy of the fish  
 722 (n=33). As described by the thin grey lines, the proportion of energy in the fish coming from  
 723 protein increased significantly with increasing energy of the fish, and likewise for lipid energy  
 724 (though not significantly), whereas the amount of energy coming from glycogen sources  
 725 decreased with energy of the fish. The grey areas represent 95% confidence interval.

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727 Figure 7 Schematic overview that summarizes possible mechanisms that suggestively can lead  
 728 to increased natural mortality of *G. morhua* with high infection densities of *C. osculatum*, a  
 729 parasitic nematode that infects the liver of the fish. Infections are associated with compromised  
 730 function of the liver and digestive system that can lead to declining nutritional condition (red  
 731 arrows) and potentially impaired swimming performance resulting in augmented susceptibility  
 732 towards predation by seal (blue arrows) and changes in prey selection (black arrows). All  
 733 mechanisms may lead to increase in natural mortality. The critical Fulton condition factor  
 734 where cod are considered dying is defined in Casini *et al.*, 2016a based on findings of Dutil  
 735 and Lambert, 2000.

736 **Tables**

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

Assessment	n	TL (cm)	BW (g)	LM (g)	Prevalence (%)	Intensity
Nutritional condition	152	39 $\pm$ 0.4 (29-53)	510 $\pm$ 14.9 (209-1098)	21 $\pm$ 1.0 (4-80)	89	32 $\pm$ 2.7 (0-203)
Aerobic performance and plasma composition	60	42 $\pm$ 0.5 (34-53)	572 $\pm$ 22.2 (260-1077)	19 $\pm$ 1.4 (4-57)	93	46 $\pm$ 5.3 (0-203)
Proximate composition of fish and liver	33	40 $\pm$ 1.0 (29-53)	532 $\pm$ 41.7 (212-1098)	25 $\pm$ 3.3 (6-80)	85	23 $\pm$ 4.3 (0-104)
Organ size	59	36 $\pm$ 0.5 (28-45)	434 $\pm$ 16.7 (209-780)	20 $\pm$ 9.2 (5-41)	86	21 $\pm$ 2.5 (0-72)

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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<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>, 2=g, 3= g L<sup>-1</sup>, 4=%, 5= kJ g<sup>-1</sup> and 6= g g liver<sup>-1</sup>. **SMR = standard metabolic rate**. Asterisks indicate the significance level of the estimated parameters (\*p<0.05, p<\*\*0.01, \*\*\*p<0.001). R<sup>2</sup> 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.

Assessment	Parameter	$\alpha$ (INF)	$\beta$ (intercept)	$\gamma$ (gender)	$\mu$ (length)	$\lambda$ (total energy)	R <sup>2</sup>
Nutritional condition	Fulton Condition	-0.02(0.003)***	-0.17(0.01)				0.30
Aerobic performance	SMR <sup>1</sup>	-0.01(0.003)**	3.98(0.02)	-			0.14
Organ size	Pyloric caeca <sup>2</sup>	-0.02(0.01)*	-3.44(0.55)	-	2.60(0.36)***		0.47
	Intestine <sup>2</sup>	-0.02(0.01)*	-2.94(0.54)	0.08(0.03) *	2.22(0.35)***		0.43
Plasma composition	Total protein <sup>3</sup>	-0.01(0.005)**	3.49(0.03)	-	-		0.13
	Globulins <sup>4</sup>	0.01(0.003)***	4.35(0.02)	-	-		0.19
	A/G	-0.16(0.03)***	-1.5(0.17)	-	-		0.33
	Prealbumin <sup>4</sup>	-0.08(0.02)***	2.35(1.42)	0.64(0.01)**	0.06(0.03)*		0.21
	Gamma <sup>4</sup>	0.02(0.008)*	2.16(0.04)	-	-		0.07
Proximate composition of fish	Albumin <sup>4</sup>	-0.15(0.03)***	2.84(0.16)	-	-		0.33
	Total energy <sup>5</sup>	-0.03(0.01)**	1.28(0.01)	-	-		0.18
	Water <sup>4</sup>	0.003(0.001)*	4.39(0.002)	-	-		0.14
	Protein <sup>5</sup>	-0.04(0.01)**	1.03(0.02)	-	-		0.25
	Glycogen <sup>5</sup>	0.05(0.01)**	0.36(0.02)	-	-		0.20
	Ash <sup>4</sup>	0.04(0.01)*	1.25(0.02)	-	-		0.14
	Dry matter <sup>4</sup>	-0.01(0.006)*	2.95(0.01)	-	-		0.14
	Protein <sup>5</sup>		-0.43(0.09)	-	-	0.40(0.02)***	0.89
Glycogen <sup>5</sup>		1.71(0.20)	-	-	-0.38(0.06)***	0.57	
Proximate composition of liver	Lipid <sup>6</sup>	-0.11(0.03)***	3.59(0.24)	0.15(0.07)*	-		0.41
	Water <sup>4</sup>	0.10(0.03)***	3.76(0.06)	-0.15(0.07)*	-		0.37

	Dry mat <sup>4</sup>	-0.10(0.03)***	4.00(0.06)	0.15(0.07)*	-		0.41
	Ash <sup>4</sup>	0.10(0.03)**	-0.49(0.06)	-	-		0.18

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762 Table 3: Results of post hoc analysis Tukey HSD test for eight variables where **infection density**  
 763 is divided in to 3 groups. For the first 4 variables **infection density** (number of nematodes per  
 764 gram liver tissue) in each group is: non=0, medium=4 and high>4, and for the 4 latter variables  
 765 **infection density** in each group is: non=0, medium=2 and high>2 as a result of different range  
 766 in **infection densities** between the two batches of fish. **Units of parameters: 1= mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>,**  
 767 **2= g L<sup>-1</sup>, 3= kJ g<sup>-1</sup>, 4=%, and 5= g g liver<sup>-1</sup>.** P-value = overall significance level between the  
 768 groups, letters = groups that do not differ statistically from each other. \* visualizes how group  
 769 b or c differ significantly from group a where: \*p<0.05, \*\*p<0.01 and \*\*\*p<0.001. N  
 770 represents number of fish within each infection groups (Non, medium, high). SMR = standard  
 771 metabolic rate. All numbers are mean ± s.e.

Parameter	Non	Medium	High	P value	N
Fulton Condition	0.88±0.2(a)	0.81±0.1(b**)	0.68±0.2(c***)	<0.001	(17,115,20)
SMR <sup>1</sup>	56.3±0.5(a)	54.6±0.2(a)	44.7±0.3(b**)	0.002	(4,39,15)
Total protein <sup>2</sup>	32.1±0.6(ab)	32.4±0.2(a)	27.8±0.3(b*)	0.02	(4,39,15)
A/G	0.33±0.9(a)	0.18±0.4(a)	0.04±1.3(b**)	<0.001	(4,39,15)
Total energy <sup>3</sup>	3.67±0.5(a)	3.49±0.2(ab)	3.32±0.4(b*)	0.05	(5,21,7)
Water fish <sup>4</sup>	80.2±0.4(a)	81.2±0.2(ab)	81.4±0.4(b*)	0.03	(5,21,7)
Lipid liver <sup>5</sup>	0.58±0.5(a)	0.45±0.3(a)	0.30.1±0.6(b**)	0.03	(5,21,7)
Water liver <sup>4</sup>	36.6±0.5(a)	44.2±0.3(ab)	54.0±0.5(b**)	0.02	(5,21,7)

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1 **Electronic Supplementary Material for:**

2 Physiological condition of Eastern Baltic cod, *Gadus morhua*, infected with the parasitic nematode

3 *Contracaecum osculatum*

4 Marie Plambech Ryberg, Peter V. Skov, Niccolò Vendramin, Kurt Buchmann, Anders Nielsen,  
5 Jane W. Behrens

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## 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:  $VIF = \left(\frac{1}{1 - R_i^2}\right)$  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.

Batch number of fish	Assessment	INF+length	Sum worm + Length + Liver weight	Length +Total energy
VIF				
Batch 1+2+3	Nutritional condition	1.05 & 1.05	1.3 & 1.8 & 1.5	-
Batch 1	Aerobic performance + plasma composition	1.0 & 1.0	1.2 & 1.6 & 1.4	-
Batch 2	Organ size	1.0 & 1.0	1.5 & 2.5 2.1	-
Batch 3	Prox com fish + liver	1.0 & 1.0	1.2 & 2.2 & 2.0	1.0 & 1.0



## 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).

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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.

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

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### 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 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.

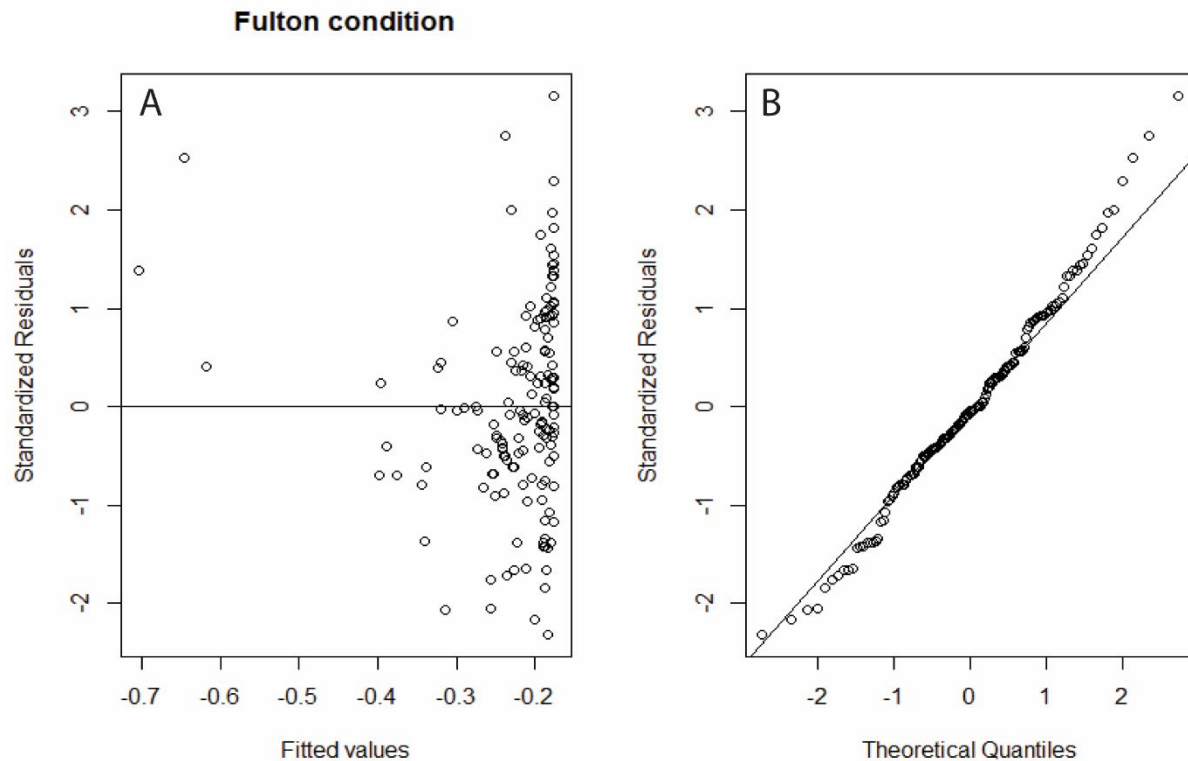
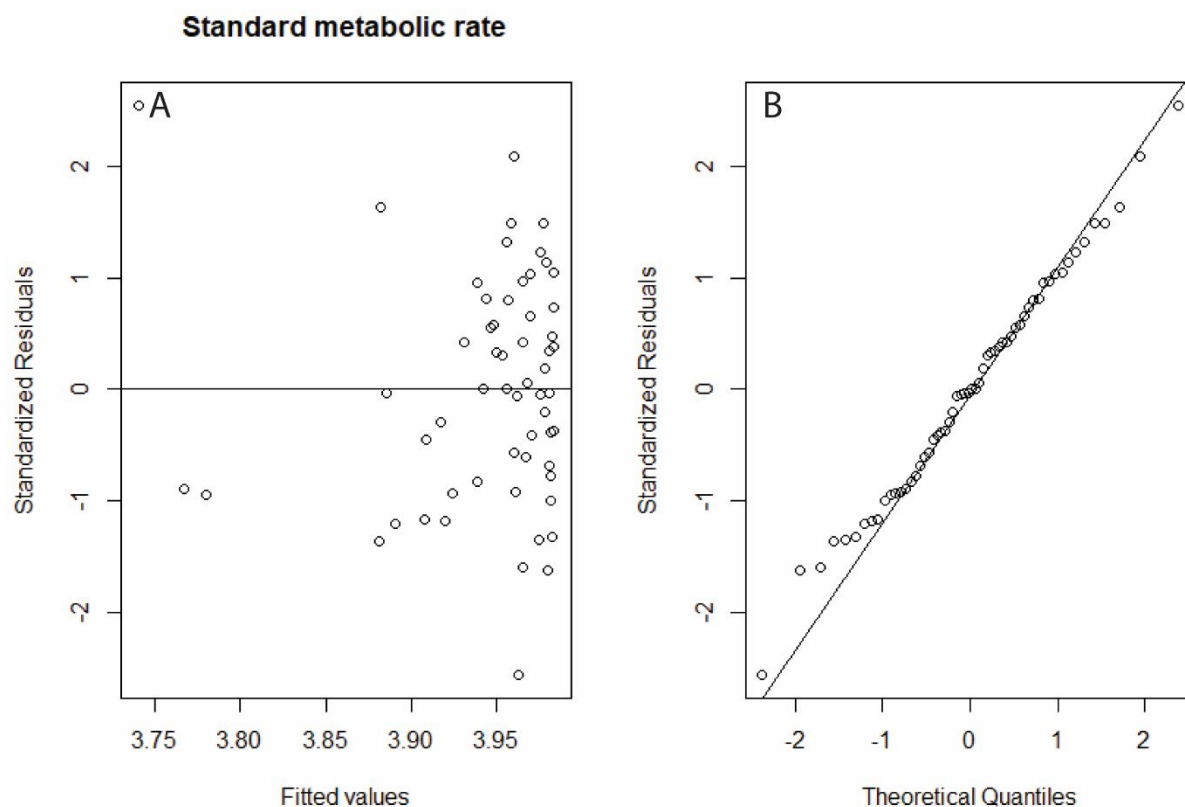
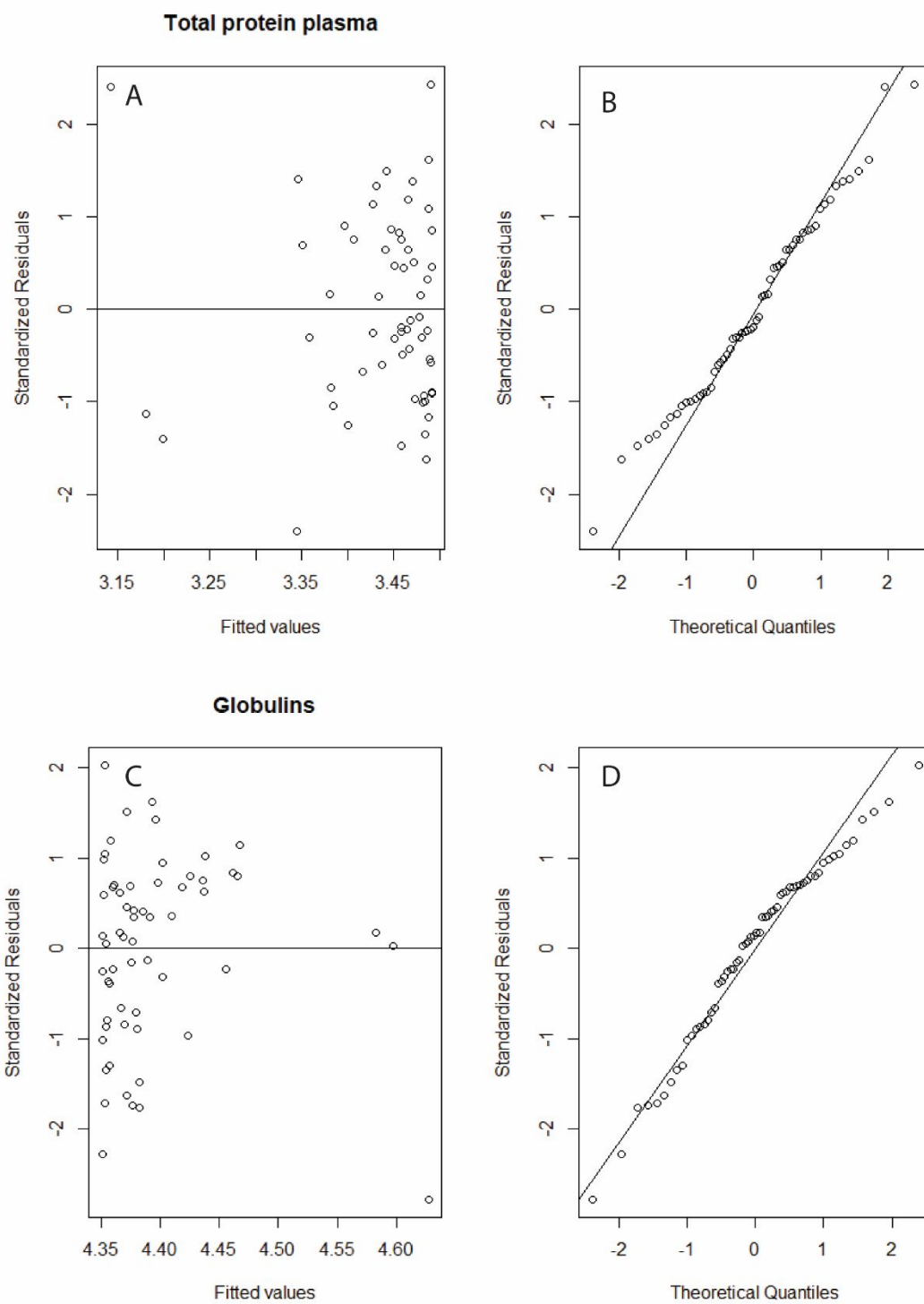


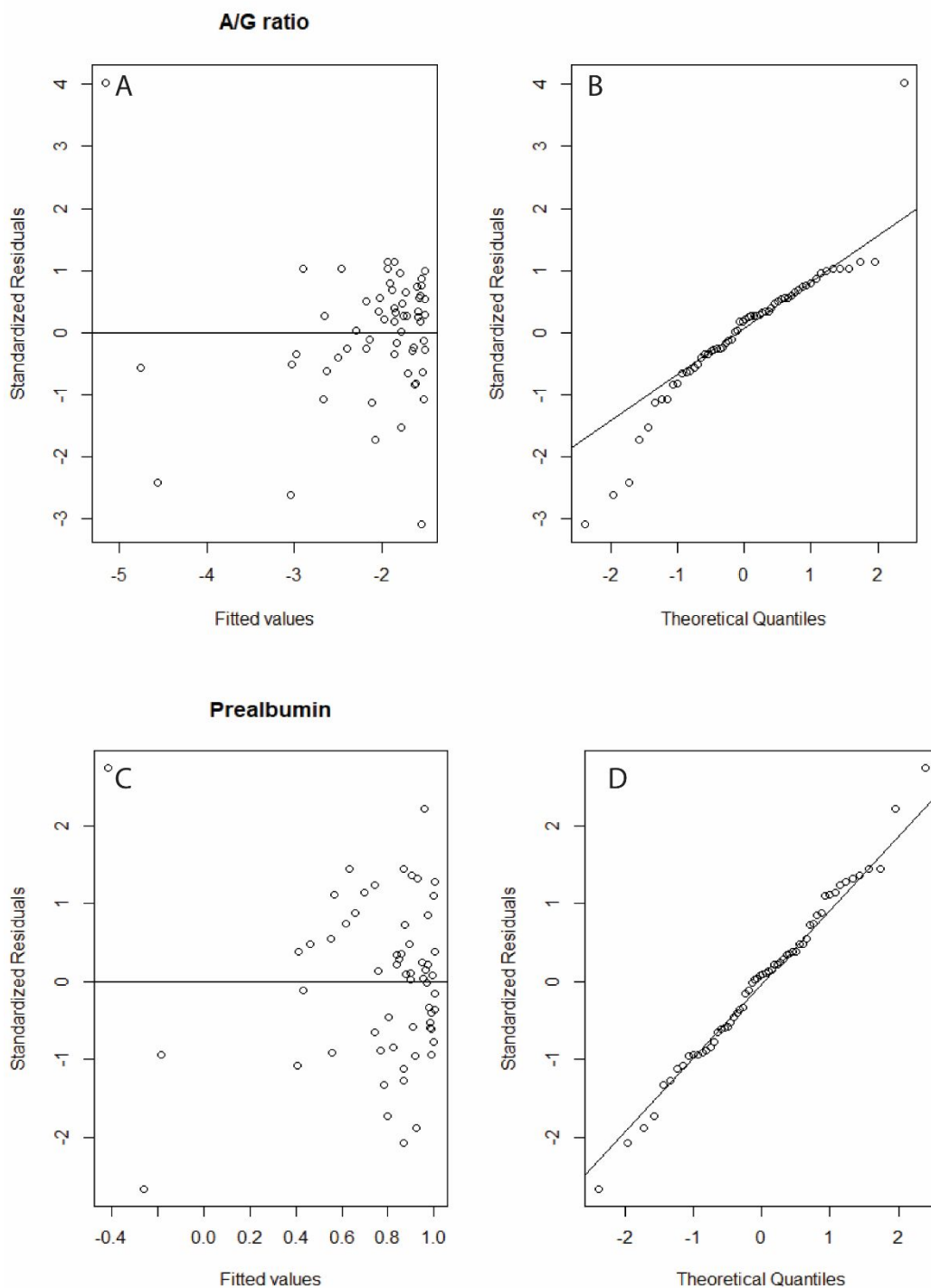
Figure S1. Model diagnostic plots for the final model of Fulton condition factor showing A) standardized residuals and fitted values and B) Q-Q plot for normal distribution of residuals.



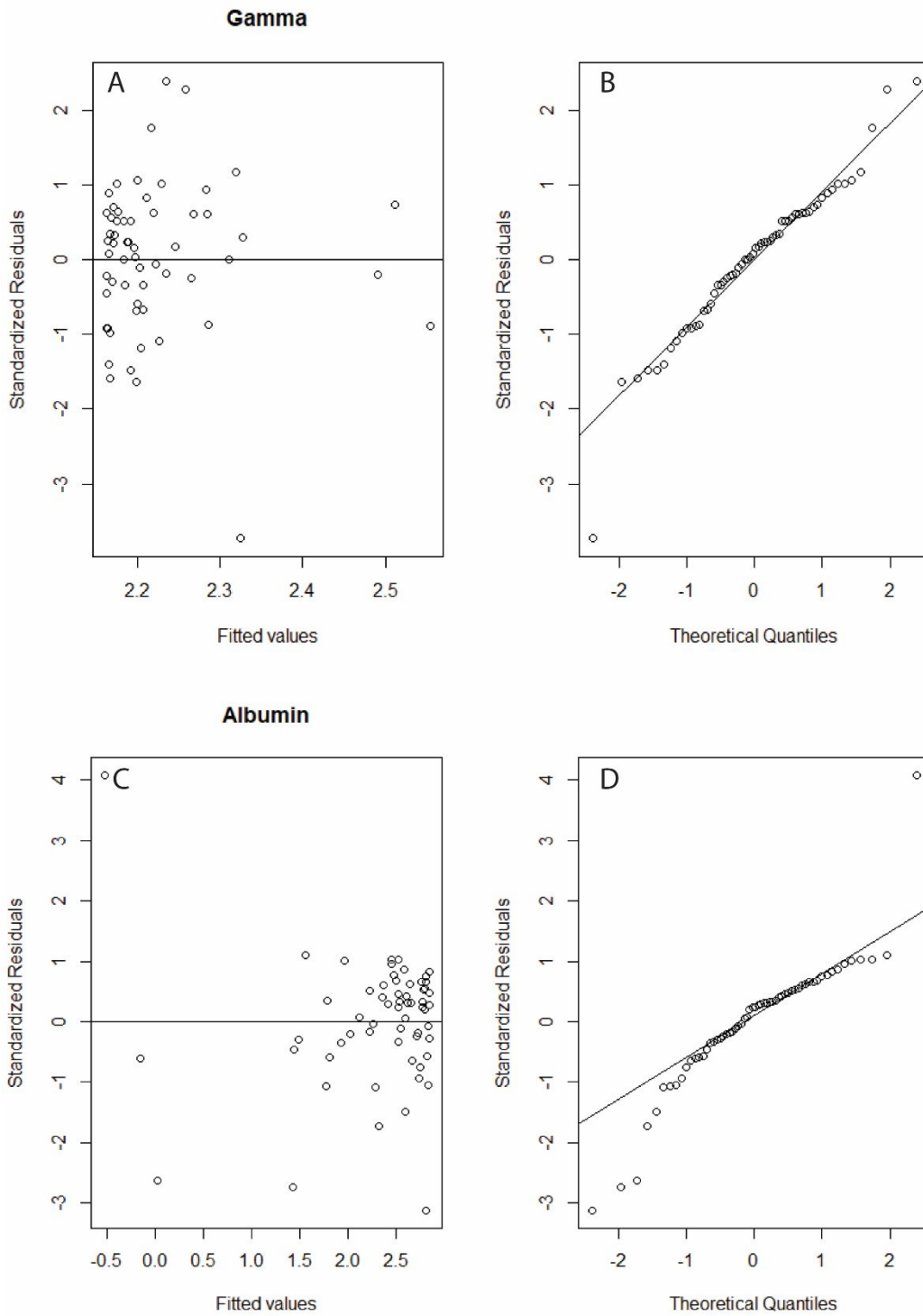
86  
 87 Figure S2. Model diagnostic plots for the final model of standard metabolic rate (SMR) showing  
 88 A) standardized residuals and fitted values and B) Q-Q plot for normal distribution of residuals.



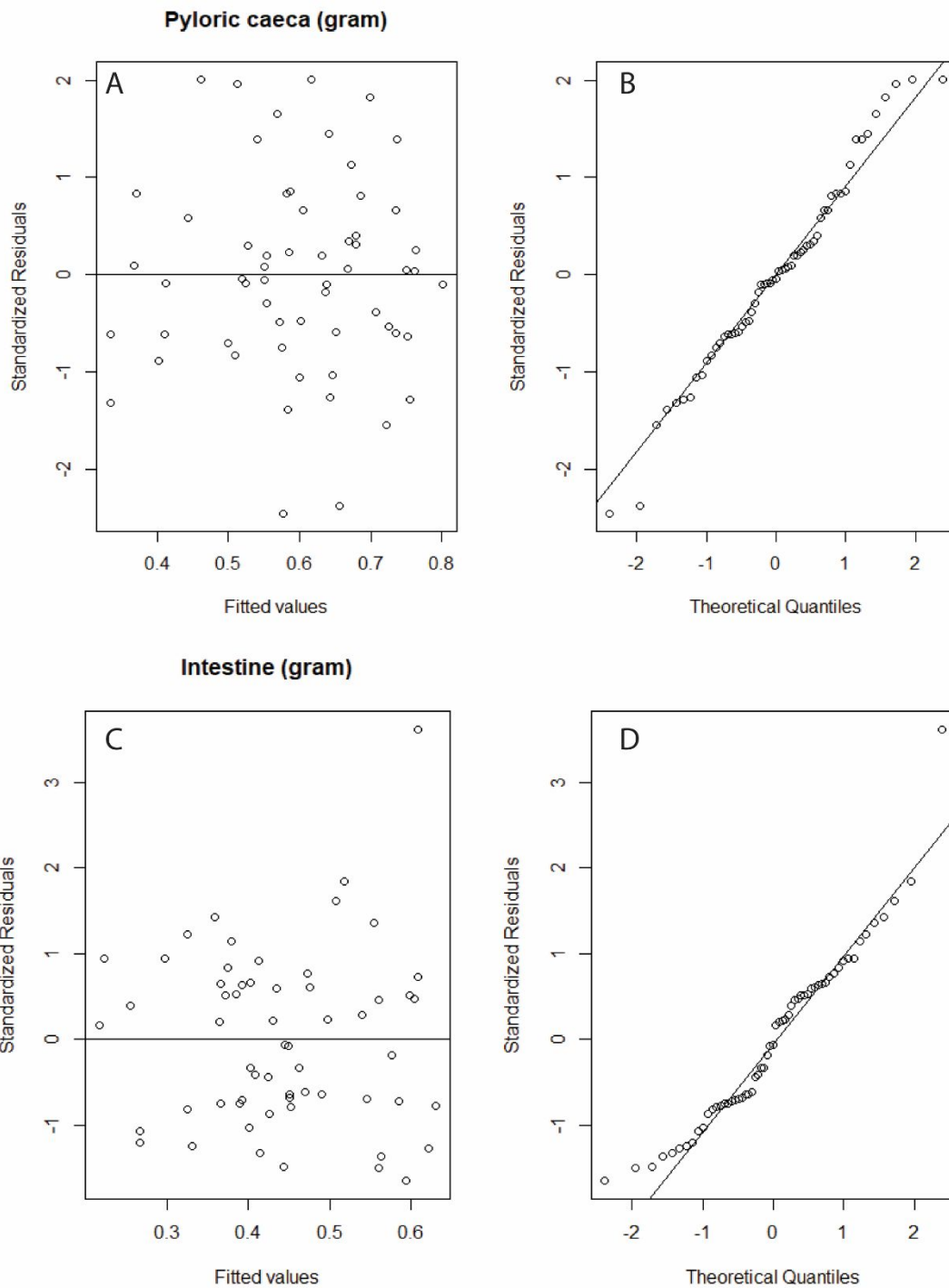
89 Figure S3. Model diagnostic plots for the final model of total protein (A+B) and globulins (C+D)  
90 in blood showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal  
91 distribution of residuals.



92 Figure S4. Model diagnostic plots for the final model of A/G ratio (A+B) and prealbumin (C+D)  
 93 showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution  
 94 of residuals.

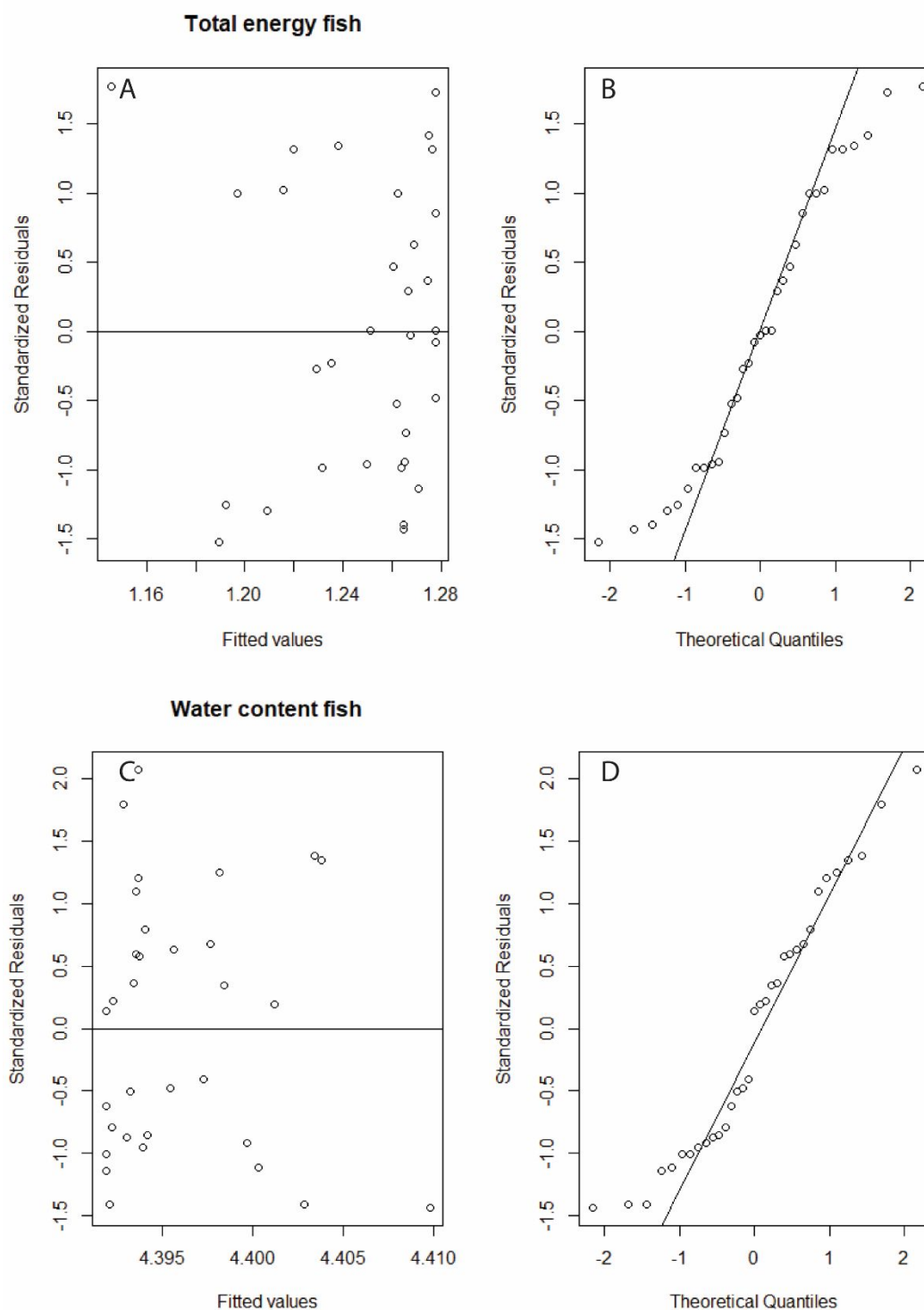


95 Figure S5. Model diagnostic plots for the final model of gamma (A+B) and albumin (C+D)  
96 showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution  
97 of residuals.

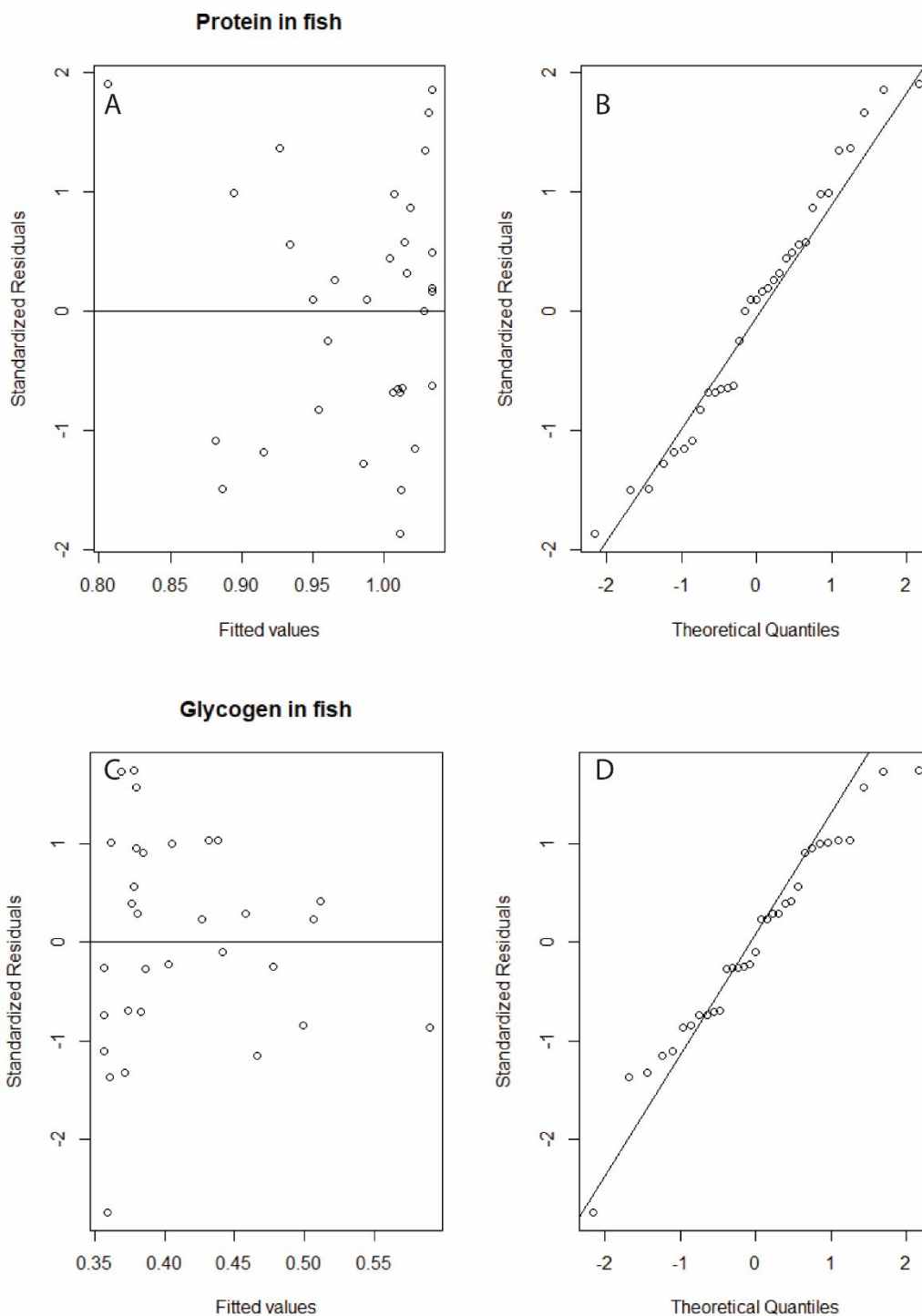


98 Figure S6. Model diagnostic plots for the final model of pyloric caeca (A+B) and intestine (C+D)  
 99 showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution  
 100 of residuals.

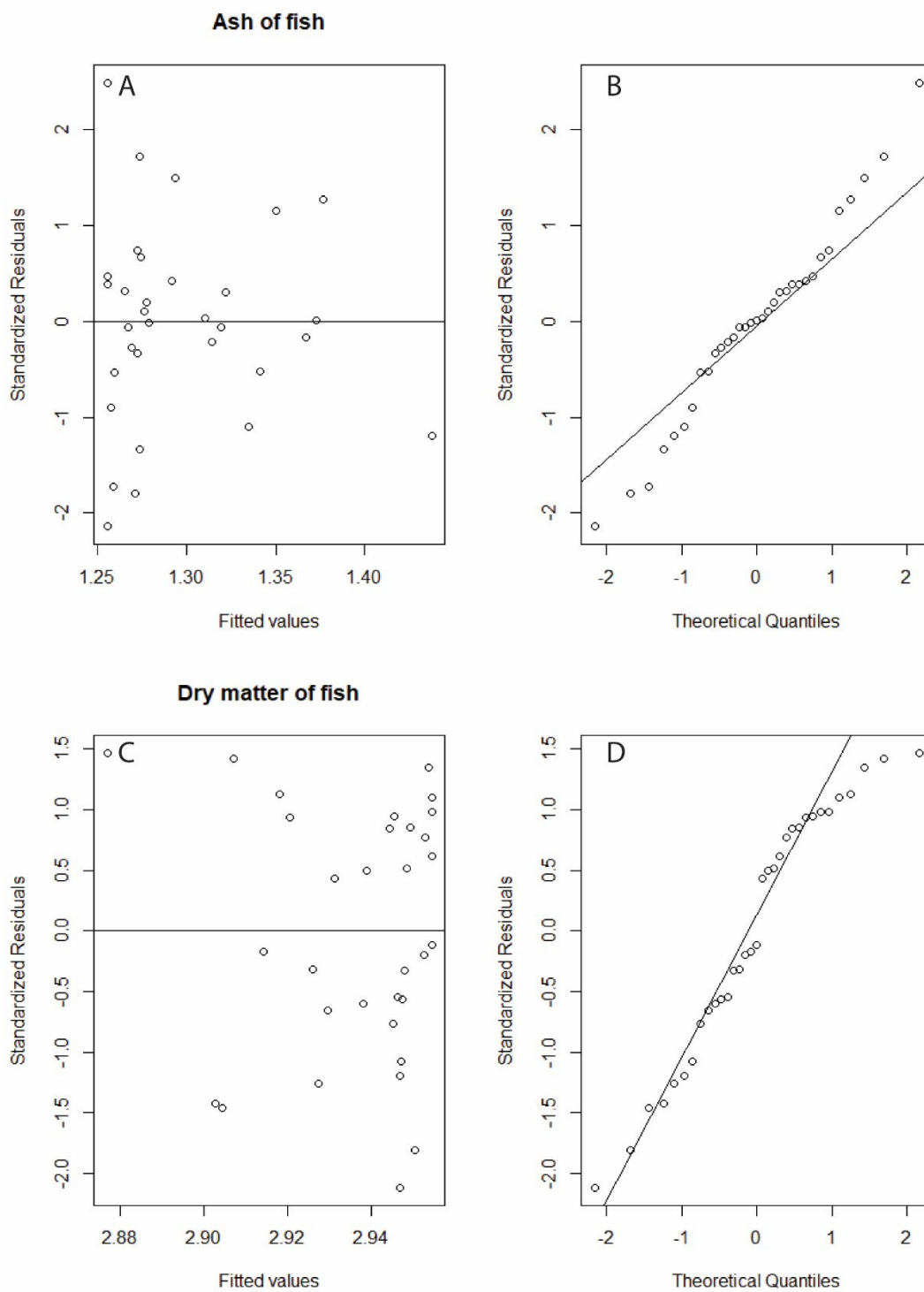




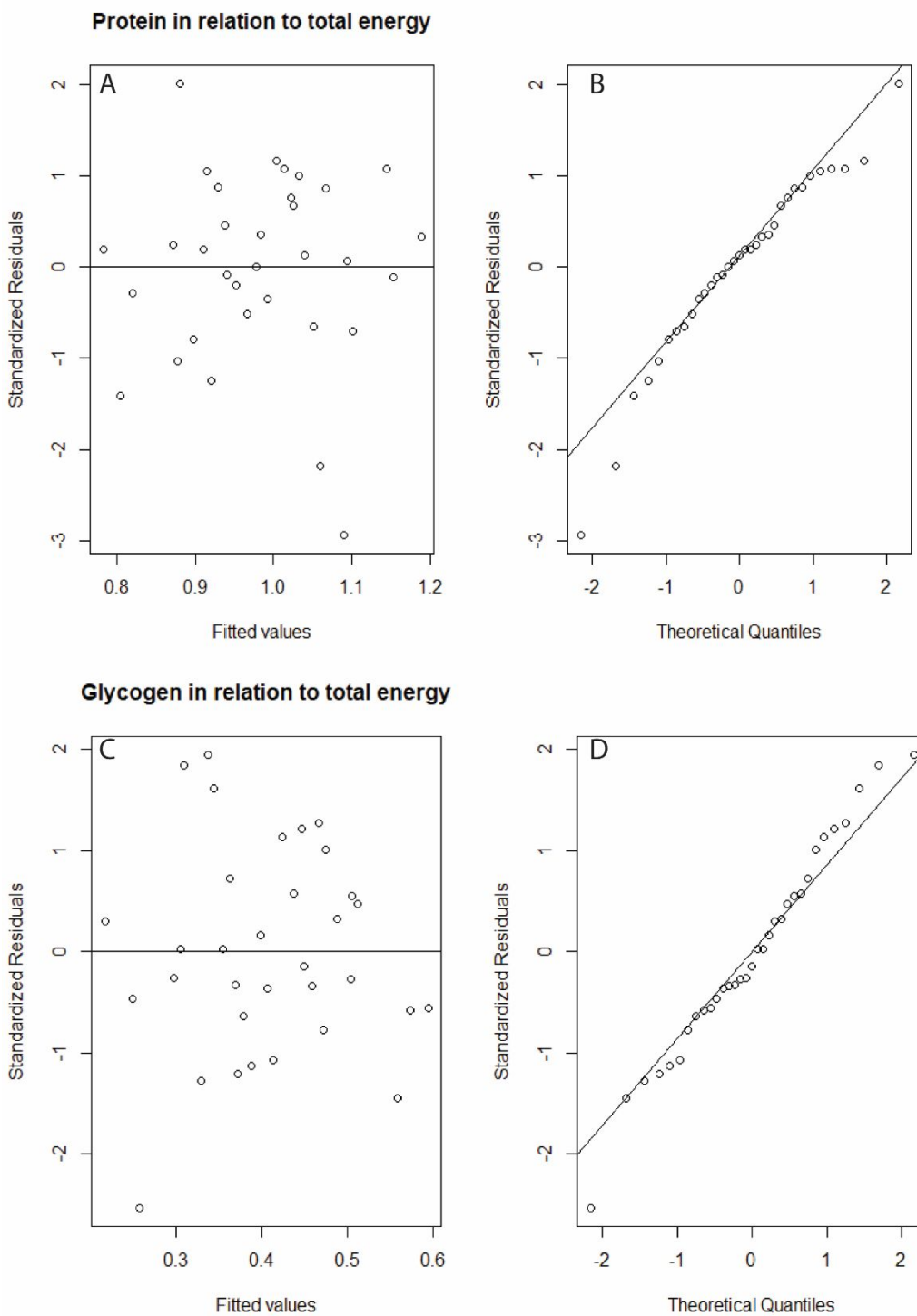
101 Figure S7. Model diagnostic plots for the final model of total energy (A+B) and water of fish  
 102 (C+D) showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal  
 103 distribution of residuals.



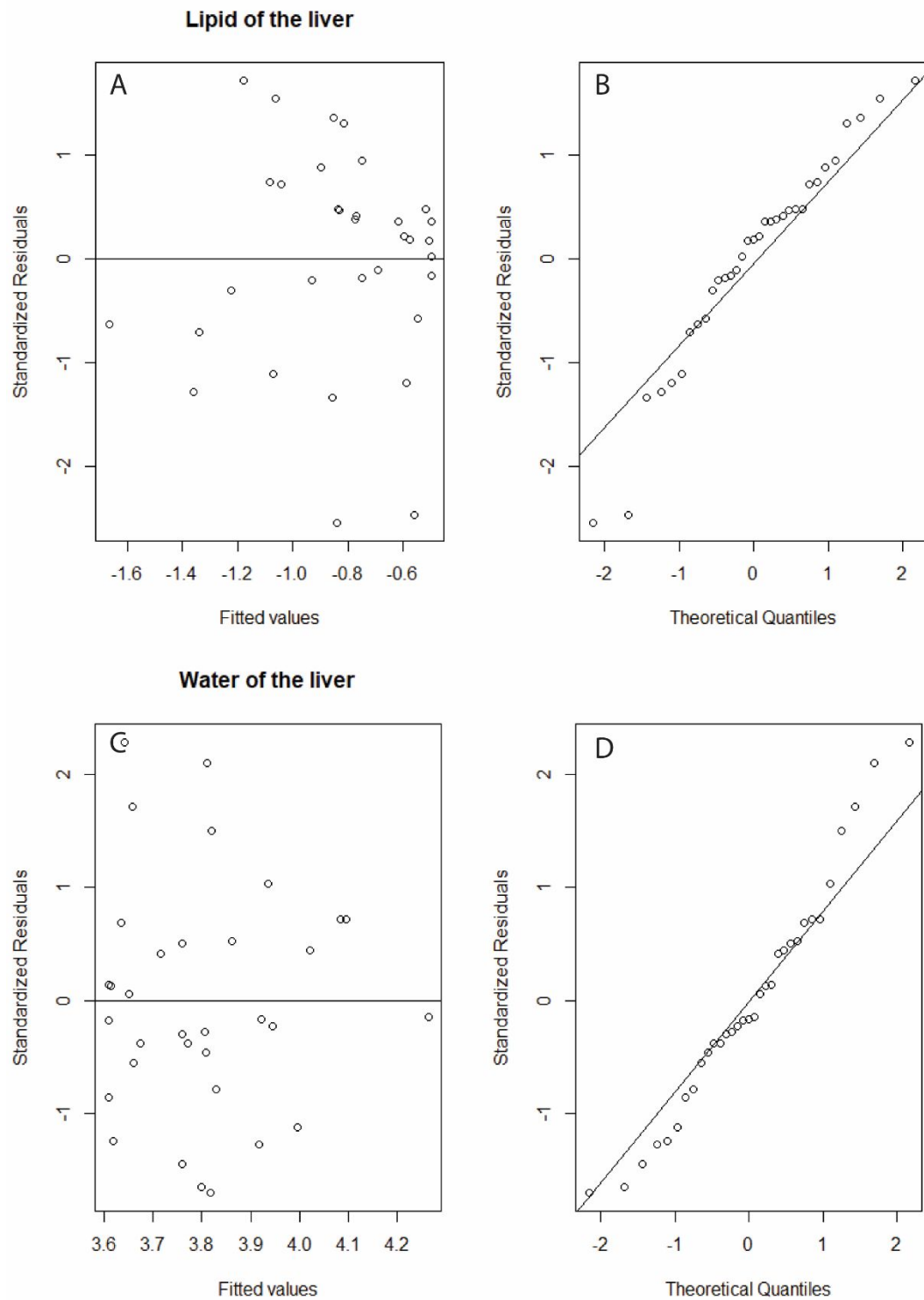
104 Figure S8. Model diagnostic plots for the final model of protein (A+B) and glycogen in fish (C+D)  
 105 showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution  
 106 of residuals.



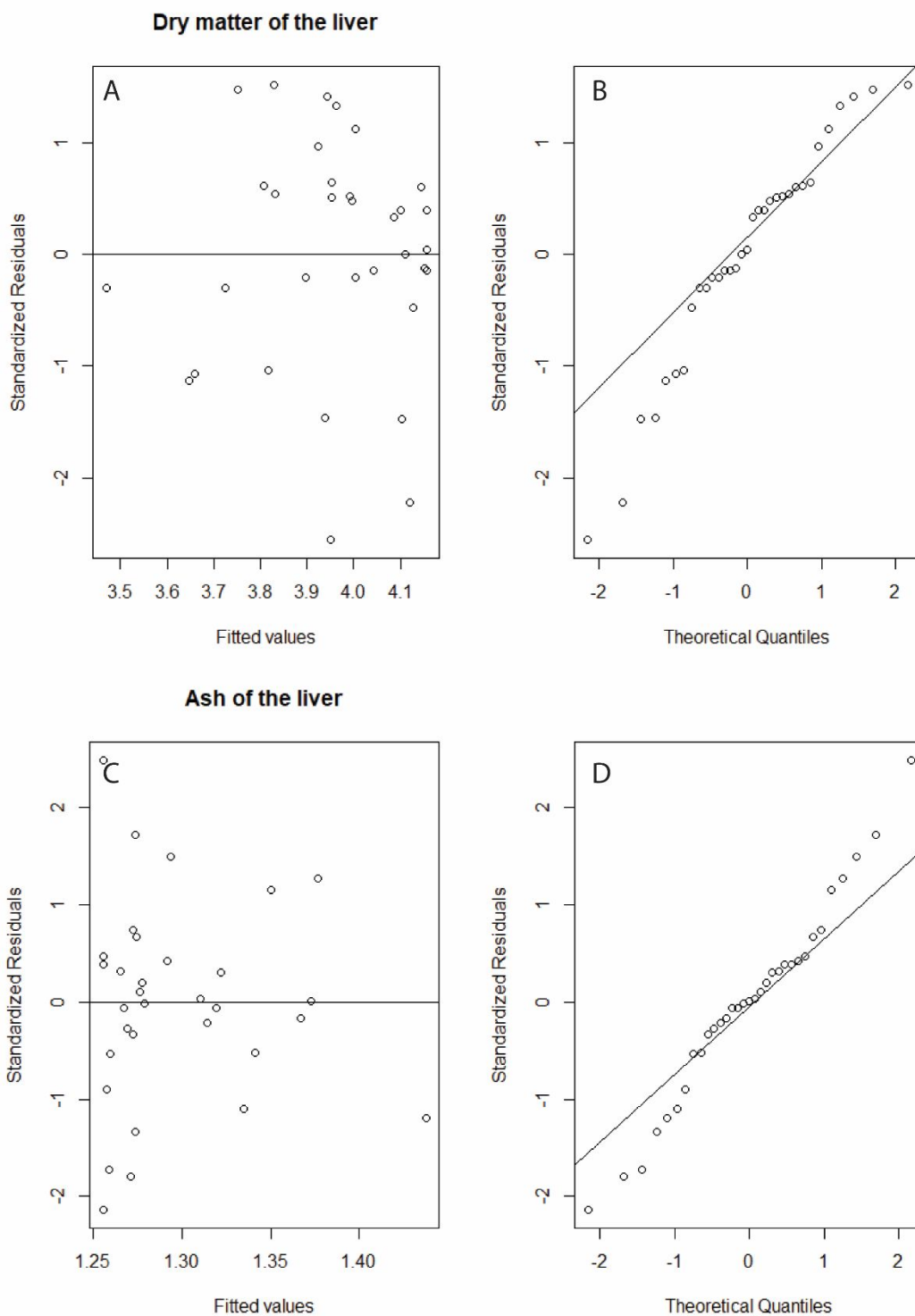
107 Figure S9. Model diagnostic plots for the final model of Ash (A+B) and dry matter of fish (C+D)  
108 showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution  
109 of residuals.



110 Figure S10. Model diagnostic plots for the final model of protein (A+B) and glycogen versus total  
 111 energy (C+D) showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for  
 112 normal distribution of residuals.



113 Figure S11. Model diagnostic plots for the final model of lipid (A+B) and water of liver (C+D)  
114 showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution  
115 of residuals.



116 Figure S12. Model diagnostic plots for the final model of dry matter (A+B) and ash of liver (C+D)  
 117 showing A+C) standardized residuals and fitted values and B+D) Q-Q plot for normal distribution  
 118 of residuals.