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Faculty of Biosciences, Fisheries and Economics
Department of Arctic and Marine Biology

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Bio-3950 Master thesis in Biology
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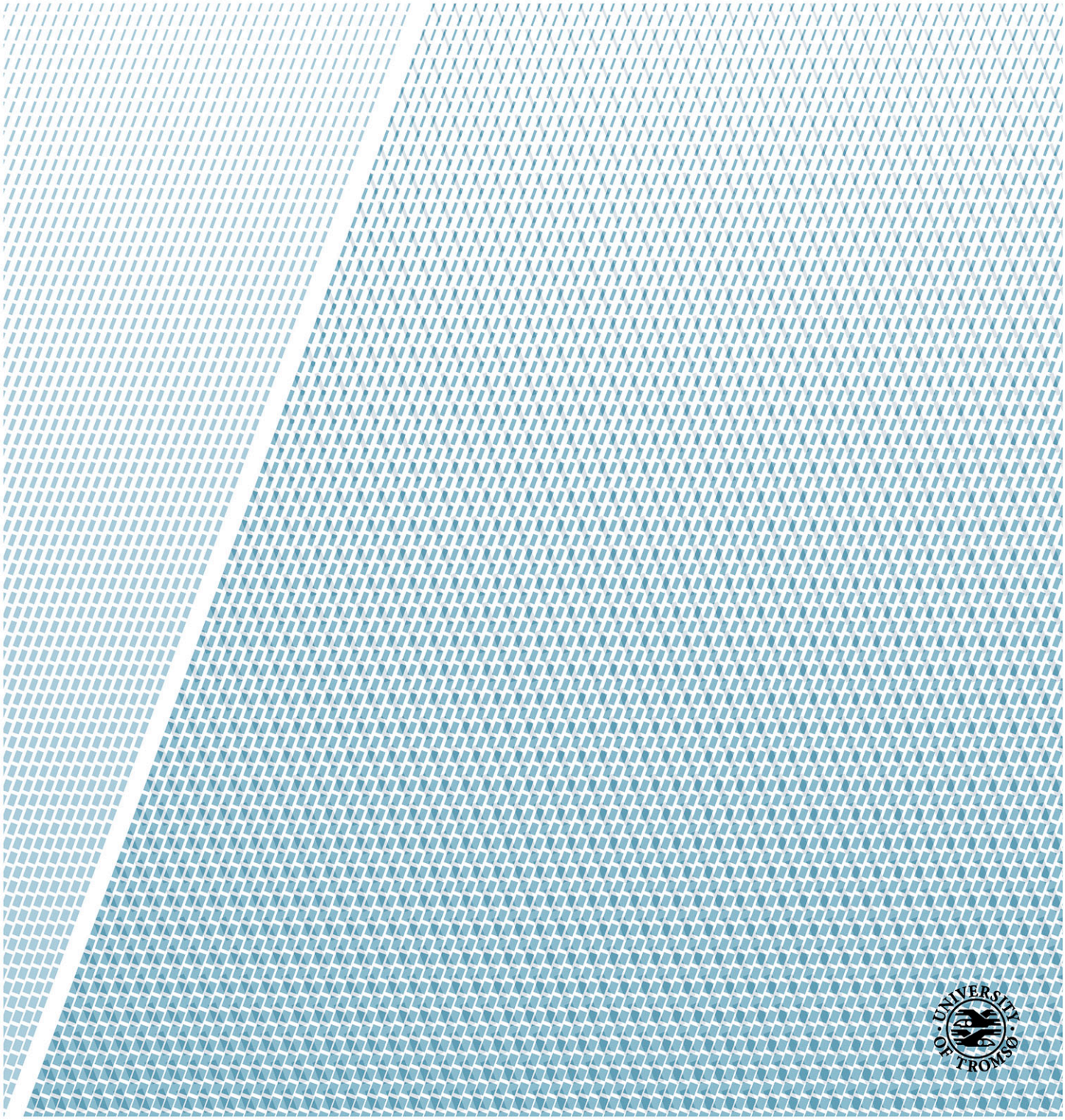

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Acknowledgement

First of all, I would like to show my appreciation to my main supervisors Per and Willy, for your patience, motivation, enthusiasm, and endless knowledge. Thank you for all the opportunities I have been given during the course of my thesis.

To everyone that has contributed to make this project happen, your help has been invaluable. You include, but are not limited to: Egil Karlsbakk (UiB) for having shaped this study. Olav Kjesbu (IMR), Jon Egil Skjæraasen (IMR) and Merete Fonn (IMR) for completing all the gonad analyses. Emma Källgren (UiT), for excellent expertise in the lab. As well as the technicians at the IMR, for being most helpful in collecting the samples for this study.

And a special acknowledgement to my dear friend and mentor, Ken MacKenzie. for your incredible hospitality and for igniting in me a passion for this field of study.

Lastly, a great thanks goes out to friends, family and fellow biology students, for your support, encouragement, ideas and inspiration. I am forever grateful!

Tromsø, July 2017
Anja Helene Alvestad.

Abstract

Parasites play a key role in the biodiversity and dynamics of an ecosystem, either by affecting host mortality and/or host reproduction. Spawning comes with a great cost in time and energy, and in times of poor conditions this time and energy is better directed into growth and survival to increase future success. The phenomenon of skipped spawning, where sexually mature fish skip a reproductive event, is a common occurence in the NEAC. Previous studies suggest that as much as 24-30% of females skip annual spawning, and instead remain at the feeding grounds. The causes for this is still unclear but it seems to occur in response to poor condition. As parasites, by definiton, have a negative effect on host fitness it is of finterest to see if parasites could affect the decision to skip spawning. In this study, we explore the effects of parasites on reproductive parameters in the Northeast Arctic cod (NEAC), the largest cod stock in the world. By comparing intensity of infestation in skippers and spawners we found that parasites do not seem to have an effect on the reproductive abilities of the NEAC. No significant differences in parasite intensity could be detected between skippers and spawners in any of the species included in our study. Nor were there any evidence to show that increase in parasite intensity were associated with decreased fecundity. Even an attempt to look at the effects of the whole parasite community by applying an intensity rank index did not find any correlation between infestation and reproductive parameters. However, caution must be made when making conclusions about parasites effect on host population dynamics. This demonstrates the importance of experimental studies of relationships between parasites and reproductive success. As well as look at parasite-induced host mortality in NEAC.

Table of content

Introduction

Parasitism is one of the most successful life strategies of all living systems, as shown by its impressive diversity and sheer numbers (Poulin & Morand, 2000). By definition, parasites have a negative effect on host fitness, reducing growth, reproduction and survival (Holmstad et al., 2005). On a large scale, this could mean regulation of populations or altering of community composition, thus playing a key role in the dynamics of an ecosystem (Tompkins et al., 2011). Dobson et al (2008) estimated that as much as 75 % of the links in food webs involve parasitic species, thereby considerably influencing food web structure.

The world's oceans cover 71% of the surface of the planet, making the marine ecosystems of great interest for ecological studies. The Barents Sea covers approximately 1.4 mill km^2 and extends from the Norwegian Sea in the west, to the coasts of Norway and Russia in the south, and the coast of Novaya Zemlya in the east (Jakobsen et al., 2011). It is a rather shallow shelf-area with input of temperate waters from the Gulf Stream and colder waters from the Arctic. This makes the Barents Sea one of the most productive oceans in the world and therefor also an area of great importance for the Norwegian fishing industry (Jakobsen et al, 2011).

One of the more valuable key fish species in this ecosystem is the Atlantic cod, *Gadus Morhua*. This is one of the most abundant and widespread piscivorous species in the North Atlantic, with a distribution ranging from Greenland to Cape Hatteras, in the western Atlantic Ocean, and from Svalbard to the British Isles and the northern Bay of Biscay, in the northeast Atlantic Ocean. The cod has been of great historic significance for many North Atlantic countries, and is to date still among the most important of all commercial fishes (Hemmingsen & MacKenzie, 2001).

In this study we examine the hypothesis that both the parasite communities as well as single parasite species are important for host population dynamics in North East Arctic cod (NEAC) , the largest cod stock in the world. To do this one can look at parameters that describe either host reproduction or mortality. We have chosen the former as the focus of this study, as it is more feasible to measure central parameters that describes reproduction than mortality on an individual level.

Reproduction is a costly feature of life. The process of spawning comes with a great demand for energy for marine fish, requiring a sufficient store of energy for vitellogenesis, as well as for potential migration. While it is commonly assumed that iteroparous fish spawn annually after having reached sexual maturity, this may not always be the case. Life history models of the NEAC, for instance, focusing on energy allocation, predict that as much as 30% of sexually mature biomass skip annual spawning (Jørgensen et al., 2006). This has been confirmed by a field study conducted by Skjæraasen et al (2012), that approximated that skippers and spawners were equally abundant, constituting 24% and 25% respectively, of the total biomass (the remainder being immatures). This showing that skipped spawning is a common occurrence in NEAC. The NEAC resides most of the year mainly on the warm side of the polar front in the Barents Sea but it's distribution ranges all the way up north of Spitsbergen and east to Novaya Zemlya (McBride et al., 2016). They undertake a long annual migration to their spawning grounds off Lofoten in January (Bergstad et al., 1987). This migration comes with a cost in energy, time and possible mortality, therefore the potential benefit of reproduction has to be traded off against migration costs. A trade-off between reproduction, growth and survival must be made, as life-long energy allocation - to growth, egg production or energy storage - affects an organism's life history. Due to phenotypic plasticity of life history, strategies vary in response to physiological and ecological factors (Jørgensen et al., 2006). If an individual is in poor condition, the time and energy it would normally invest in reproduction is better redirected into growth and survival.

Hemmingsen & MacKenzie (2001) reported a rich and diverse parasite fauna associated with Atlantic cod. More than 107 species of protozoan and metazoan parasites have been identified. The omnivorous diet of cod, occurrence at both high and low salinities, and status as one of the most abundant piscivorous species in the North Atlantic are indicated as factors influencing this diversity.

Many empirical studies have been conducted on the impact parasite species may have on their host (Patterson et al., 2013; Lutterschmidt et al., 2007; Hudson et al., 1998, Longshaw et al., 2010; Krkosek et al., 2012), but most seem to focus on a single species of parasite. The effect of multiple infections is only just beginning to be understood, despite the fact that most hosts are subjected to a whole community of parasite species. Holmstad et al (2005) showed that

the parasite community was negatively correlated with host parameters in willow ptarmigans (*Lagopus lagopus*), specifically reduced host body mass and increased breeding mortality.

The approach taken here is an observational study of parasites and reproduction in NEAC, in the feeding grounds in the Barents Sea and the spawning grounds off the Lofoten islands. We ask two questions. The first is whether parasites affect skipped spawning. As parasites can have a profound impact on host fitness, one would expect to see a negative correlation between parasite intensity and skipping, i.e. individuals with a heavier parasite load will skip spawning and instead will remain at the feeding grounds in the Barents Sea. The second question is whether parasites affect fecundity, i.e. would we find a negative correlation between estimated fecundity and parasite intensity in the spawning population.

Material and methods

Cod samples

The study is based on female fish sampled in two regions of the North Atlantic in 2015 as part of an annual survey conducted by the Institute of Marine Research (IMR), from the research vessel Johan Hjort. The first sampling took place in February in the Barents Sea (Fig 1). The second sampling was conducted in March, at the main spawning grounds of the NEAC, close to the Norwegian coast, near Vesterålen, Lofoten islands (Fig 1, map insert).

Figure 1**:** Map of sampling areas in the Barents Sea and, outside Vesterålen, Lofoten islands (map inset), highlighted in blue. The fish examined in this study was collected from a total of 115 stations in the Barents Sea, and 36 stations in Lofoten.

Each of the fish were measured at fork length and weighed. In an attempt to target individuals with a high probability of having spawned before, a selection of females in the size range 65 cm and upwards were collected for further examination (Barents Sea: N=123, Lofoten islands: N=121). The age of the individuals was later assessed by otolith readings. These readings also confirmed that all samples used in this study were from NEAC and not adjacent coastal cod stocks. In addition, liver and gonads were weighed separately to the nearest gram. These measurements were done by the technicians from IMR, Bergen.

Parasite samples

For this study I have chosen parasite species that are thought to be common in the NEAC, especially pathogenic or both. The following 12 species were therefore selected (Willy Hemmingsen & Ken MacKenzie pers. comm.). The nematodes *Anisakis simplex*, *Contracaecum osculatum*, *Hysterothylacium aduncum* and *Pseudoterranova decipiens*, the copepods *Laernaeocera branchialis* and *Cresseyus confuses,* the cestode *Abothrium gadi* and *Pyramicocephalus phocarum*, the acanthocephala *Echinorhynchus gadi,* myxosporean *Myxidium oviforme* and *Myxidium gadi* and metacercarian *Prosorhynchoides borealis.*

The gills, nasal cavity, cranial cavity and internal organs (intestines, pyloric caeca, liver and gall bladder) were examined separately. Parasites were extracted, identified down to species level based on external morphology and counted. Due to a tight schedule, some of the intestines and pyloric caeca were labeled and frozen for later examination. The rest were examined on board the research vessel using a dissecting microscope at 20 to 40 X magnification, or a compound microscope at 200 to 400 X for the smaller myxosporeans. To examine the cranial cavity for *P. borealis*), and the gills, for *L. branchialis*, a microscope was not needed, as these could be easily inspected with the naked eye.

The pyloric caeca were gently removed from the stomach and the intestine. An extra precaution was made when separating the caeca from the intestine, as to not fracture specimen of the cestode *A. gadi,* commonly found with the scolex (head) embedded inside the pyloric caeca with the body extending out into the intestine. The outside was then examined for nematode larvae (*A. simplex*, *C. osculatum*, *H. aduncum*) and cestodes (*P. phocarum)*.

The liver was examined externally first, then pulped by hand using tweezer, looking for nematode larvae (*A. simplex*, *C. osculatum, P. decipiens*).

The intestine was cut open longitudinally from the anal opening to the pyloric caeca and content was extracted and inspected. Here we expected to find adult nematodes (*H. aduncum*), acanthocephalans (*E. gadi*) and cestodes (*A. gadi*). After being extracted and counted, *H.aduncum* and *E.gadi* were preserved separately in 96% ethanol for future weighing. In addition, nematode larvae (*A. simplex*, *C. osculatum*) can often be found on the outer wall of the intestines, in the mesenteries, or embedded in the wall itself. Of these, only parasites in the wall was counted and made note of.

Slides were made of the gall from the gall bladder and examined under 200 to 400 X magnification for myxosporean (*M. oviforme*, *M. gadi*). These were noted as present (1) or absent (0). The nasal cavity was removed and examined for the parasitic copepod *C. confuses*. An estimate of number of individuals was made by counting the egg sacs, and prescribing 2 egg sacks per individual.

Parasites from the external body surface, skin and eyes, were not included in this study due to the handling of the fish. Stomachs were not available for examination as they were required for content analysis by the IMR.

Parasite weighing

The intestinal parasites *H. adundum* and *E. gadi* were dried and weighed to estimate the effect of biomass as well as intensity.

Parasites retrieved from individual fish were placed in individual pans; *H.aduncum* in one pan, *E.gadi* in another; and given a category based on the size of the sample; small or large. A trial run in the drying cabinet determined the time needed to thoroughly dehydrate the samples. Three samples from both species were placed in a 60°C drying cabinet, and monitored hourly until they no longer lost weight. Our findings indicate that small specimens require a minimum of 24hours to dry out, while large ones require 48.

By keeping the cabinet temperature steady at 60 degrees, the specimens remain undamaged if kept in the cabinet after thoroughly drying out.

After drying, each of the samples were weighed on a scale 3 consecutive times and an average was estimated for each of the samples. For samples with a wet weight of less than 0.01 g, a micro scale was used to measure dry weight.

Gonad analysis

A small subsample was taken from the right ovarian lobe and immediately fixed in formalin (4%) to determine which ones were skippers and which were spawners, as well as estimating the fecundity of the spawning part of the sample. These samples were subjected to image analysis as described by Thoresen & Kjesbu (2001), a method for estimating oocyte density by determining the average diameter of yolk containing (vitellogenic) oocytes. Potential fecundity, i.e. the standing stock of maturing oocytes in the pre-spawning stock, could then be calculated as

$$F = OW * OD^{-2.70} * 2.503 * 10^{11} \quad (1)$$

where F is potential fecundity, OW is ovary weight (g) and OD is mean oocyte diameter (μm).

Only spawning individuals in the vitellogenic stage of gonad development (N=46) can be used to calculate such estimates.

In addition, image analysis is applied to separate the developing from the nondeveloping individuals. By then conducting follicle analysis on the nondeveloping portion of the Barents Sea-sample, skippers could be separated from immatures by looking for postovulatory follicles (POF's). After spawning, POF's become apparent in female gonads, persisting for more than a year in cod. Hence, the presence of these follicles reveals that an individual has spawned before.

Both these analyses were performed by professor Olav Kjesbu at the IMR, Bergen.

Statistical analyses

Statistical parameters

There are a number of statistical parameters used in describing parasite data. Among them the terms; mean intensity, mean abundance and prevalence appear frequently.

Bush et al. (1997) define the parameters as follows:

Prevalence (P) is the percentage of hosts infected with a particular parasite species. A numeric value is given by the formula:

(2) $P = (a / N) * 100$,

where a is the number of hosts with the parasite species and N is the total number of hosts examined.

Intensity (I) is the number of a particular parasite species found in a single infected host. For the purpose of this study the mean, or average, intensity is calculated. In other words, it is the average number of parasites species, found on an infected individual within infected hosts in the host population. It is given by the formula:

(3) $I = b / n$,

where b is the total number of parasites within the host sample, and n is the total number of infected hosts.

Abundance (A) is the number of a particular parasite species among all the members of a particular host population, regardless of infection-status (infected/non-infected).
Here again we use the mean, or average values, to analyze the sample. Meaning, the average number of a parasite species among all members of a particular host population.

(4) $A = b / N$

Spearman's rank correlation

A Spearman's rank-order correlation was used to estimate the correlation between the variables. The coefficient, or Spearman's rho, obtain from such an analysis is a non-parametric measure of rank correlation, and measures the strength and direction of this monotonic relationship; a value of +1/-1 representing a perfect correlation (Sokal et al. 2012). A p-value smaller than 0.05 indicate a significant result.

Mann-Whitney U test

A Mann-Whitney U-test is a non-parametric test that compares two sample means, originating from the same population, to see if they are equal or not (Sokal et al. 2012). It is used when normal distribution criteria is not met. A p-value smaller than 0.05 indicate a significant result.

Community index

To examine the effects of total parasite community on its host we created a community index by ranking each parasite species according to Holmstad et al (2005). A score was assigned to each parasite intensity; 1 being the lowest count and then increasing scores for higher intensities. Zero values were excluded. For individuals with equal intensities, a mean value was assigned. The sum of all ranks for the parasite species within an individual host was then calculated, to reflect the relative intensity of infection in that particular host. A Mann-Whitney U-test was conducted to compare the sum of ranks in skippers versus spawners. A Spearman's rank-order correlation was used to test for correlations between sum of ranks and fecundity estimates for the spawners.

Ranking was only performed on hosts with complete data sets for all parasite species (skippers N=47, spawners N=56).

Software used for computing statistical analysis and creating graphs include R studio (version 0.98.1091) and Microsoft Excel (2011). The map was created by graphic designer from UiT, Tove Midtun.

Results

Weight and age distribution

A total of 244 fish were examined; 123 skippers and 121 spawners. They varied in age from 5 to 12 years, with a mean age of 6.9 years for skippers and 8.3 for spawners (Fig 2). A Mann-Whitney U-test showed that spawners were significantly older than skippers (Fig 2). The same goes for host body weight, spawners being significantly heavier than skippers, with a mean total body weight of 4383 g for spawners and 2503 for skippers (Fig 3).

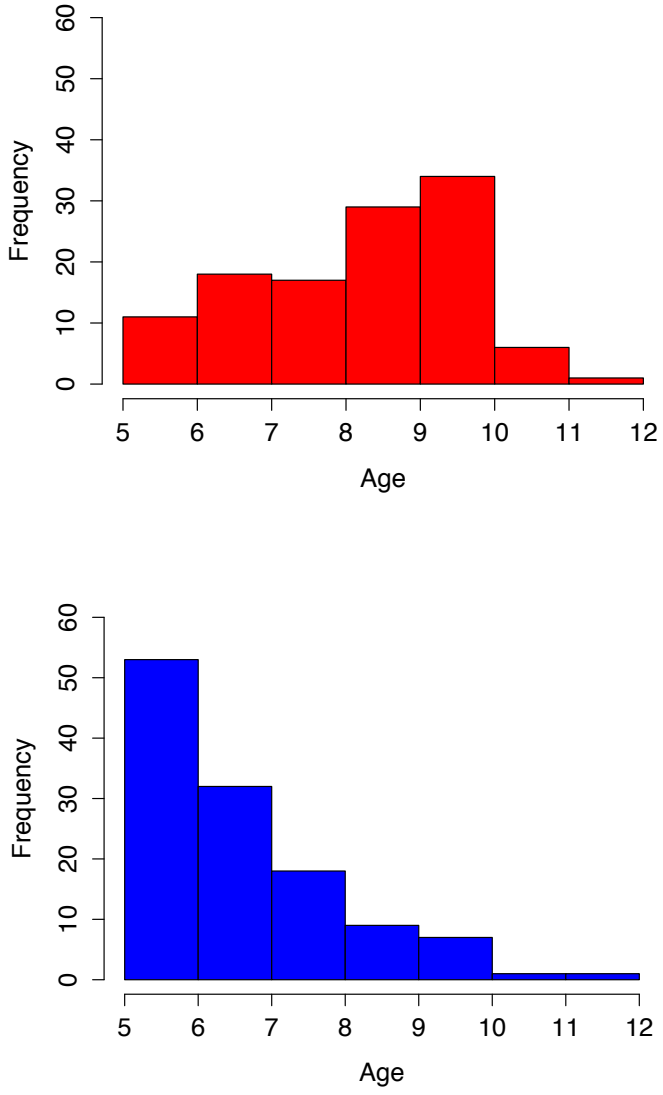

Figure 2: Age distribution of cod; spawners (top) and skippers (bottom). Results from Mann Witney U-test for difference between skippers and spawners in age are as follows W=11011, $p<<0.05$.

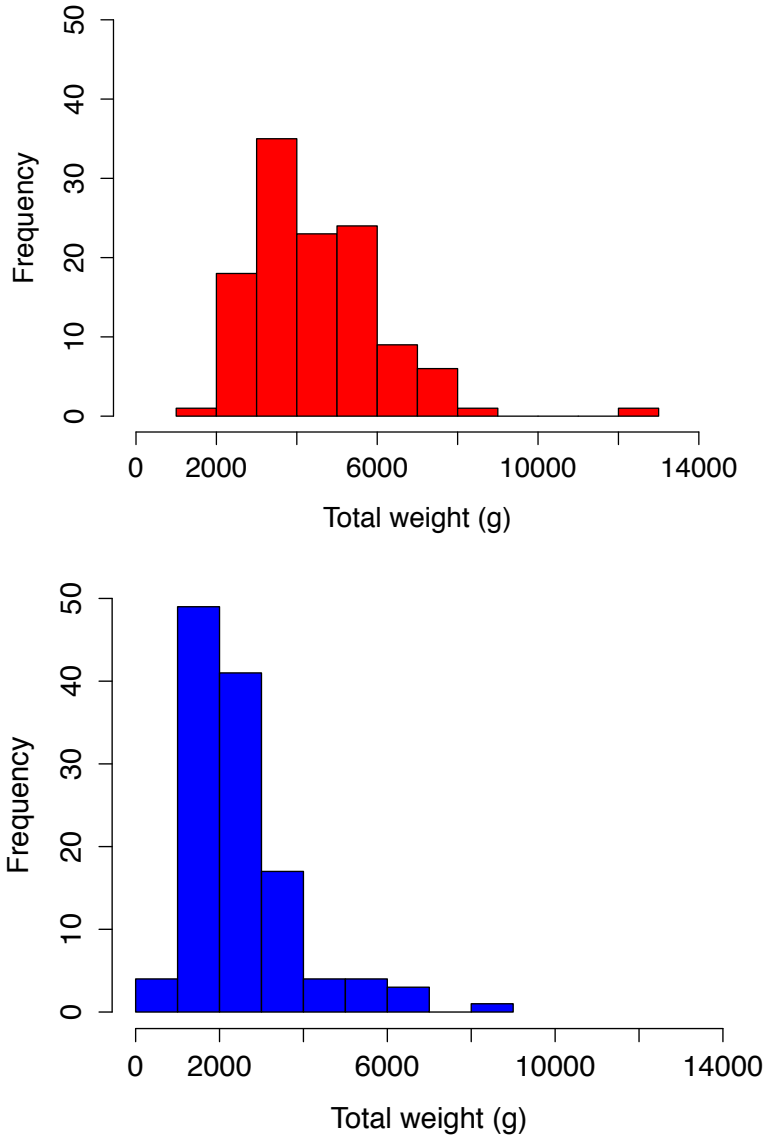

Figure 3: Total body weight distribution of cod; spawners (top) and skippers (bottom). Results from Mann Witney U-test for difference between skippers and spawners in total host body weight are as follows W=12509, p<<0.05.

Parasites

Altogether, 11 of the parasite species included in this study were found (Table 1). The metacercarian *P. borealis* was the only species not located. All the fish were parasitized. The dominant taxonomic group of parasites was nematodes (4 species), comprising the absolute majority of the samples; 94% of the total number of individuals in the skipper-group and 96%

of the spawners. Overall an equal number of parasite species were represented in adult and larval form. However, there were more parasite individuals in larval than in adult form; 56% for skippers and 62% for spawners. The majority of these were anisakid nematodes, which made up 43% (skippers) and 47%(spawners) of the total number of individuals. Two of the nematode species, the adult *H. aduncum* and the larval *A. simplex* showed the highest prevalence (95% and 98/96% respectively).

Table 1: The parasites collected in this study, and their location in/on the host and their life stage.

Parasite species	**Location in/on host**	**Life stage**
Nematoda		
Anisakis simplex (Rudolphi, 1802)	Visceral cavity Liver Pyloric caeca Musculature	Larval
Contracaecum osculatum (Rudolphi, 1802)	Visceral cavity Liver Pyloric caeca Musculature	Larval
Pseudoterranova decipiens (Krabbe, 1878)	Musculature Liver	Larval
Hysterothylacium aduncum (Rudolphi, 1802)	Visceral cavity Pyloric caeca Musculature	Larval
Hysterothylacium aduncum (Rudolphi, 1802)	Intestine Stomach	Adult
Eucestoda		
Abothrium gadi Van Beneden, 1871	Intestine Pyloric caeca	Adult
Pyramicocephalus phocarum (Fabricius, 1780)	Visceral cavity Intestine	Larval
Copepoda		
Lernaeocera branchialis (Linnaeus, 1767)	Gills	Adult
Cresseyus confusus (Stock, 1953)	Nasal cavities	Adult
Acanthocephala		

Echinorhynchus gadi Zoega in Müller, 1776	Intestine	Adult
Myxosporea		
Myxidium gadi Georgévitch, 1916	Gall bladder	Cyst
Myxidium oviforme Parisi, 1912	Gall bladder	Cyst

The prevalence, mean intensities and abundance for all the parasite species are given in Appendix table 1. Only prevalence is calculated for myxosporedeans, as the data is based on recordings of presence (1) or absence (0).

Given the close morphological resemblance between larval nematodes *A. simplex*, *C. osculatum* and larval *H.aduncum*, these were grouped together for further data analysis. As we did not obtain data from the pyloric caeca of all the individual fish, the nematode larvae were grouped according to their location in the host; as "nematode larvae caeca" (*A. simplex*, *C. osculatum* and *H.aduncum*) and "nematode larvae liver" (*A. simplex* and *C. osculatum*) respectively. In total, 47 skippers and 56 spawners had complete datasets.

Analysis of relationship between skipping and parasite intensities

When looking at the relationship between parasite intensities and total host body weight or age we see few correlations. Of all the results from a Spearman rank analysis, we revealed only one significant correlation between total host body weight and nematode larvae intensity on liver in skippers ($p=0.015$). Results from all the correlation analyses are summarized in Appendix table 2 and 3. We have therefore not controlled for the effects of these variables in the further analysis. Below, plots are shown for nematode larvae (from pyloric caeca and liver), adult *H.aduncum* and *E.gadi* in figure 4 and 5, the plots for the remainder parasite species are found in Appendix figure 1 and 2.

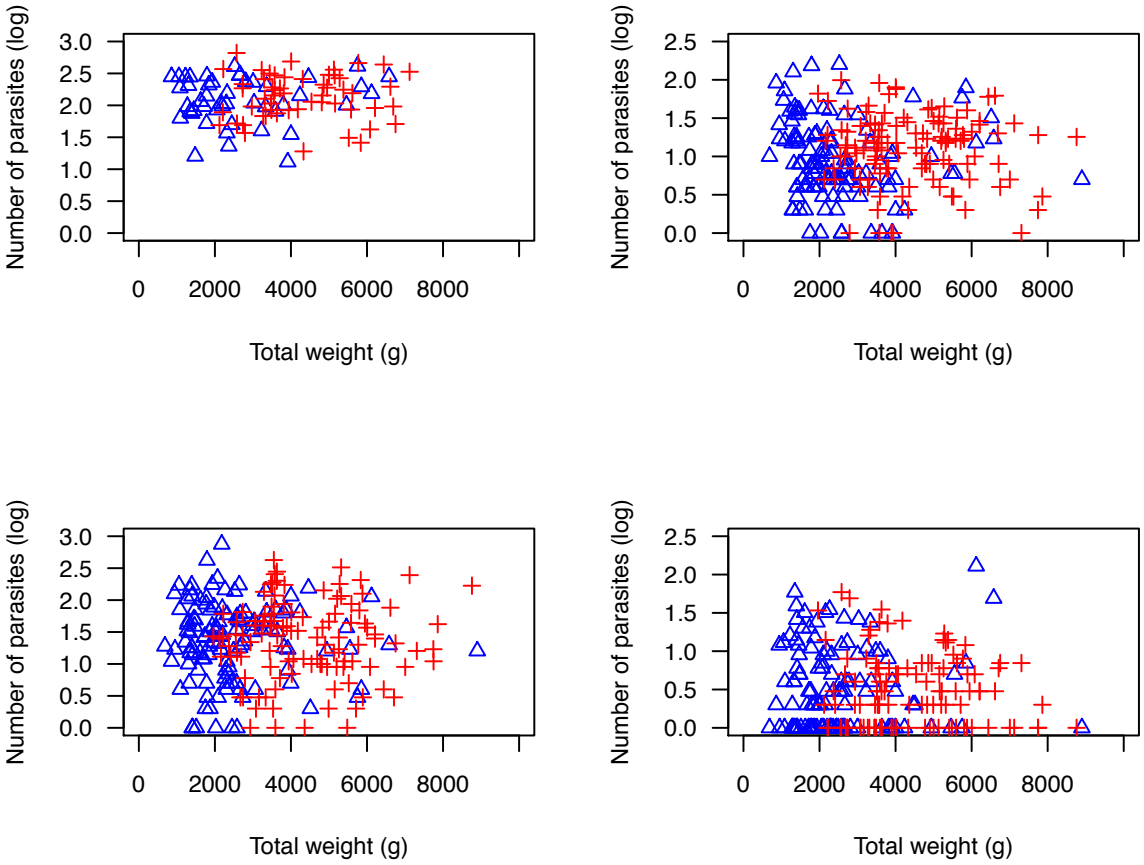

Figure 4: Distribution of number of parasites found in relation to total body weight; skippers depicted in blue, spawners in red. Results from Spearman rank analysis for correlations between parasite intensity and total host body weight are shown in brackets for each parasite variable. From top left: nematode larvae from pyloric caeca (skippers: rho=0.032, p= 0.83, spawner: rho=0.054, p= 0.70); nematode larvae from liver (skipper: rho=-0.221, p= 0.01, spawner: rho=-0.003, p= 0.98); adult *H. aduncum* (skipper: -0.016, p= 0.86, spawner: rho=-0.022, p= 0.82); *E. gadi* (skipper: rho=-0.014, p= 0.88, spawner: rho=-0.021, p= 0.82).

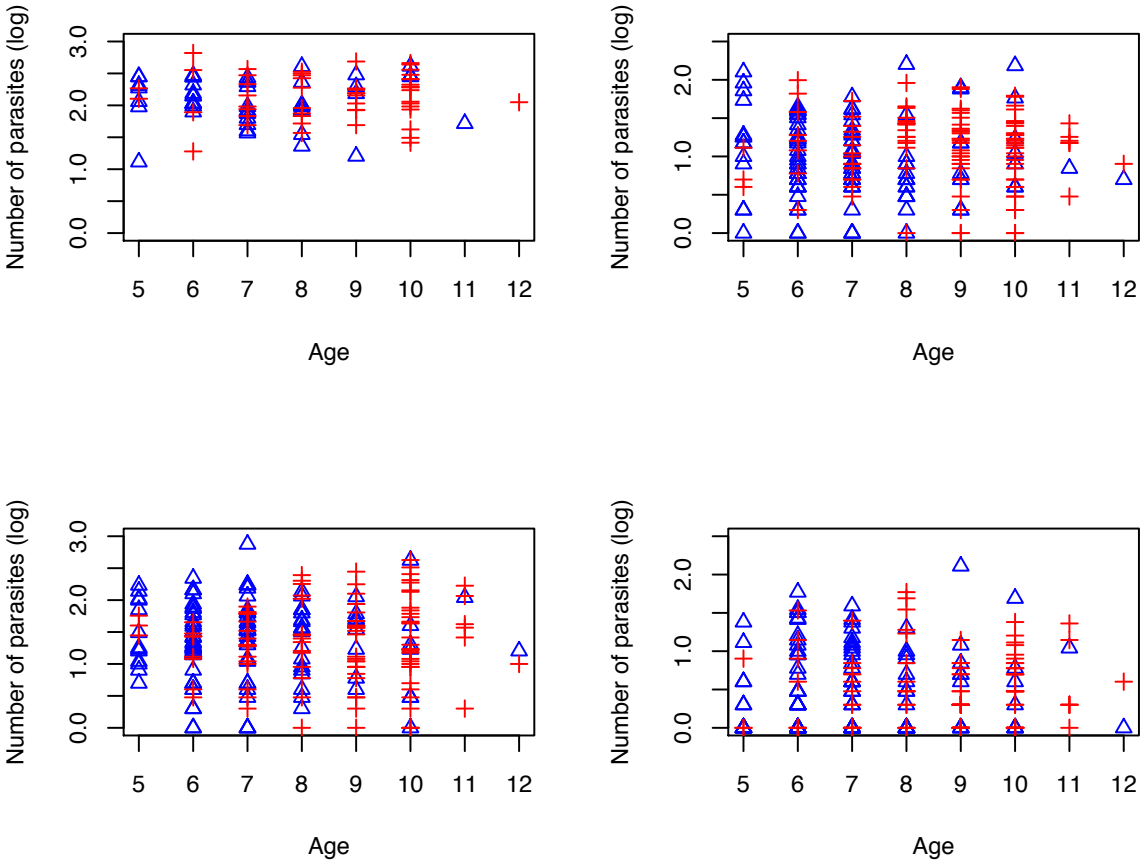

Figure 5: Distribution of number of parasites found in relation to age; skippers depicted in blue, spawners in red. Results from Spearman rank analysis for correlations between parasite intensity and host age are shown in brackets for each parasite variable. From top left: nematode larvae from pyloric caeca (skippers: rho=-0.034, p= 0.82, spawner: rho=0.042, p= 0.77); nematode larvae from liver (skipper: rho=-0.152, p= 0.10, spawner: rho=-0.048, p= 0.61); adult *H. aduncum* (skipper: rho=0.011, p= 0.91, spawner: rho= 0.049, p= 0.61); *E. gadi* (skipper: rho=0.038, p= 0.68, spawner: rho=0.072, p= 0.46).

To be able to study how a parasite's population dynamics can affect its host as a whole community we must evaluate how the parasite species co-vary; to define whether the effects of the parasites should be considered as a whole or to look at the individual parasites. A Spearman's rank-order correlation revealed few significant correlations between the parasite species. Results are shown for nematode larvae (from pyloric caeca and liver), adult *H.aduncum* and *E.gadi* in Table 2. For the other macroparasite species, the results are listed in Appendix table 4. Of these, there were only significant correlations ($p<0.05$) between *E. gadi*

and *A. gadi*, and between *C. confusus* and adult *H. aduncum* as well as liver nematodes in spawners. These results indicate that intensities of the different parasite species do not co-vary to a large extent, which supports the notion that they can be analyzed separately.

Nevertheless, we also analyzed the rank sum of parasite intensities between skippers and spawners to see if there was a cumulated effect of all parasite species. This revealed no significant differences between skippers and spawners (Mann-Whitney U-test, W=1404, p-value>0.05).

Tabell 2: Results from Spearman's rank analysis for correlations between parasite intensities of the different parasite species. Skippers are depicted in blue, spawners in red. The table provides the rank coefficient, rho, with significant values ($p<0.05$) marked *.

	Nematodes (caeca)	Nematodes (liver)	*H.aduncum* adult	*E.gadi*
Nematodes (caeca)		0.570*	0.131	0.036
Nematodes (liver)	0.702*		0.276*	0.225*
H.aduncum adult	0.243*	0.237*		0.215*
E.gadi	-0.209	0.044	0.095	

Intensities of the different parasite species did not differ between the two groups. The result was shown by a Mann-Whitney U-test comparing the parasite intensities of individual species included in this study of skippers and spawners, which showed no significant values (Fig 6). Boxplots are shown for nematode larvae (from pyloric caeca and liver), *H. aduncum* and *E. gadi* in figure 6. For the other species, the boxplots and results from Mann-Whitney U-test are placed in Appendix table 5.

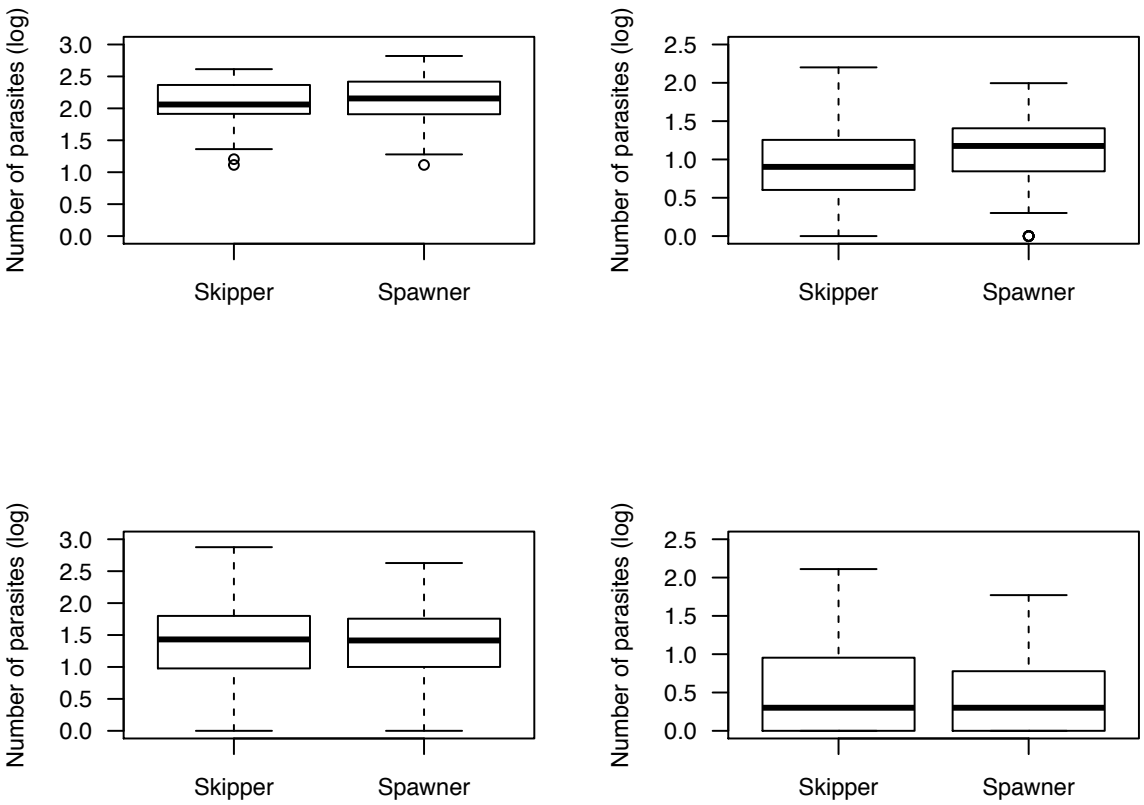

Figure 6: Box plots showing median parasite intensity and border for upper and lower quartile in skippers and spawners. Results from Mann Witney U-test for difference between skippers and spawners in parasite intensity are shown in brackets for each parasite variable. From top left: nematode larvae from pyloric caeca (W = 1373, p=0.71), nematode larvae from liver(W = 8616, p=0.012), Adult *H. aduncum* (W = 6584, p=0.786) and *E. gadi* (W = 6681, p=0.932).

When comparing weight of the intestinal parasites between skippers and spawners, the trends are the same. The dry weight of the two major intestinal parasites, the nematode *H.aduncum* and the acanthocephala *E.gadi*, do not differ between the two groups (Fig 7).

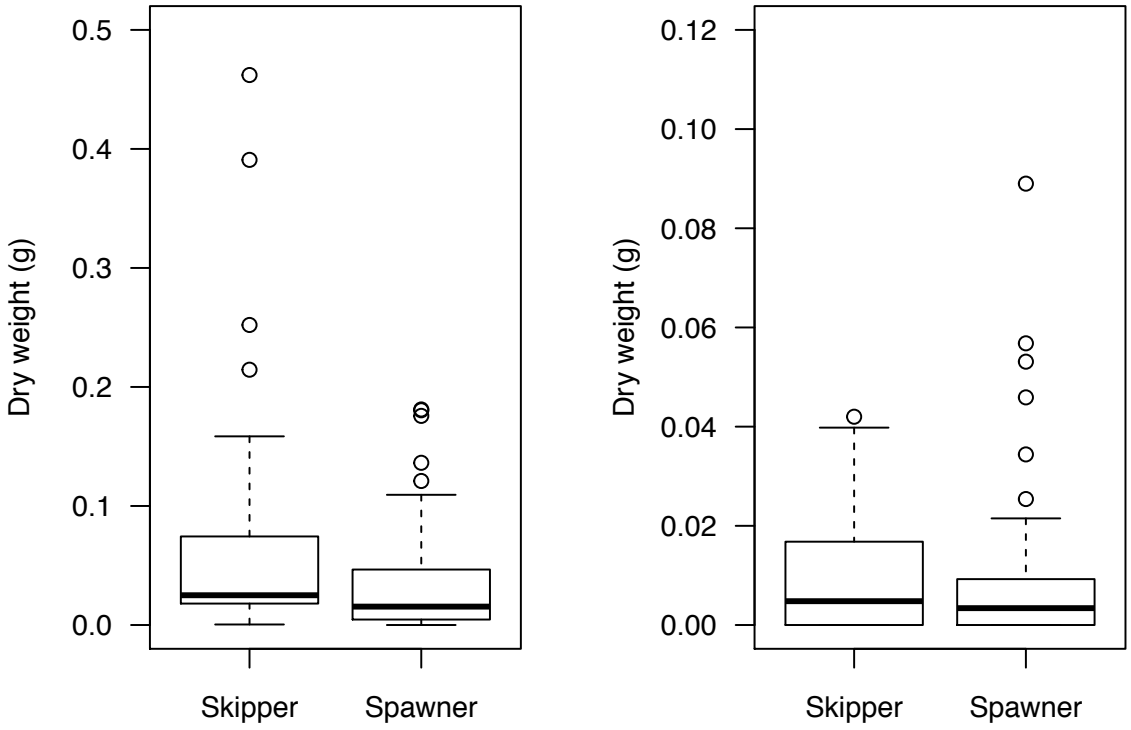

Figure 7: Box plots showing median dry weight (g) of intestinal parasites, and border for upper and lower quartile in skippers and spawners. Results from Mann Whitney U-test for difference between skippers and spawners in parasite intensity are shown in brackets for each parasite variable. From left: Adult *H. aduncum* (W = 860, p=0.06) and *E. gadi* (W = 1052, p=0.55).

Analyses of relationships between fecundity estimates and parasite intensities

When testing for relationships between parasite intensities and cod fecundity, one needs to control for the effects of host body weight, as fecundity increases with body weight (Fig 8). By comparing the residuals from a weight-fecundity regression against the parasite intensities, we see that there is no significant relationship between relative fecundity and intensities of any of the parasite species. Here, plots are presented for nematode larvae (from pyloric caeca and liver), adult *H. aduncum* and *E. gadi* in figure 9. Results for the other parasite species are found in Appendix table 6.

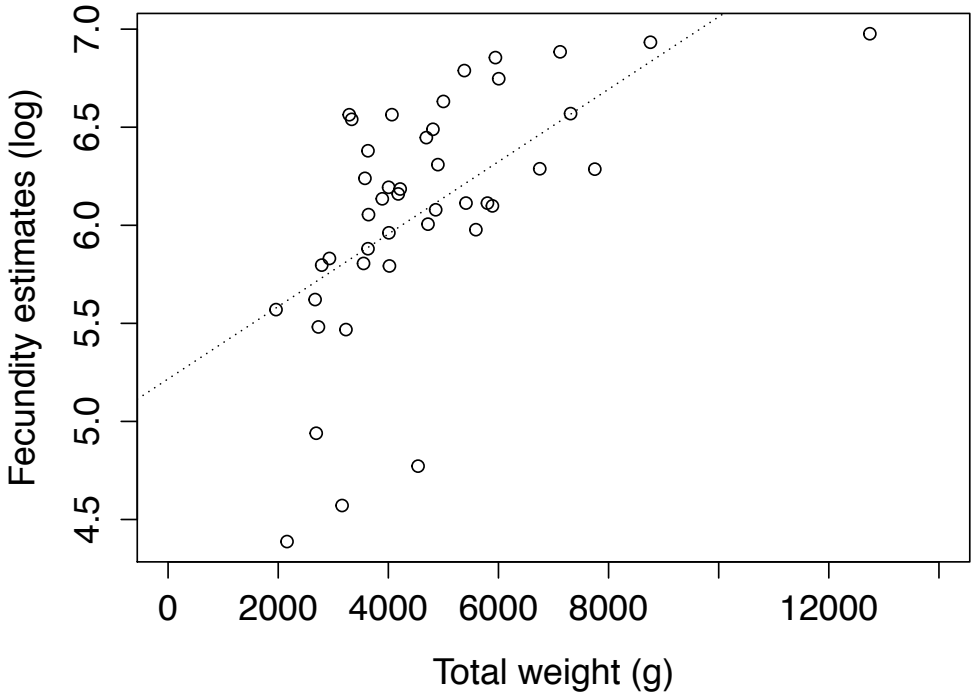

Figure 8: Fecundity estimates plotted against total host body weight (g).

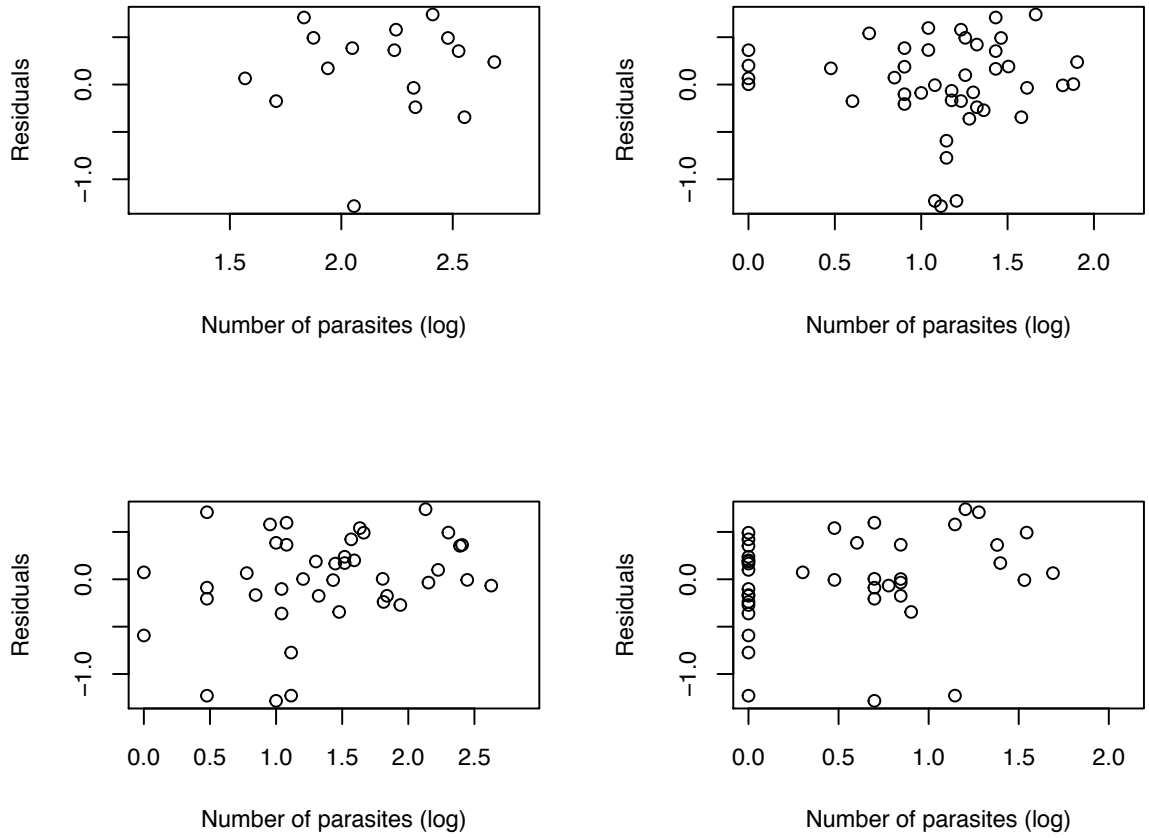

Figure 9: Residuals of fecundity estimates in relation to parasite intensity. Results from Spearman rank analysis for correlations between residuals and parasite intensity are shown in brackets for each parasite variable. From top left: nematode larvae from pyloric caeca (rho= -0.05, p=0.87), nematode larvae from liver (rho= 0.049, p=0.76), adult *H. aduncum* (rho= 0.233, p= 0.14) and *E. gadi* (rho= 0.248, p= 0.12).

Also, no significant results were found when comparing the dry weight of the intestinal parasites to the residuals from the weight-fecundity regression (Fig 10).

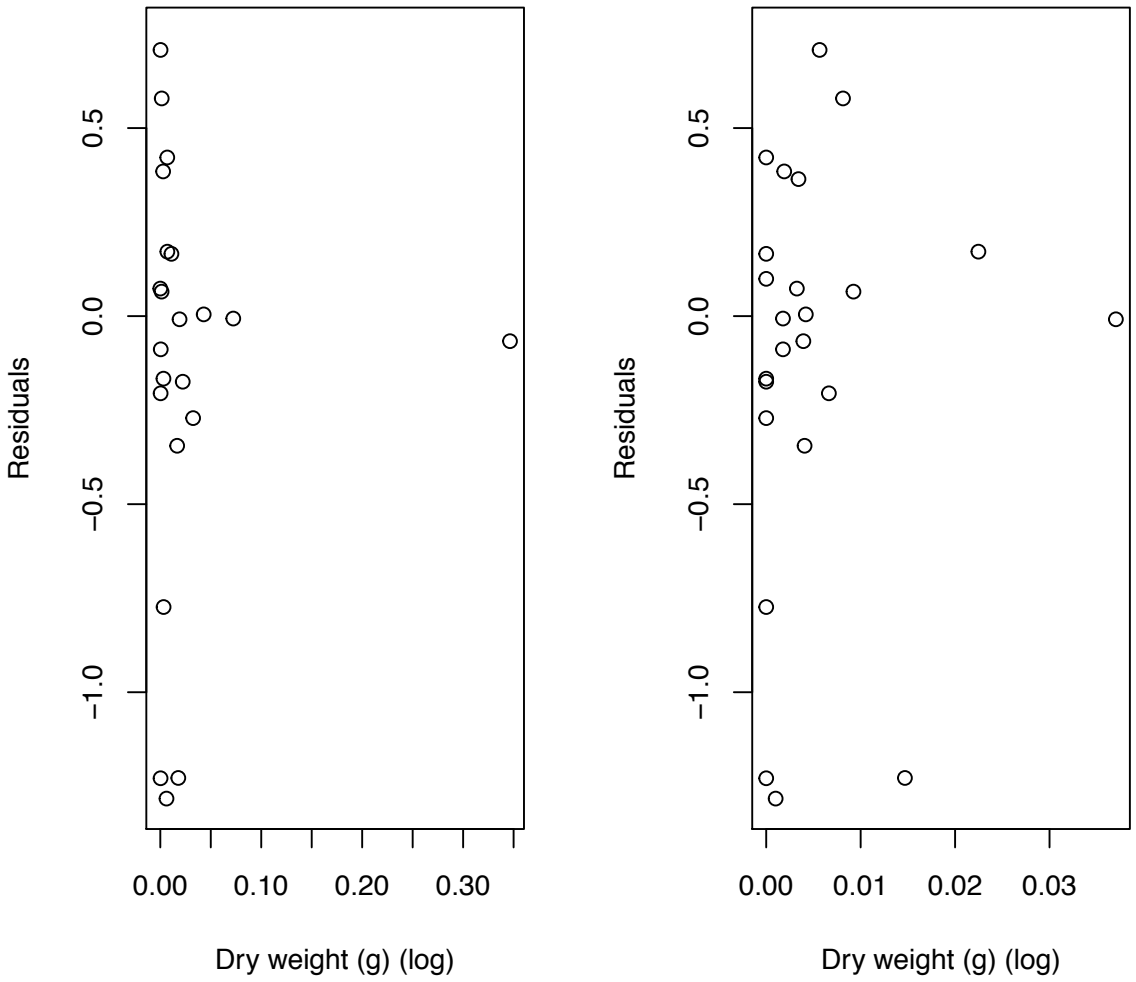

Figure 10: Residuals of fecundity estimates in relation to intestinal parasite dry weight. Results from Spearman rank analysis for correlations between residuals and dry weight are shown in brackets for each parasite variable. From left: adult *H. aduncum* (rho= -0.17, p=0.44) and *E. gadi* (rho= 0.21, p=0.32).

Analysis based on the community index, comparing sum of parasite intensity ranks to fecundity estimates, did not reveal any significant results (Spearman's rank correlation, rho=0.097, p-value=0.72).

Discussion

During the course of this study we did not find any significant difference in parasite intensity between skippers and spawners (Fig. 6 and Table 2). Nor were there any evidence to show that increases in parasite intensity were associated with decreased fecundity (Fig. 9). This was the case both for individual parasite species, as well as the parasite community composition. Intestinal parasite dry weight also did not differ between skippers and spawners (Fig 7), and showed no correlation to estimated fecundity (Fig 10). Age and total body weight of the host did not appear to be a factor in parasite intensities in our study, even though there were significant differences between the two groups (Fig. 2 and 3)

Thus, our results do not support the hypothesis that parasites affect reproduction in the NEAC. However, could it be that there is an effect that could not be detected by our study design?

Our assumption states that skippers will allocate the energy they would otherwise spend on reproduction fighting off parasites, while the opposite is true for the spawners. Hence, we assume the spawners will be the ones with the highest parasite load. However, since the "decision" to initiate vitellogenesis or to skip the reproductive event is made well ahead of the spawning event, as indicated by experimental studies conducted on cod (Skjæraasen et al., 2009), this effect might be undetectable with our study design. As spawning is associated with a great migrational cost for cod, it makes sense to make this decision ahead of the migration, leaving skippers in the Barents Sea. Should this be the case the opposite effect could be expected. As skippers spend their energy rather on fighting off parasites their load could be reduced, while as the spawners invest most of their energy into spawning, they will potentially accumulate more parasites. Hence the spawners might be expected to have a higher parasite load at the time of our sampling, not the other way around. But, as it was, no such effect was detected with our study.

To accurately determine how parasites regulate or limit host population dynamics we see a need for an experimental study. Ideally one requires comparison of populations of hosts with and without parasitic infection, which could be greatly facilitated by experimental design. In fact, when comparing results from experimental and observational tests on parasites and host reproductive success, the results are not equivocal.

Several studies where parasites have been removed experimentally from the host have indicated that parasites do in fact play a vital part in host reproduction. Either by increasing juvenile mass and enhancing survival probability of the young (Patterson et al., 2013), by increasing reproductive success of females (Neuhaus, 2003; Hillegass et al., 2010) or by prevention of population crashes (Hudson et al., 1998). These experimental studies give a great insight into directly assessing the effects of ectoparasites on host reproductive parameters. However, observational studies do not necessarily draw the same conclusions. A study conducted on muskrats (*Ondatra zibethicus*) documented that reproduction and nutritional conditions were not substantially influenced by abundance of ectoparasitic mites (Prendergast et al., 2011). Another study even suggested a positive correlation between ectoparasite intensities and male reproductive success, however a negative relationship was found to be associated with endoparasite richness (Godderham et al., 2011). What this tells us is that effects of parasites on host reproductive success seems to be ambiguous, and that the negative effects that we are looking for might be easier detectable with experimental studies.

We might also look at another factor for the parasites apparent low impact on the NEAC; the Barents Sea ecosystem. Preliminary results from studies conducted as a part of the TIBIA-project reveal that the Barents Sea ecosystem appears to be characterized by relatively low infection levels of parasites and low transmissions rates. As an example, in this study the parasite intensity of *E. gadi* per individual host varied from 0 to 128 for skippers and up to 58 for spawners, however the mean values did not vary greatly, with 7 and 5 respectively. When comparing these infections to other populations of cod infected with *E. gadi* these are relatively low levels of infection. A study by Buchman (1994) looking at Baltic cod infected with the same parasite revealed mean intensities of up to 124.3 (with variance to mean ratios ranging from 47.3 to 183.2 for all length groups included). Buchman's results suggest that cod are able to withstand much higher levels of infection by this acanthocephalan than what we observe in the Barents Sea. Observational studies comparing parasite loads in Barents Sea cod of coastal water versus open water systems have revealed that both total number of parasites species per host, as well as parasite intensities, were higher in coastal water systems (Unpublished master thesis Ann-Beate Løvland). These results suggest that the roles parasites play is less important in the population dynamics of open oceans compared to coastal regions. Løvland theorized two scenarios to explain this difference. The first is related to availability of hosts. A shortage, or lack of, intermediate or final hosts will prevent parasites in

completing their life cycle, yielding a lower parasite diversity. The second theory suggests another important factor for parasites to prevail; the contact-rate between hosts. It is natural to assume that population densities and corresponding contact between individuals are lower in open water masses than those in coastal waters.

Similar evidence has been found in other observational studies, comparing parasite communities in coastal versus open water systems. Hermida et al (2015) revealed that two closely related species of mackerel *Trachurus picturatus*, a coastal fish, and *T.trachurus,* an open ocean fish, differed in relation to parasite community; with the continental-shelf localities having higher infection levels of digenean trematodes. Their discussion reached a possible explanation similar to Løvland (2017); differences in intermediate host abundance of these parasites, being caused by differences in habitat.

Another unexpected finding of our study was the apparent lack of correlation between age or weight in relation to the intensity of any of the parasite species included (Fig. 4 and 5). This result does not support the general hypothesis that there tends to be a positive correlation between parasite intensity and fish size and age, as large, older hosts consume more and larger prey, thus increasing their potential exposure to parasites. This could have implications for the effects of the infestation. However, this effect tends to differ between species, reflecting the life-span of the parasite and changes in feeding behavior of the host. Long-lived species, such as *A.simplex*, has shown to increase significantly in abundance with increasing age, as they are able to remain encysted in their hosts for longer periods of time, leading to accumulations. Therefor, heavy infections with nematodes could pose as having severe pathogenic effects; such as causing significantly smaller liver and a lower liver fat content (Hemmingsen & MackKenzie, 2001). This effect is particularly prominent in these long-lived species. Another common parasite of cod, *E. gadi,* also shows variation in intensity with host size. Buchman (1995) reported a decrease in infection from small to medium length fish followed by an increase in the largest cod of over 61 cm. Changes in food preference with increasing size have reflected the shift in intensity of *E.gadi* in cod. High intensities of this species have been indicated to cause emaciation in its host, however acanthocephalans are not typically associated with affecting pathology (Hemmingsen et MacKenzie 2001). In fact, although cod harbors an impressive amount and diversity of parasites, Hemmingsen et MacKenzie (2001) indicate that only a few of these are actually pathogenic to NEAC. The copepod *L.branchialis* is listed as one of the more severe pathogens of cod, as cause of its

invasive infestation (Hemmingsen et MacKenzie, 2001), but it will not be of great importance to this study, as only 1 individual was infected with this parasite. However, other field experiments conducted looking the effects of parasitic copepods showed that they pose as a significant limiting factor in recruitment of Atlantic salmon (Kroksek et al., 2013).

To get a complete image of parasites effect on host population dynamics one needs to address the possible effects on mortality as well as reproduction. Anderson and May (1978) base their population model of host-parasite interaction on the assumption that it is parasite-induced death rate of the host, rather than reproductive rate, which stands for the majority of host-parasite associations. According to their paper, mortality play a crucial role in regulating and controlling the growth of host populations, specifically stabilizing the dynamics of the host-parasite relationship and the regulatory role of the parasite. Meta-analysis conducted on experimental studies of parasite-associated host mortality indicated that the likelihood of host mortality was 2.6 times higher for infected individuals (Robar et al., 2010).

In summary, we see no indication of parasites induced effect on reproduction in NEAC. However, one must be cautious about making conclusions about parasites effect on host population dynamics. There is still a need to conduct an experimental demonstration of relationships between parasites and reproductive success. As well as look at parasite-induced host mortality in NEAC.

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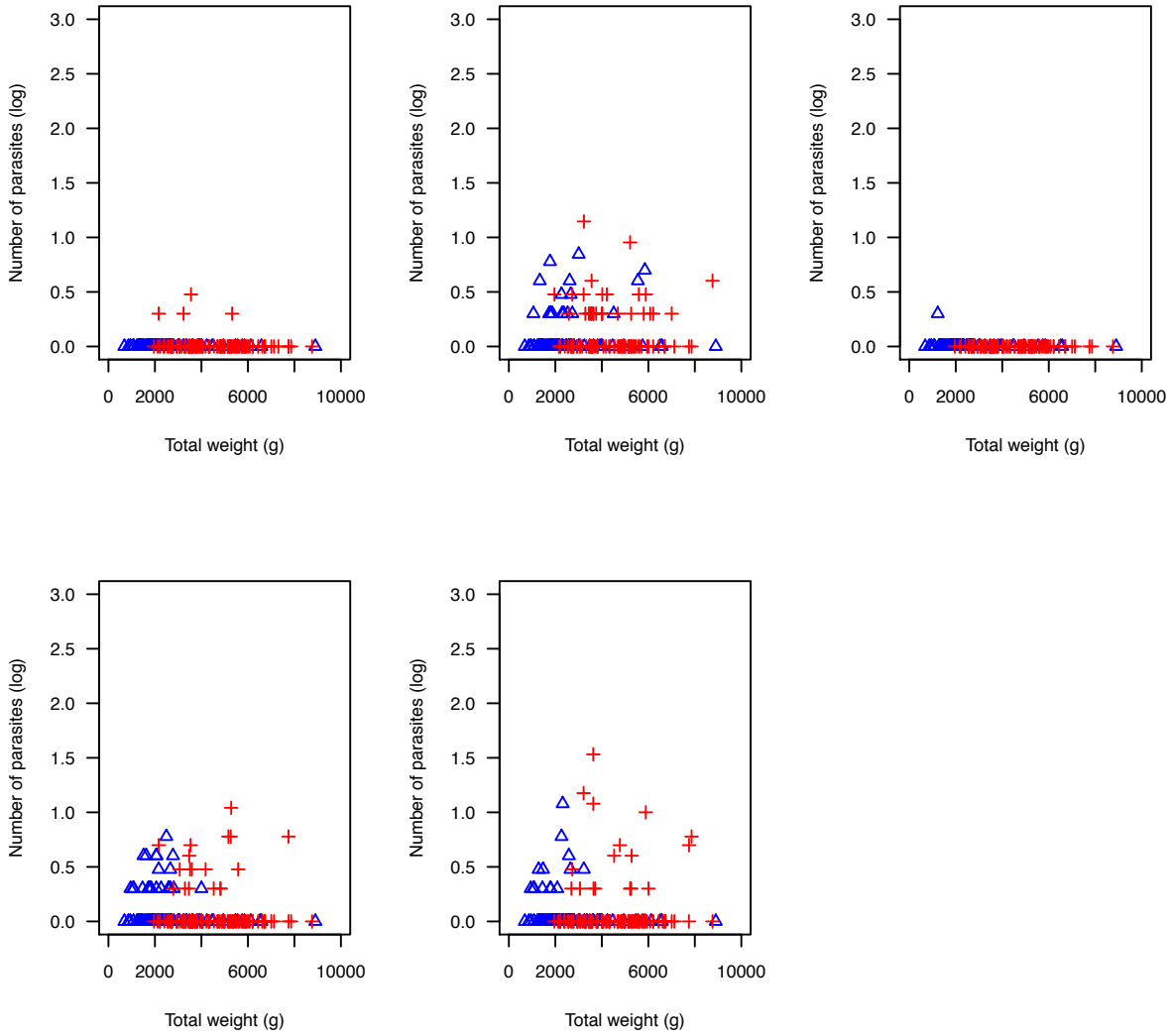
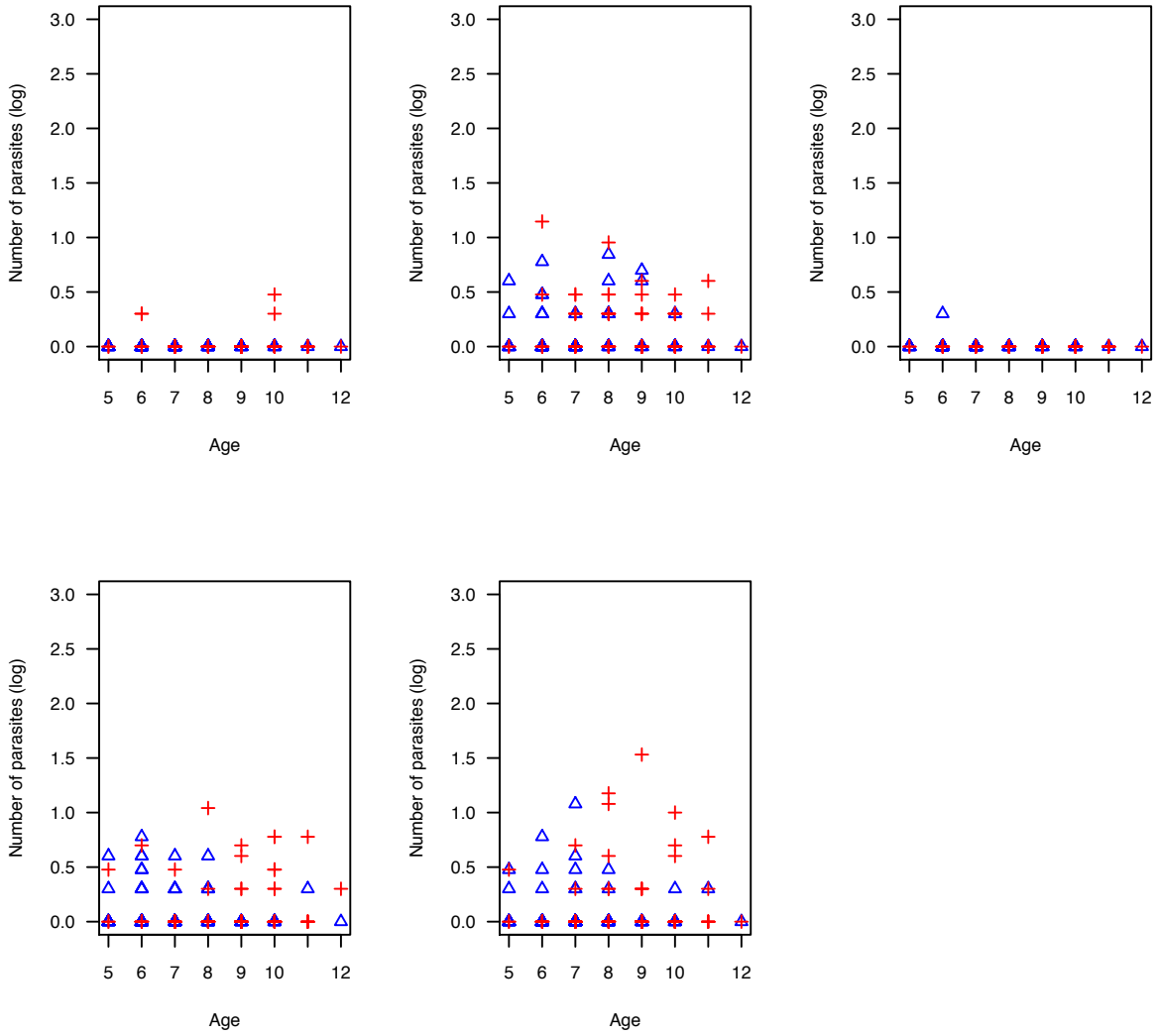
Appendix

Appendix table 1: Infections of the parasites included in this study. The nematode larvae *A. simplex*, *C. osculatum* and *H.aduncum* were grouped together according to their location in the host, as ”larvae (caeca)” and ”larvae (liver)”. Only prevalence was calculated for the myxosporeans *M. gadi* and *M. oviforme*, as these were recorded as present (1) or absent (0).

	Spawner (N=121)			**Skipper (N=123)**		
Parasite species	**Prevalence %**	**Mean intensity**	**Mean abundance**	**Prevalence %**	**Mean intensity**	**Mean abundance**
Larvae (caeca)	96	149	144	100	136	136
Larvae (liver)	98	19	19	94	18	17
***H. aduncum* (adult)**	96	54	51	95	54	51
E. gadi	65	7	5	60	11	7
P. decipiens	15	6	1	12	2	0.3
P. phocarum	16	3	0.5	19	2	0.3
L. branchialis	-	-	-	1	1	0.01
C. confusus	30	2	1	17	2	0.4
A. gadi	4	1	0.05	-	-	-
M. gadi	-	-	-	2	-	-
M. oviforme	-	-	-	1	-	-



Appendix figure 1: Distribution of number of parasites found in relation to total host body weight; skippers depicted in blue, spawners in red. From top left: *A. gadi, C. Confusus* and *L. branchialis.* From bottom left *P. phocarum* and *P. decipiens.*



Appendix figure 2: Distribution of number of parasites found in relation to host age; skippers depicted in blue, spawners in red. From top left: *A. gadi, C. Confusus* and *L. branchialis.* From bottom left *P. phocarum* and *P. decipiens*

Appendix table 2: Results from Spearman's rank analysis for correlations between parasite intensity and total host body weight of the different parasite species. Skippers are depicted in blue, spawners in red. The nematode larvae *A. simplex*, *C. osculatum* and *H.aduncum* were grouped together according to their location in the host, as "nematode larvae (caeca)" and "nematode larvae (liver)".The table provides the rank coefficient, rho, with significant values ($p<0.05$) marked *.

Parasite	**Skippers**	**Spawners**
Nematode larvae (caeca)	0.032	0.054
Nematode larvae (liver)	-0.221*	-0.003
***H.aduncum* (adult)**	-0.016	-0.022
E.gadi	-0.014	-0.021
A.gadi	-	-0.124
C.confusus	0.138	-0.029
L.branchialis	-0.146	-
P.phocarum	-0.082	-0.067
P. decipiens	-0.164	0.049

Appendix table 3: Results from Spearman's rank analysis for correlations between parasite intensity and age of the different parasite species. Skippers are depicted in blue, spawners in red. The nematode larvae *A. simplex*, *C. osculatum* and *H.aduncum* were grouped together according to their location in the host, as "nematode larvae (caeca)" and "nematode larvae (liver)".The table provides the rank coefficient, rho, with significant values ($p<0.05$) marked*.

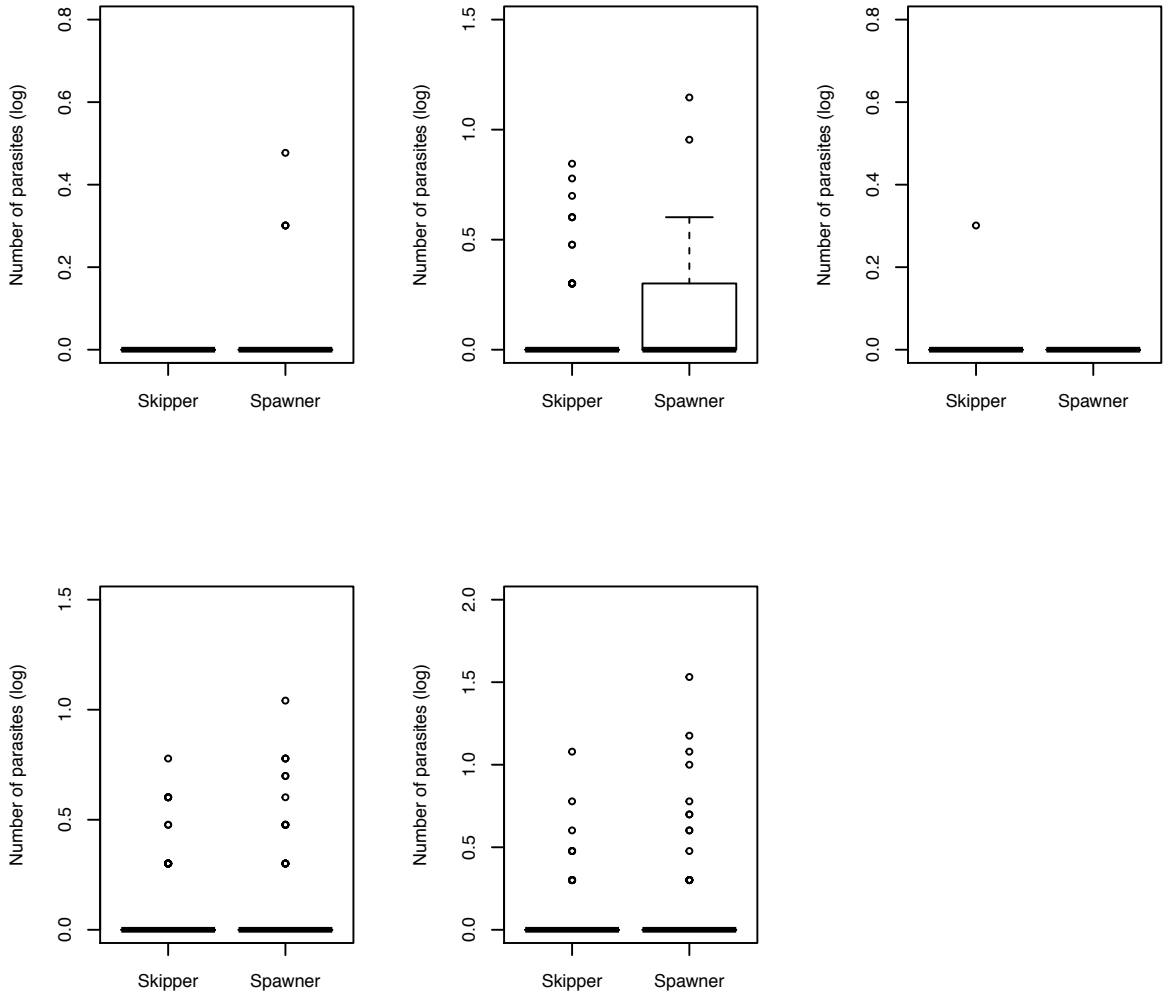
Parasite	**Skippers**	**Spawners**
Nematode larvae (caeca)	-0.034	0.042
Nematode larvae (liver)	-0.152	-0.048
***H.aduncum* (adult)**	0.011	0.049
E.gadi	0.038	0.072
A.gadi	-	-0.055
C.confusus	0.061	-0.135
L.branchialis	-0.075	-
P.phocarum	-0.059	0.120
P. decipiens	0.017	-0.003

Appendix table 4: Results from Spearman's rank analysis for correlations between parasite intensities of the different parasite species. Skippers are depicted in blue, spawners in red. The nematode larvae *A. simplex*, *C. osculatum* and *H.aduncum* were grouped together according to their location in the host, as "larvae (caeca)" and "larvae (liver)".The table provides the rank coefficient, rho, with significant values ($p<0.05$) marked *.

	Larvae (C)	**Larvae (L)**	***H.aduncum* adult**	***E.gadi***	***P.phocarum***	**P. decipiens**	**C.confusus**	**L.branchialis**	**A.gadi**
Larvae (C)		0.570*	0.131	0.036	-0.175	-0.188	0.262	0.119	-
Larvae (C)	0.702*		0.280*	0.225*	-0.058	0.120	0.065	0.117	-
***H.aduncum* adult**	0.243*	0.240*		0.215*	0.069	0.030	-0.095	0.007	-
E.gadi	-0.209	0.044	0.095		-0.019	0.127	0.127	0.147	-
P.phocarum	-0.054	-0.169	0.031	0.193		0.129	0.058	-0.05	-
P. decipiens	0.037	0.054	0.107	0.033	0.026		0.053	-0.038	-
C.confusus	0.032	0.261*	0.257*	0.038	-0.199	0.094		-0.045	-
L.branchialis	-	-	-	-	-	-	-		-
A.gadi	0.182	0.083	0.156	0.229*	0.050	-0.08	0.055	-	

Appendix table 5: Results from Mann-Whitney U-test comparing parasite intensities of individual species in skippers and spawners. The nematode larvae *A. simplex*, *C. osculatum* and *H.aduncum* were grouped together according to their location in the host, as ”nematode larvae (caeca)” and ”nematode larvae (liver)”. This test could only be applied to species that were present in both groups, meaning that *A. gadi, M. oviforme, M. gadi L. branchialis* had to be excluded.

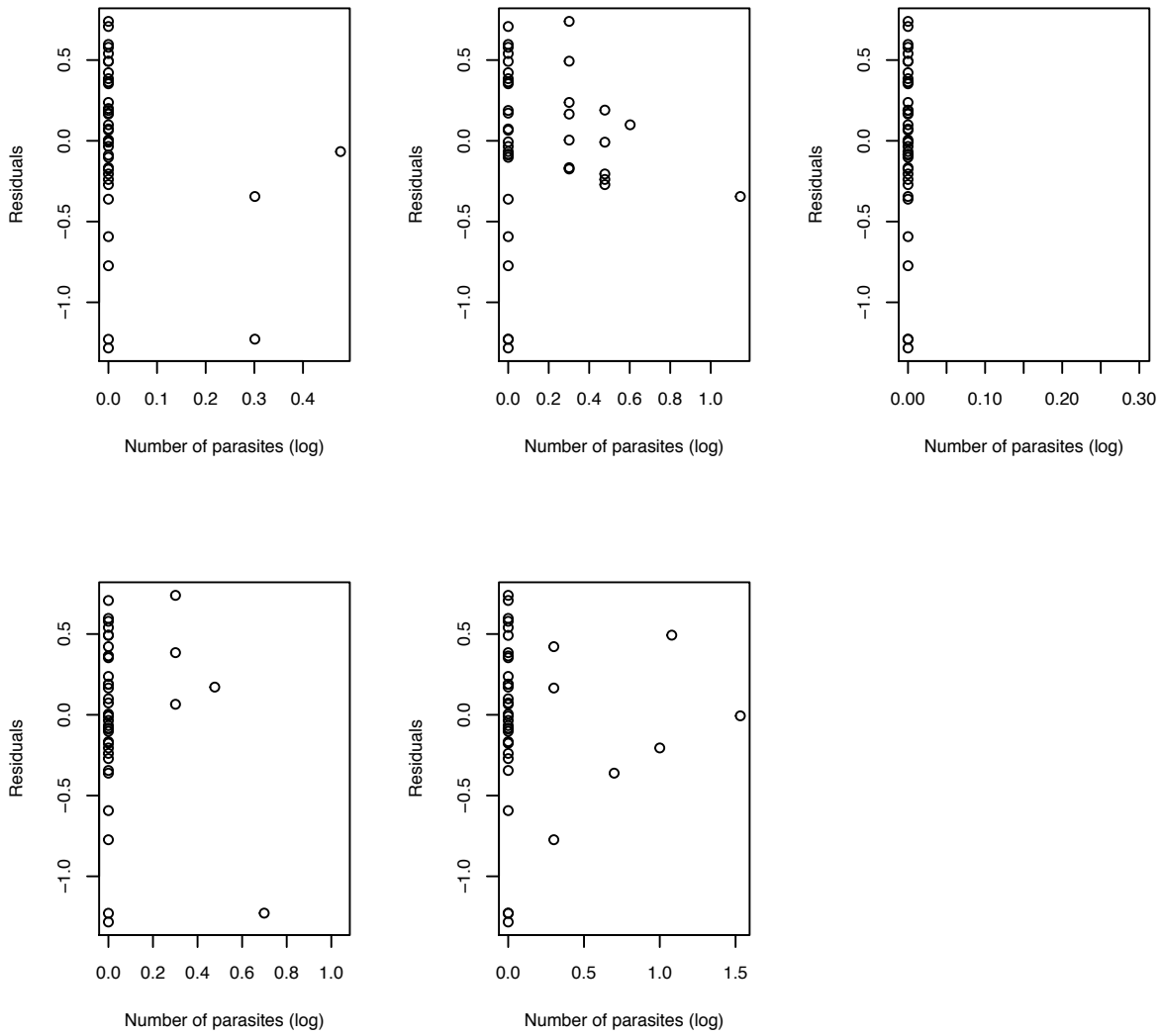
Parasite	**p-value**
Nematode larvae caeca	0.71
Nematode larvae liver	0.012
***H.aduncum* (adult)**	0.786
E.gadi	0.932
C.confusus	0.057
P.phocarum	0.762
P. decipiens	0.429



Appendix figure 3: Box plots showing median parasite intensity and border for upper and lower quartile in skippers and spawners. From top left: *A. gadi, C. Confusus* and *L. branchialis.* From bottom left *P. phocarum* and *P. decipiens*

Appendix table 6: Results from Spearman's rank analysis for correlations between parasite intensity and residuals of fecundity estimates of the different parasite species. The nematode larvae *A. simplex*, *C. osculatum* and *H.aduncum* were grouped together according to their location in the host, as "nematode larvae (caeca)" and "nematode larvae (liver)".The table provides the rank coefficient, rho, with significant values ($p<0.05$) marked *.

Parasite	**rho**
Nematode larvae (caeca)	-0.050
Nematode larvae (liver)	0.049
***H. aduncum* (adult)**	0.233
E. gadi	0.248
A. gadi	-0.288
C. confusus	-0.120
P. phocarum	0.106
P. decipiens	-0.055



Appendix figure 4: Residuals of fecundity estimates in relation to parasite intensity. From top left: *A. gadi, C. Confusus* and *L. branchialis.* From bottom left *P. phocarum* and *P. decipiens*