Effects of chronic crude oil exposure on the fitness of polar cod (*Boreogadus saida*) through changes in growth, energy reserves and survival

Ireen Vieweg[1], Morgan Lizabeth Bender[1], Philipp Robert Semenchuk[1, 2], Haakon Hop[3], Jasmine Nahrgang[1]

[1] Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries and Economics, UiT The Arctic University of Norway, N-9037 Tromsø, Norway

[2] Division of Conservation Biology, Vegetation Ecology and Landscape Ecology, Department of Botany and Biodiversity Research, Rennweg 14, 1030 Vienna

[3] Norwegian Polar Institute, Fram Centre, N-9296 Tromsø, Norway

Corresponding author: Ireen Vieweg (ivi006@uit.no)

Abstract

Climate models predict extended periods with sea-ice free Arctic waters during the next decade, which will allow more shipping activity and easier access to petroleum resources. Increased industrial activities raise concerns about the biological effects of accidental petroleum release on key species of the Arctic marine ecosystem, such as the polar cod (*Boreogadus saida*). This study examines effects on physiological traits related to the fitness of adult polar cod, such as growth, survival, and lipid parameters. Fish were exposed to environmentally-relevant crude oil doses through their diet over an 8-month period, concurrent with reproductive development. In liver tissue, lipid class composition differed between treatments while in gonad tissue, lipid class composition varied between sexes, but not treatments. Crude oil did not affect growth and survival, which indicated that polar cod were relatively robust to dietary crude oil exposure at doses tested (0.11 – 1.14 µg crude oil/g fish/day) in this study.

Key words: Arctic, crude oil, polar cod, growth, survival, lipid classes

1. Introduction

Arctic regions are becoming more accessible for year-round industrial activities due to the strong decline in sea-ice extent (Kwok, 2018). New drilling and production sites for oil and gas exploitation are expanding northwards and into more sea ice-associated regions, thereby exposing these ecosystems to the risk of accidental release of petroleum compounds from oil extraction activities. The projected sea ice decline will also expand the opportunities for Arctic-transit shipping with less sea ice on shipping routes (Melia et al., 2016). This prospect demands a thorough investigation of environmental consequences related to the accidental release of petroleum compounds during exploration, production, and transport in Arctic waters. Oil spills are generally known to cause detrimental effects in marine organisms with regard to acute toxicity as well as long-term, chronic effects (reviewed by Beyer et al., 2016; Pasparakis et al., 2019; Peterson et al., 2003). The polar cod (*Boreogadus saida*) has been commonly used in exposure studies of petroleum compounds on fish in the Arctic marine ecosystem, since it provides an essential trophic link between organisms of lower and higher trophic levels (Hop and Gjøsæter, 2013; Welch et al., 1992) and has a circumpolar occurrence in high abundance across Arctic shelf seas (Benoit et al., 2008; Geoffroy et al., 2011; Hop and Gjøsæter, 2013; Majewski et al., 2017; Marsh et al., 2020). Experimental studies have shown effects of petroleum compound exposure on physiological processes that are crucial for polar cod fitness, such as reduced somatic growth (Bender et al., 2018; Christiansen and George, 1995; Nahrgang et al., 2019), depressed metabolism (Christiansen et al., 2010; Dussauze et al., 2014) and alterations in lipid metabolism of exposed fish (Andersen et al., 2015; Vieweg et al., 2018).

The objective of the present study was to examine how chronic crude oil exposure would affect the accumulation of energy reserves and growth of polar cod during their sexual maturation. The reproductive period was chosen as a potential sensitive period in the life cycle of polar cod. High lipid reserves and balanced lipid metabolism are assumed to be fundamental for polar cod reproduction during winter months (Hop et al., 1995) and are also regarded as essential for successful overwintering and post-spawning survival. The gonadal development starts in late summer and gonads reach their maximum size close to final spawning in February-March (Hop et al. 1995). The timing of gonad growth differs distinctly between the sexes and male gonads reach maximum gonad size earlier than female gonads (Hop et al. 1995). Energy reserves are needed to fuel this gonad growth and provide the basis for successful reproduction. Exposure to petroleum compounds has been shown to alter lipid metabolism with the consequence of reduced lipid energy reserves in exposed fish (Amara et al., 2007; Claireaux et al., 2004; Dey et al., 1983; Gilliers et al., 2012; Meador et al., 2006). This is thought to be related to the mobilization of energy reserves to fuel detoxification processes and stress responses (Calow, 1991; Claireaux et al., 2004; Meador et al., 2006). Alterations in phospholipid levels in Atlantic cod (*Gadus morhua*) muscle and liver are found to be related to the exposure to petroleum compounds (Balk et al., 2011; Dey et al., 1983; Meier et al., 2007), presumably caused by the direct interaction between

petroleum compounds and the cell membranes. In Arctic environments, petroleum compounds released from oil spills may persist in the environment and expose organisms over a long time due to slower decomposition processes in cold compared to temperate waters (Vergeynst et al., 2018). Relatively little is known about the long-term effects of petroleum compound exposure on the lipid dynamics of key Arctic fish species such as the polar cod, despite the crucial role of energy reserves in form of lipids for its overwinter survival and reproduction.

The uptake of contaminated prey is an important exposure route in fish, and therefore, dietary exposure was chosen for this study in contrast to exposure via water used in most other crude oil exposure experiments on polar cod (e.g. Andersen et al., 2015; Christiansen et al., 2010; Nahrgang et al., 2010). The study was designed to mimic environmentally relevant crude oil doses that pelagic fish could encounter after an oil spill in their food (Carls et al., 2006; Salas et al., 2006). The overall aim was to investigate sublethal effects, hence we chose exposure doses of crude oil that were distinctly lower than those in previous polar cod studies that found adverse physiological effects in shorter experiments outside the period of reproductive development (Christiansen and George, 1995; Nahrgang et al., 2019). In the present experiment, fish were chronically exposed to one of four crude oil doses (0.11 – 1.14 µg crude oil/g fish/ day), all of which were distinctly lower than those used in previous polar cod studies (2.1 - 28 ug crude oil/ g fish/ day [Christiansen and George, 1995; Nahrgang et al., 2019]) in part to mitigate of food avoidance behavior. In terms of measured polycyclic aromatic hydrocarbon (PAH) concentrations, these equate to exposure doses (0.8 – 13.1 ng Σ 26 PAH /g fish/day) that can be expected in copepods (*Calanus finmarchicus)* chronically exposed to petroleum compounds (231 ng ∑44 PAH/ g wet weight, Hansen et al., 2020). Petroleum compounds have been shown to accumulate and only slowly depurate from lipid-rich *Calanus* sp. (Agersted et al., 2018; Hansen et al., 2018), an important part of the natural zooplankton diet of polar cod (Cusa et al., 2019). Slow depuration rates increase the likelihood for petroleum compounds to be transferred to their predators (Øverjordet et al., 2018) and highlight the exposure to petroleum compounds via food as a highly relevant exposure route, however little investigated in fish exposure studies.

The 8-month exposure experiment was conducted from July 2014 to February 2015, a time period concurrent with the development of gonads from early gonad growth towards final maturation of gonads prior to spawning in February-March. The present experiment was divided into two parts: to study the effects of chronic crude-oil exposure on the reproductive development of adult polar cod (Bender et al., 2016) and to examine the effects of chronic crude oil exposure on the overall fitness of polar cod through changes in growth, energy reserves and survival (present study). Bender et al. (2016) showed increased levels of biliary PAH metabolites (1-OH phenanthrene) in exposed polar cod, which verified the induction of PAH biotransformation upon crude oil exposure. Male fish showed a decrease in sperm motility as response to chronic crude oil exposure, which could cause reduction in overall fertility.

2. Materials and methods

2.1 Fish husbandry and experimental set-up

The field sampling, fish husbandry, and experimental methodology have been described in detail by Bender et al. (2016). Briefly, wild polar cod were caught by bottom trawl at the northwest coast of Svalbard (Norway, 79°N) in January 2014. Fish were kept in tanks on deck of the research vessel until their transfer to the research station of the UiT The Arctic University of Norway in Kårvika (Norway, 69°N). Polar cod were kept in a 4000 L tank for 5 months of acclimation and fed daily *Calanus* sp. (purchased from Calanus AS, Norway, 68°N) distributed by hand until satiation. Seawater and light conditions in the experiment tanks were adjusted to simulate natural habitat conditions retrieved from mooring data in Kongsfjorden (see details in Bender et al., 2016, Table S1). Twenty-five days prior to the experiment start (from hereon "day -25"), fish were selected based on similar size (mean fork length [±SE]: 149 ± 2 mm, total weight: 17.2 ± 0.2 g) and anaesthetized (5 mg/L Finquel®) consecutively in order to implant a pit-tag (Biomark®) in each fish. Subsequently, 544 fish were distributed randomly into eight experimental tanks (300 L) and acclimated until the exposure started on June 30, 2014 (day 0). The exposure lasted for a total of 218 days (31 weeks) until February 3, 2015 (day 218). Polar cod were exposed through their diet to four different crude-oil treatments (2 tanks per treatment) corresponding to: 0 (*Control*), 20 (*Low*), 100 (*Medium*) and 200 µg crude oil/ g fish/ meal (*High*). The preparation of crude-oil treatments and detailed PAH composition of the fish food can be found in Bender et al. (2016). The exposure experiment was performed in accordance with the Animal Welfare Act and regulations of the Norwegian Animal Research Authorities, which granted permission for the performance of this experiment (ID 6571). Persons with the relevant training and certificate (FELASA Category C) performed the experimental work.

2.2 Growth measurements, fish sampling, and monitoring of survival

Somatic growth of polar cod was measured in all fish (referred to as "live fish") over the course of the experiment by measuring changes in total weight (± 0.1 g) and fork length (± 1 mm) at five events between the day of tag insertion (day -25) and the final sampling of the experiment (day 218). For the analyses of growth, weight measurements were included on exposure day: -25, 72, 120, 152 and 193. Day 0 and day 218 were not included due to a different weighing procedure used at these dissection days, preventing a direct comparison with the other weight measurements. Prior to measurements, fish were anaesthetized (5 mg/L Finquel®), identified by scanning the transponder tag, dried on a paper towel, and measured carefully for total weight (± 0.1 g) and fork length (± 1 mm). Following measurements, the fish were kept under observation in a bucket with clean seawater to ensure that each individual regained consciousness and could be returned to the original experiment tank. Based on weight measurements made at these monitoring timepoints (days 72, 120, 152, and 193), the amount of

food given to each tank was repeatedly adjusted to the actual mean fish weight measured over the course of the experiment.

Growth of adult polar cod approximated linear growth in the present study, hence relative growth rates (RGR) and absolute growth rates (AGR) were calculated based on the total weight of the individual fish (Hop et al., 1997), as follows:

$RGR = AGR/ W_1*100$ and $AGR = (W_2 – W_1)/ (t_2 – t_1)$

where W_x is the total weight of the individual fish at the respective timepoint in the experiment t_x (number of days after June 5, 2014). Growth rates were calculated for four growth intervals: day -25 – 72 (Gi I), day 72 – 120 (Gi II), day 120 – 152 (Gi III), day 152 – 193 (Gi IV).

Fish were sampled at days 0, 12, and 218 and are from hereon referred to as "sampled fish", following the sampling procedure outlined in Bender et al. (2016). Briefly, individual fish was anaesthetized and given a sharp blow to the head prior to tissue sampling. Total length, fork length, somatic weight, sex, and liver and gonad weights of each fish were recorded and further dissection of the fish was performed on ice. Approximately 1 g of liver and gonad tissue was wrapped in aluminum foil and kept at -80°C until further lipid class analyses. The middle section of the gonads was transferred to 4% buffered formalin and used to determine the maturity status of each fish (see Bender et al. [2016] for further details). Fish somatic indices, gonadosomatic index (GSI), and hepatosomatic index (HSI) were calculated as follows:

GSI = (gonad weight/ somatic weight) x 100

HSI = (liver weight/ somatic weight) x 100

where the somatic weight refers to the weight of the fish after all internal organs were removed.

Fish mortality was monitored daily for each tank and dead fish are referred hereafter as "perished fish". Perished fish were collected, the presence of ecto- and endoparasites was noted and the fish were then frozen at -20°C until further analysis. If parasites were present in perished fish, they were most commonly one of two types of endoparasites present in the body cavity of the fish: cestode larvae *Pyramicocephalus phocarum* and *Contracaecum osculatum*. These cestodes are commonly found in polar cod and are assumed to enter the fish through their copepod diet (Køie, 2009). Morphometric information (fish sex, total and somatic weight, fork length, liver and gonad weight) of perished fish was obtained on frozen fish shortly after the experiment was completed.

2.3 Total lipid extraction and lipid-class analyses

Lipid analyses were performed following the analytical procedure described by Sprague et al. (2012). Total lipids were prepared according to the method of Folch et al. (1957). Briefly, lipids were extracted from liver and gonad tissue by homogenising the sample in chloroform/methanol (2:1 v/v) with non-lipid impurities removed by washing with 0.88% (w/v) KCl. The weight of lipids was determined gravimetrically after evaporation of the solvent (henceforth referred to as total lipid content) and overnight desiccation under vacuum before being reconstituted to a concentration of 10 $mg \cdot ml^{-1}$ concentration using chloroform/methanol (2:1, v/v) containing 0.01 % butylated hydroxy toluene (w/v).

Tissue lipid-class compositions were determined by single-dimension, double-development, high-performance thin-layer chromatography (HPTLC). A total lipid aliquot (~ 10 µg) was applied to 3-mm origins on 10 × 20 cm HPTLC plates (Silica gel 60; Merck KGaA, Darmstadt, Germany) and developed to half- and full-distance, respectively, using methyl acetate/propan-2-ol/chloroform/methanol/0.25% aqueous KC1 (25:25:25:10:9, by vol.) and hexane/diethyl ether/acetic acid (85:15; 1.5, by vol.), as the polar and neutral lipid mobile solvents (Henderson and Tocher, 1992). Lipid classes were quantified by charring plates at 160 °C for 15 min after spraying with 3% (w/v) aqueous cupric acetate containing 8% of phosphoric acid and followed by calibrated scanning densitometry using a Camag 3 Scanner (Version Firmware 1.14.16, Camag, Switzerland) with winCATS Planar Chromatography Manager. Identification of individual classes was confirmed by comparing reference values of known standards run alongside samples, which yield the proportional composition of the total lipid aliquot.

2.4. Statistical analyses

All statistical analyses were conducted with R 4.1.0 (R Core Team, 2021). Differences in growth rates were analyzed with linear models (stats package) with growth interval, crude oil treatment, sex, and presence of parasites as predictor variables. Parasite presence was introduced in these and below models to correct for potential confounding factors caused by reduced fitness in case of parasite presence. While both fork length and total weight measurements were collected, we decided to use weight to calculate growth rate as several fish studies depicted fish weight as a more accurate variable to assess growth effects of oil compounds (Meador et al., 2006).

Fish survival was tested with Cox's proportional hazards test (survival package, Therneau, 2015) with crude oil treatment, fish sex, parasite presence/absence, and initial weight as predictor variables. The somatic indices of perished fish were tested with a linear model with the predictors: crude oil treatment, day of death, fish sex, and parasite presence/absence in the fish. The GSI and HSI data were log transformed in order to comply with normal distribution.

In the above models, initial predictor variables were added with all-ways interactions and the interactions removed step-wise using the Akaike information criterion (AIC). A more complex model was only considered if its AIC was at least two points lower per differing term compared to the more

parsimonious model (Zuur et al., 2009). The crude oil treatment was not subject to removal due to the main focus of the present study and kept in all models even when not significant. The most parsimonious (minimal) model was then used for presentation and interpretation of results. Here, we present estimated effect sizes with 95% CIs of each term retained in the minimal models in relation to the relevant intercept, which always represents the *Control* treatment and the first level of other potential predictors. Statistically significant effects implies that CIs do not contain zero, which here always coincided with $p<0.05$ from the corresponding *t*-test.

Due to the complexity of the lipid data and the factors that may influence lipid composition, we chose to execute an ANOVA simultaneous component analysis (ASCA, in R package MetaStat; Dorscheidt, 2013), which considers the possibility of multiple effects on the composition of lipid classes in polar cod liver and gonad tissue (Checa et al., 2015). We considered 12 lipid classes in this composition (wax ester, triacylglyceride [TAG], free fatty acids [FFA], diacylglycerol [DG], cholesterol, phosphatidylethanolamine [PE], phosphatidylglycerol [PG], phosphatidylinositol [PI], phosphatidylserine [PS], phosphatidylcholine [PC], lysophosphatidylcholine [LPC], and sphingomyelin [SM]), presented as raw mass lipid values (mg/g wet weight in tissue) converted from proportional compositions. The lipid class composition was tested for the effect of the factors of crude oil treatment, fish sex, maturity status (mature and immature) and HSI. This analysis was performed only for samples taken at day 218 to include the potential effects of maturity status (data received from Bender et al., 2016) and representation of the crude oil treatment groups. The ASCA test was done with 1000 permutations to assess the statistical significance of the factors under the null hypothesis of no effect of factors on lipid composition (Checa et al., 2015).

Additionally, the ASCA test provides information on which lipid classes are the most influential to the differences between factors and guided the non-parametric multiple pairwise Kruskal Wallis comparison test on significant factors from the ASCA. A post hoc Dunn`s test (Mangiafico, 2015) was performed to determine which treatment groups were different from one another for individual liver lipid classes. To correct for potential biases from multiple comparisons, a Bonferroni correction (Mangiafico, 2015) was implemented together with the Dunn`s test.

3. Results

3.1 Relative growth rates

Polar cod RGR was significantly altered by both crude oil treatment and over time (growth interval [Gi]) in the minimal model, where these predictors were included as an additive effect (Fig. 1). The RGR was 0.06 % $\cdot$ day^{-1} higher in the *Low* treatment (95% CI 0.01-0.11) compared to *Control* conditions ($p = 0.001$, Fig. 1), while no significant effects were found on RGR for *Medium* and *High* treatment. The RGR changed over the course of the experiment, irrespective of crude oil treatment. The RGR was 0.32 and 0.29 % $\cdot$ day^{-1} higher (95% CI 0.28 - 0.36 and 0.24 - 0.33, respectively) at Gi II and III compared

to the Gi I at the beginning of the experiment ($p < 0.001$, Fig. 1). The RGR at the end of the experiment (Gi IV) was not significantly different from Gi I.

3.2 Fish survival

The crude oil exposure did not affect total fish survival nor timing of death over the course of the experiment (Fig. 2). Besides crude oil treatment, only parasite presence/absence was retained in the minimal model, which increased the likelihood of death by 1.8 (95% CI 1.4-2.4) compared to fish without parasites ($p<0.001$, Fig. 2). A *Control* fish without parasites had a median survival time of 125.5 days (95% CI 100-148 days, intercept of model) (Fig. 2), which corresponds to experiment day 126 (November 03, 2014).

Neither crude oil treatment nor parasite presence had significant effects on the HSI in perished fish (Fig. 3A). In the minimal model, day of death (i.e. day into the experiment), parasite presence and their interaction were retained (besides crude oil treatment, which effect was insignificant but not subject to model selection). While the intercept along the day of death axis was independent of parasite presence (i.e. all fish started the experiment with the same HSI values), the slope was higher for fish without parasites, i.e. the presence of parasites caused a lower increase of HSI compared to parasite-free fish (slope coefficient, 95% CI 1.007-1.009, $p < 0.001$) (Fig. S1).

The crude oil treatment had no significant effects on GSI of perished fish (Fig. 3B). Day of death, sex and their interaction were retained in the minimal model besides crude oil treatment. On average during the experiment, the GSI was 0.32 times lower for males compared to females (95% CI 0.23-0.44, $p<0.001$). However, the index increased significantly faster in male (1.010 times per day, 95% CI 1.007-1.012, $p<0.001$) compared to female fish (1.006 times per day, 95% CI 1.004-1.009, $p <0.001$), leading to a higher GSI in males at the end of the experiment (Fig. 3B). Finally, slightly higher HSI and GSI values were determined in perished fish from the *Low* treatment compared to the perished fish from other treatments (Figs. 3A, B), however not significant.

3.3 Lipid-class composition in polar cod liver and gonad

In the liver, the crude oil treatment affected lipid class composition significantly at day 218 (ANOVA-simultaneous component analysis, $p=0.014$, Fig. S2A). The factors sex, maturity status, or HSI did not show a significant effect on the lipid class composition. To further discern treatment group differences, individual lipids were tested and no significant differences were found in the TAG between the treatment groups (Fig. 4A). However, FFA and phospholipids (summed masses of PE, PG, PI, PS, PC, LPC, and SM) showed a significant inverted U-shaped dose response as *Control* and *High* exposure groups were more similar to one another relative to the elevated levels in the *Low* and *Medium* groups ($p<0.01$ for all phospholipids; Figs. 4B, C). A significant increase of diacylglycerol (DG) was found in the *High*

crude oil dose compared to the *Control* treatment (p=0.004, Fig. 4D). A selection of the lipid classes are presented by treatment and sex with statistical analysis in Table S2.

In the gonads, significant differences were observed between sexes at day 218 (p=0.001; Fig. S2B) with no effect of treatment or maturity status. Significantly higher levels of triacylglycerol and sphingomyelin were observed in females, while males exhibited higher levels of cholesterol, and the phospholipids phosphatidylserine and phosphatidylcholine (PC) ($p<0.02$ for all lipid classes; Table S2). In general, phospholipids were the major lipid class (55 - 67 % of total lipids) in polar cod gonads in February (day 218) and PC represented up to 50% of the phospholipid fraction in polar cod gonads (Table S2). Phospholipid levels increased in gonads (males and females pooled) from 37.5 % (±13.8 SEM) on day 0 to 62.3 % (±27.9 SEM) towards spawning (day 218) (Fig. S3).

4. Discussion

4.1 Somatic growth

Dietary crude oil exposure at the tested concentrations did not affect the somatic growth of adult polar cod over the course of the experiment, except for slightly increased growth rates in fish of the *Low* treatment. The lack of exposure effect on somatic growth in polar cod is opposite to what is expected based on dietary exposure studies in other marine fishes, where petroleum compounds had negative effects on the growth performance of fish (e.g., Meador et al., 2006; Saborido-Rey et al., 2007; Vignet et al., 2014). Our findings also diverge from the results of two previous dietary exposure studies on polar cod, where crude oil caused lower growth in exposed fish (Christiansen and George, 1995; Nahrgang et al., 2019). However, exposure levels were distinctly higher in the studies by Christiansen and George (1995, estimated to be at 2.1 - 2.6 µg crude oil/ g fish/ day) and Nahrgang et al. (2019, 28.6 µg crude oil/g fish/day) compared to the present study (0.11 – 1.14 µg crude oil/g fish/day). Dietary doses used in the present study seemed to have been too low to cause detectable changes in growth patterns of exposed polar cod, even though effects were found at the cellular level (EROD, biliary metabolites, reduced sperm motility) (Bender et al., 2016). Polar cod of the *Low* treatment in the present study showed a slight increase in RGR, which could be related to a slightly higher initial weight of fish in this group at experiment start (despite not being significantly different). Also, these fish showed higher HSI levels at the end of the experiment compared to the other treatment groups (Bender et al., 2016), further supporting a higher body condition and a potential increased energy allocation towards growth (Wootton, 2011). Different initial body size has been described as a relevant factor determining the growth rate of fish in exposure experiments, irrespective of the oil treatments (Bautista et al., 2019; Saborido-Rey et al., 2007; Vignet et al., 2014).

Growth rates changed significantly over the course of the experiment and were independent of crude oil exposure. The highest rates were measured in September and October (Gi II, 0.32 % /day), whereas

growth rates were low in late summer (Gi I., 0.18 % /day) and even slightly negative just before spawning in January (Gi IV, -0.03 % /day). Lower growth rates during Gi I might be related to polar cod being exposed to handling stress related to the tagging at the experiment start, a response reported in another polar cod experiment (Nahrgang et al., 2019). Stress has been shown to negatively affect fish growth (Jentoft et al., 2005). Decreased growth rates in January might be ascribed to a shifted energy allocation towards gonadal development in polar cod before spawning (Hop et al., 1995; Jensen et al., 1991). Female polar cod developed their gonads from the first stage of oogenesis in June towards oocytes reaching the vitellogenic stage 2b (i.e. oocytes being close to spawning) in February, while male gonads progressed from an early maturing stage to being able to be stripped at the end of the experiment (Bender et al., 2016). Gonadal development has been shown to require a high energy investment in other cold-water gadids such as the Atlantic cod (Lambert and Dutil, 2000).

4.2 Fish survival

The mortality rates of polar cod were not related to the crude oil treatment; rather, fish mortality was related to intrinsic biological factors of the experimental fish. In a similar exposure study on adult polar cod during the same time of the year (June 2015 – January 2016), Bender et al. (2018) measured cumulative mortality of up to 35% that was also not correlated to the crude oil exposure. Increased mortality is also commonly observed in captive polar cod after spawning in early spring (February/ March) (Bender et al., 2018; Nahrgang et al., 2019) and might be related to a high energy investment into the gonadal development and the release of eggs and sperm during the reproductive period (Hop et al., 1995). After spawning, polar cod can have extremely low HSI (3.4 %) in March – April (Nahrgang et al., 2019), hence fish might be more prone to mortality.

The presence of parasites in polar cod was a significant factor in fish mortality during our experiment, however independent of crude oil exposure. Higher intensity of parasite presence has been observed during the reproductive period of fish (Šimková et al., 2005; 2008), which might be explained by an energy trade-off between the gonadal development and the immune defense against parasites (Sheldon and Verhulst, 1996). Reproductive activity has been shown to cause the suppression of the immune system in fish (Šimková et al., 2008; Skarstein et al., 2001), which can facilitate parasite infection and eventually lower survival of infested organisms. Bender et al. (2016) showed that polar cod were undergoing active development of their gonads during the course of the present experiment (July to January), hence energy allocated to gonadal development might have been the cause for a higher parasitic infection of polar cod during the experiment. The parasitic infection of polar cod was, however, not a major endpoint measured during the present experiment, which limits our conclusion. We would recommend including parasite prevalence as a factor for the survival of experimental fish populations of feral origin in future experimental studies.

4.3 Lipids

Most phospholipids showed an inverted U-shaped dose-response, as polar cod of the *Low* and *Medium* treatment had higher phospholipid levels compared to fish of the *Control* and *High* group. This response might be referred to as hormesis, where low exposure doses cause a stimulatory effect on physiological endpoints in contrast to an inhibitory response upon higher exposure doses (Calabrese, 2013). Higher levels of phospholipids in liver tissue of exposed polar cod might reflect an increased investment into structural lipids, which could be an indication of increased metabolic activity upon exposure. Polar cod have been suggested to shift towards enhanced energy mobilization upon crude oil exposure, indicated by an alteration in blood serum parameters (Vieweg et al., 2018) and increased metabolic activity (Nahrgang et al., 2019). The increase of diacylglycerol (DG) in exposed polar cod liver of the present study verifies the mobilization of energy reserves, as this compound is a metabolic intermediate of lipolysis and generated by TAG lipase in adipose tissue (Carrasco and Mérida, 2007). Diacylglycerol also has an important function as precursor for the synthesis of phospholipids (Carrasco and Mérida, 2007), hence increased DG-levels in crude-oil exposed polar cod might be related to an increased allocation into phospholipid synthesis, reflected by increased PE and PC levels (Table S2). Phospholipids are required for the formation of mixed micelles and lipoproteins in fish, which facilitate the metabolism and extracellular transport of lipids, respectively (Tocher et al., 2008). Phospholipids have also an important function as regulation molecule for metabolic processes, for instance in the immune response of fish (Tocher et al., 2008). It is important to consider that the lipid class data of the present study are expressed relative to the total lipid content of the fish, hence we observed lower TAG levels in exposed fish that showed significantly higher phospholipid levels. The total lipid levels did, however, not change between the treatment groups (Table S2), which might indicate that we have a true increase in phospholipids of exposed fish.

The crude oil treatment did not affect the lipid class composition in the gonad tissue, however total lipid content and lipid class composition in the gonads differed significantly between male and female fish on day 218. The phospholipids PC and PE are reported as the predominant phospholipids in fish ovaries (Cejas et al., 2003; Nelson and Cox, 2005; Wiegand, 1996), also in polar cod (Kohlbach et al., 2017). Bender et al. (2016) showed that polar cod ovaries had reached secondary vitellogenesis by day 218, implying a yolk accumulation of more than 50 % within the oocyte cytoplasm. Vitellogenesis requires an increased accumulation of yolk lipoproteins rich in TAGs (Wiegand, 1996) and phospholipids (Tocher, 2003) in oocytes, which explains the main differences in lipid class composition between female ovaries and male testes. The phospholipid levels increase in gonads towards spawning confirms findings by a previous experimental study on adult polar cod by Hop et al. (1995). This indicates that polar cod of the present study underwent a natural gonadal development and were acclimated to the experimental conditions.

Conclusion

The present study showed that chronic crude oil exposure did not affect the somatic growth and survival of polar cod during their gonadal development. Exposed fish appeared as relatively robust against the dietary chronic exposure to crude oil at doses tested in this study, which were defined as background levels with uptake in zooplankton after an oil spill. These environmentally relevant doses were distinctly lower than in previous oil exposure studies on polar cod. However, our exposure levels caused sublethal effects on the lipid composition of liver tissue after 8 months, presumably indicating an altered allocation of energy upon exposure. Pollutants have been shown to cause compensatory changes in the allocation of energy in marine organisms in order to fuel detoxification and repair mechanisms (Calow, 1991). Sublethal effects, despite not affecting growth and survival, are still significant and may induce long-term effects on the gonadal development of polar cod close to spawning and reduce the overall reproductive success. This notion is supported by the findings of Bender et al. (2016), who showed reduced sperm motility in exposed polar cod of the present experiment. The Arctic environment is characterized by a high seasonality in food availability, hence Arctic organisms such as polar cod need to balance the consumption and storage of energy during an annual cycle to enable overwinter survival and reproduction. An oil spill scenario during the Arctic winter would expose polar cod both to sublethal effects of chronic crude oil exposure and reduced food accessibility, which could cause long-term effects on the sustainability of polar cod populations. Further studies are needed to examine the implication of seasonality in food availability and reproductive stage for the sensitivity of adult polar cod toward crude oil exposure, with a special focus on spawning and post-spawning fish.

Acknowledgments

The authors thank Appoline Laenger for her technical assistance with the perished fish data collection and Willy Hemmingsen (UiT The Arctic University of Norway) for his support with the taxonomy of polar cod parasites. Furthermore, we would like to thank James R. Dick and Matthew Sprague (University of Stirling, Scotland) for the lipid analyses and the technical support during and after the analyses. For the lead author, experimental work, data analyses and first work on the manuscript was funded by the Research Council of Norway through the project POLARISATION (RCN#214184), whereas the final work was performed during the Postdoc-position funded through The Nansen Legacy (RCN #275730).

References

Agersted, M.D., Møller, E.F., Gustavson, K., 2018. Bioaccumulation of oil compounds in the high-Arctic copepod *Calanus hyperboreus*. Aquatic Toxicology 195, 8-14. https://doi.org/10.1016/j.aquatox.2017.12.001.

Amara, R., Meziane, T., Gilliers, C., Hermell, G., Laffargue, P., 2007. Growth and condition indices in juvenile sole *Solea solea* measured to assess the quality of essential fish habitat. Marine Ecology Progress Series 351, 201-208. https://doi.org/10.3354/meps07154.

Andersen, O., Frantzen, M., Rosland, M., Timmerhaus, G., Skugor, A., Krasnov, A., 2015. Effects of crude oil exposure and elevated temperature on the liver transcriptome of polar cod (*Boreogadus saida*). Aquatic Toxicology 165, 9-18. https://doi.org/10.1016/j.aquatox.2015.04.023.

Balk, L., Hylland, K., Hansson, T., Berntssen, M.H.G., Beyer, J., Jonsson, G., Melbye, A., Grung, M., Torstensen, B.E., Borseth, J.F., Skarphedinsdottir, H., Klungsøyr, J., 2011. Biomarkers in natural fish populations indicate adverse biological effects of offshore oil production. Plos One 6, 10. https://doi.org/10.1371/journal.pone.0019735.

Bautista, N.M., Pothini, T., Meng, K., Burggren, W.W., 2019. Behavioral consequences of dietary exposure to crude oil extracts in the Siamese fighting fish (*Betta splendens*). Aquatic Toxicology 207, 34-42. https://doi.org/10.1016/j.aquatox.2018.11.025.

Bender, M.L., Frantzen, M., Vieweg, I., Falk-Petersen, I.B., Johnsen, H.K., Rudolfsen, G., Tollefsen, K.E., Dubourg, P., Nahrgang, J., 2016. Effects of chronic dietary petroleum exposure on reproductive development in polar cod (*Boreogadus saida*). Aquatic Toxicology 180, 196-208. https://doi.org/10.1016/j.aquatox.2016.10.005.

Bender, M.L., Frantzen, M., Camus, L., Le Floch, S., Palerud, J., Nahrgang, J., 2018. Effects of acute exposure to dispersed oil and burned oil residue on long-term survival, growth, and reproductive development in polar cod (*Boreogadus saida*). Marine Environmental Research 140, 468-477. https://doi.org/10.1016/j.marenvres.2018.09.005.

Benoit, D., Simard, Y., Fortier, L., 2008. Hydroacoustic detection of large winter aggregations of Arctic cod (*Boreogadus saida*) at depth in ice-covered Franklin Bay (Beaufort Sea). Journal of Geophysical Research-Oceans 113. https://doi.org/10.1029/2007jc004276.

Beyer, J., Trannum, H.C., Bakke, T., Hodson, P.V., Collier, T.K., 2016. Environmental effects of the Deepwater Horizon oil spill: A review. Marine Pollution Bulletin 110, 28-51. https://doi.org/10.1016/j.marpolbul.2016.06.027.

Calabrese, E.J., 2013. Biphasic dose responses in biology, toxicology and medicine: Accounting for their generalizability and quantitative features. Environmental Pollution 182, 452-460. https://doi.org/10.1016/j.envpol.2013.07.046.

Carls, M.G., Short, J.W., Payne, J. 2006. Accumulation of polycyclic aromatic hydrocarbons by Neocalanus copepods in Port Valdez, Alaska. Marine Pollution Bulletin 52, 1480-1489. https://doi.org/10.1016/j.marpolbul.2006.05.008.

Calow, P., 1991. Physiological costs of combating chemical toxicants – ecological implications. Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology 100, 3-6. https://doi.org/10.1016/0742-8413(91)90110-f.

Carrasco, S., Mérida, I., 2007. Diacylglycerol, when simplicity becomes complex. Trends Biochemical Science 32, 27-36. https://doi.org/10.1016/j.tibs.2006.11.004.

Cejas, J.R., Almansa, E., Villamandos, J.E., Badia, P., Bolanos, A., Lorenzo, A., 2003. Lipid and fatty acid composition of ovaries from wild fish and ovaries and eggs from captive fish of white sea bream (*Diplodus sargus*). Aquaculture 216, 299-313. https://doi.org/10.1016/s0044-8486(02)00525-2.

Checa, A., Bedia, C., Jaumot, J., 2015. Lipidomic data analysis: Tutorial, practical guidelines and applications. Analytica Chimica Acta 885, 1-16. https://doi.org/10.1016/j.aca.2015.02.068.

Christiansen, J.S., George, S.G., 1995. Contamination of food by crude-oil affects food selection and growth-performance, but not appettite, in an Arctic fish, the polar cod (*Boreogadus saida*) Polar Biology 15, 277-281. https://doi.org/10.1007/BF00239848.

Christiansen, J.S., Karamushko, L.I., Nahrgang, J., 2010. Sub-lethal levels of waterborne petroleum may depress routine metabolism in polar cod *Boreogadus saida* (Lepechin, 1774). Polar Biology 33, 1049-1055. https://doi.org/10.1007/s00300-010-0783-2.

Claireaux, G., Desaunay, Y., Akcha, F., Auperin, B., Bocquene, G., Budzinski, F.N., Cravedi, J.P., Davoodi, F., Galois, R., Gilliers, C., Goanvec, C., Guerault, D., Imbert, N., Mazeas, O., Nonnotte, G., Nonnotte, L., Prunet, P., Sebert, P., Vettier, A., 2004. Influence of oil exposure on the physiology and ecology of the common sole *Solea solea*: Experimental and field approaches. Aquatic Living Resources 17, 335-351. https://doi.org/10.1051/alr:2004043.

Cusa, M., Berge, J., Varpe, Ø., 2019. Seasonal shifts in feeding patterns: Individual and population realized specialization in a high Arctic fish. Ecology and Evolution 9, 11112-11121. https://doi.org/10.1002/ece3.5615.

Dey, A.C., Kiceniuk, J.W., Williams, U.P., Khan, R.A., Payme, J.F., 1983. Long term exposure of marine fish to crude petroleum - I. Studies on liver lipids and fatty acids in cod (*Gadus morhua*) and winter flounder (*Pseudopleuronectes americanus*). Journal of Comparative Physiology C 75, 93 - 101. https://doi.org/10.1016/0742-8413(83)90016-6.

Dorscheidt, T., 2013. MetStaT: Statistical metabolomics tools. URL https://rdrr.io/cran/MetStaT/.

Dussauze, M., Camus, L., Le Floch, S., Pichavant-Rafini, K., Geraudie, P., Coquille, N., Amerand, A., Lemaire, P., Theron, M., 2014. Impact of dispersed fuel oil on cardiac mitochondrial function in polar cod *Boreogadus saida*. Environmental Science and Pollution Research 21, 13779-13788. https://doi.org/v10.1007/s11356-014-2618-0.

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226, 497-509.

Geoffroy, M., Robert, D., Darnis, G., Fortier, L., 2011. The aggregation of polar cod (*Boreogadus saida*) in the deep Atlantic layer of ice-covered Amundsen Gulf (Beaufort Sea) in winter. Polar Biology 34, 1959-1971. https://doi.org/10.1007/s00300-011-1019-9.

Gilliers, C., Claireaux, G., Galois, R., Loizeau, V., Pape, O.L., 2012. Influence of hydrocarbons exposure on survival, growth and condition of juvenile flatfish: A mesocosm xxperiment. Journal of Life Sciences 4, 113-122. https://doi.org/10.1080/09751270.2012.11885203.

Hansen, B.H., Olsen, A.J., Salaberria, I., Altin, D., Øverjordet, I.B., Gardinali, P., Booth, A., Nordtug, T., 2018. Partitioning of PAHs between crude oil microdroplets, water, and copepod biomass in oil-in-seawater dispersions of different crude oils. Environmental Science & Technology 52, 14436-14444. https://doi.org/10.1021/acs.est.8b04591.

Hansen, B.H., Sørensen, L., Størseth, T.R., Altin, D., Gonzalez, S.V., Skancke, J., Rønsberg, M.U., Nordtug, T., 2020. The use of PAH, metabolite and lipid profiling to assess exposure and effects of produced water discharges on pelagic copepods. Science of The Total Environment 714, 136674. https://doi.org/10.1016/j.scitotenv.2020.136674.

Henderson, R.J., Tocher, D.R., 1992. Thin-layer chromatography. In: Hamilton, R.J., Hamilton, S. (Eds.), Lipid Analysis: a Practical Approach. IRL Press, Oxford, pp. 65 - 111.

Hop, H., Graham, M., Trudeau, V.L., 1995. Spawning energetics of Arctic cod (*Boreogadus saida*) in relation to seasonal development of the ovary and plasma sex steroid levels Canadian Journal of Fisheries and Aquatic Sciences 52, 541-550. https://doi.org/10.1139/f95-055.

Hop, H., Tonn, W.M., Welch, H.E., 1997. Bioenergetics of Arctic cod (*Boreogadus saida*) at low temperatures. Canadian Journal of Fisheries and Aquatic Sciences 54, 1772-1784. https://doi.org/10.1139/cjfas-54-8-1772.

Hop, H., Gjøsæter, H., 2013. Polar cod (*Boreogadus saida*) and capelin (*Mallotus villosus*) as key species in marine food webs of the Arctic and the Barents Sea. Marine Biology Research 9, 878-894. https://doi.org/10.1080/17451000.2013.775458.

Jensen, T., Inne Ugland, K., Anstensrud, M., 1991. Aspects of growth in Arctic cod, *Boreogadus saida* (Lepechin 1773). Polar Research 10, 547-552. https://doi.org/10.3402/polar.v10i2.6765.

Jentoft, S., Aastveit, A.H., Torjesen, P.A., Andersen, O., 2005. Effects of stress on growth, cortisol and glucose levels in non-domesticated Eurasian perch (*Perca fluviatilis*) and domesticated rainbow trout (*Oncorhynchus mykiss*). Comparative Biochemistry and Physiology-Part A Molecular &Integrative Physiology 141, 353-358. https://doi.org/10.1016/j.cbpb.2005.06.006.

Kohlbach, D., Schaafsma, F.L., Graeve, M., Lebreton, B., Lange, B.A., David, C., Vortkamp, M., Flores, H., 2017. Strong linkage of polar cod (*Boreogadus saida*) to sea ice algae-produced carbon: Evidence from stomach content, fatty acid and stable isotope analyses. Progress in Oceanography 152, 62-74. https://doi.org/10.1016/j.pocean.2017.02.003.

Kwok, R., 2018. Arctic sea ice thickness, volume, and multiyear ice coverage: losses and coupled variability (1958–2018). Environmental Research Letters 13, 105005. https://doi.org/10.1088/1748-9326/aae3ec.

Køie, M., 2009. *Boreogadus saida* (Lepechin) (Gadidae): a review of its metazoan parasite fauna from Greenland, eastern Canada, Alaska and the Russian Arctic. Polar Biology 32, 1399-1406. https://doi.org/10.1007/s00300-009-0650-1.

Lambert, Y., Dutil, J.D., 2000. Energetic consequences of reproduction in Atlantic cod (*Gadus morhua*) in relation to spawning level of somatic energy reserves. Canadian Journal of Fisheries and Aquatic Sciences 57, 815-825. https://doi.org/10.1139/cjfas-57-4-815.

Majewski, A.R., Atchison, S., MacPhee, S., Eert, J., Niemi, A., Michel, C., Reist, J.D., 2017. Marine fish community structure and habitat associations on the Canadian Beaufort shelf and slope. Deep Sea Research Part I: Oceanographic Research Papers 121, 169-182. https://doi.org/10.1016/j.dsr.2017.01.009.

Mangiafico, S.S., 2015. An R Companion for the Handbook of Biological Statistics. Version 1.32. rcompanion.

Marsh, J.M., Mueter, F.J., Quinn, T.J., 2020. Environmental and biological influences on the distribution and population dynamics of polar cod (*Boreogadus saida*) in the US Chukchi Sea. Polar Biology 43, 1055-1072. https://doi.org/10.1007/s00300-019-02561-w.

Meador, J.P., Sommers, F.C., Ylitalo, G.M., Sloan, C.A., 2006. Altered growth and related physiological responses in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) from dietary exposure to polycyclic aromatic hydrocarbons (PAHs). Canadian Journal of Fisheries and Aquatic Sciences 63, 2364-2376. https://doi.org/10.1139/f06-127.

Meier, S., Andersen, T.C., Lind-Larsen, K., Svardal, A., Holmsen, H., 2007. Effects of alkylphenols on glycerophospholipids and cholesterol in liver and brain from female Atlantic cod (*Gadus morhua*). Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 145, 420-430. https://doi.org/10.1016/j.cbpc.2007.01.012.

Melia, N., Haines, K., Hawkins, E., 2016. Sea ice decline and 21st century trans-Arctic shipping routes. Geophysical Research Letters 43, 9720-9728. https://doi.org/10.1002/2016GL069315.

Nahrgang, J., Camus, L., Carls, M.G., Gonzalez, P., Jönsson, M., Taban, I.C., Bechmann, R.K., Christiansen, J., Hop, H., 2010. Biomarker response in polar cod (*Boreogadus saida*) exposed to the water soluble fraction of crude oil. Aquatic Toxicology 97, 234-242. https://doi.org/10.1016/j.aquatox.2009.11.003.

Nahrgang, J., Bender, M.L., Meier, S., Nechev, J., Berge, J., Frantzen, M., 2019. Growth and metabolism of adult polar cod (*Boreogadus saida*) in response to dietary crude oil. Ecotoxicology and Environmental Safety 180, 53-62. https://doi.org/10.1016/j.ecoenv.2019.04.082.

Nelson, D.L., Cox, M.M., 2005. Lehninger-Principles of biochemistry. W.H. Freeman and Company, New York, U.S.

Øverjordet, I.B., Nepstad, R., Hansen, B.H., Jager, T., Farkas, J., Altin, D., Brönner, U., Nordtug, T., 2018. Toxicokinetics of Crude Oil Components in Arctic Copepods. Environmental Science & Technology 52, 9899-9907. https://doi.org/10.1021/acs.est.8b01812.

Pasparakis, C., Esbaugh, A.J., Burggren, W., Grosell, M., 2019. Impacts of deepwater horizon oil on fish. Comp Biochem Physiol C Toxicol Pharmacol 224, 108558. https://doi.org/10.1016/j.cbpc.2019.06.002.

Peterson, C.H., Rice, S.D., Short, J.W., Esler, D., Bodkin, J.L., Ballachey, B.E., Irons, D.B., 2003. Long-term ecosystem response to the Exxon Valdez oil spill. Science 302, 2082-2086. https://doi.org/10.1126/science.1084282.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Saborido-Rey, F., Dominguez-Petit, R., Tomas, J., Morales-Nin, B., Alonso-Fernandez, A., 2007. Growth of juvenile turbot in response to food pellets contaminated by fuel oil from the tanker 'Prestige'. Marine Ecology Progress Series 345, 271-279. https://doi.org/10.3354/meps06961.

Salas, N., Ortiz, L., Gilcoto, M., Verela, M., Bayona, J.M., Groom, S., Álvarez-Salgado, X.A., Alnaigés, J., 2006. Fingerprinting petroleum hydrocarbons in plankton and surface sediments during the spring and early summer bloom in the Galician coast (NW Spain) after the Prestige oil spill. Marine Environmental Research 62, 388-413. https://doi.org/10.1016/j.marenvres.2006.06.004.

Sheldon, B.C., Verhulst, S., 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends Ecol Evol 11, 317-321. htpps://doi.org/10.1016/0169-5347(96)10039-2.

Šimková, A., Jarkovský, J., Koubková, B., Barus, V., Prokes, M., 2005. Associations between fish reproductive cycle and the dynamics of metazoan parasite infection. Parasitology Research 95, 65-72. https://doi.org/10.1007/s00436-004-1261-y.

Šimková, A., Lafond, T., Ondračková, M., Jurajda, P., Ottová, E., Morand, S., 2008. Parasitism, life history traits and immune defence in cyprinid fish from Central Europe. BMC Evolutionary Biology 8, 29. https://doi.org/10.1186/1471-2148-8-29.

Skarstein, F., Folstad, I., Liljedal, S., 2001. Whether to reproduce or not: immune suppression and costs of parasites during reproduction in the Arctic charr. Canadian Journal of Zoology 79, 271-278. https://doi.org/10.1139/z00-193.

Sprague, M., Dick, J.R., Medina, A., Tocher, D.R., Bell, J.G., Mourente, G., 2012. Lipid and fatty acid composition, and persistent organic pollutant levels in tissues of migrating Atlantic bluefin tuna (*Thunnus thynnus*, L.) broodstock. Environmental Pollution 171, 61-71. https://doi.org/10.1016/j.envpol.2012.07.021.

Therneau, T.M., 2015. A Package for survival analysis. URL https://CRAN.R-project.org/package=survival https://CRAN.R-project.org/package=survival.

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science 11, 107-184. https://doi.org/10.1080/713610925.

Tocher, D.R., Bendiksen, E.Å., Campbell, P.J., Bell, J. G., 2008. The role of phospholipids in nutrition and metabolism of teleost fish. Aquaculture 280, 21-34. https://doi.org/10.1016/j.aquaculture.2008.04.034.

Vergeynst, L., Wegeberg, S., Aamand, J., Lassen, P., Gosewinkel, U., Fritt-Rasmussen, J., Gustavson, K., Mosbech, A., 2018. Biodegradation of marine oil spills in the Arctic with a Greenland perspective. Science of The Total Environment 626, 1243-1258. https://doi.org/10.1016/j.scitotenv.2018.01.173.

Vieweg, I., Bilbao, E., Meador, J.P., Cancio, I., Bender, M.L., Cajaraville, M.P., Nahrgang, J., 2018. Effects of dietary crude oil exposure on molecular and physiological parameters related to lipid homeostasis in polar cod (*Boreogadus saida*). Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 206 -207, 54 -64. https://doi.org/10.1016/j.cbpc.2018.03.003.

Vignet, C., Le Menach, K., Mazurais, D., Lucas, J., Perrichon, P., Le Bihanic, F., Devier, M.H., Lyphout, L., Frere, L., Begout, M.L., Zambonino-Infante, J.L., Budzinski, H., Cousin, X., 2014. Chronic dietary exposure to pyrolytic and petrogenic mixtures of PAHs causes physiological disruption in zebrafish - part I: Survival and growth. Environmental Science and Pollution Research 21, 13804-13817. https://doi.org/10.1007/s11356-014-2629-x.

Welch, H.E., Bergmann, M.A., Siferd, T.D., Martin, K.A., Curtis, M.F., Crawford, R.E., Conover, R.J., Hop, H., 1992. Energy-flow through the marine ecosystem of the Lancaster Sound region, Arctic Canada. Arctic 45, 343-357. https://doi.org/10.14430/arctic1413

Wiegand, M.D., 1996. Composition, accumulation and utilization of yolk lipids in teleost fish. Reviews in Fish Biology and Fisheries 6, 259-286. https://doi.org/10.1007/BF00122583.

Wootton, R.J., 2011. Energy utilization in growth. In: Farrell, A.P. (Ed.), Encyclopedia of Fish Physiology. Elsevier Inc., pp. 1623 - 1628.

Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed effects models and extensions in ecology with R. Springer Science & Business Media.

Figure captions

Figure 1. Effect size estimates from the minimal model on relative growth rate (RGR) in polar cod including crude oil treatment (low, medium, high) and growth interval (Gi II: day 72 – 120, Gi III: day 120 – 152, Gi IV: day 152 – 193) as additive terms. The RGR is the difference of relative growth compared to the intercept that represents the treatment level *Control*, Gi I (day -25 -72) and has an RGR of 0.18 % day^{-1} (95% CI 0.14-0.22).

Figure 2. Model estimates from Cox's proportional hazards test. Risk of death is the difference of likelihood of death compared to the intercept, which represents the treatment level *Control* (in the case of Low, Medium and High) and absence of parasites (in the case of individuals with parasites) and has a median survival time of 125.5 days (95% CI 100-148 days).

Figure 3. Treatment differences in hepatosomatic index (HSI) (A) and gonadosomatic indices (GSI) (B) of perished fish at days of death over the course of the experiment. For HSI (A), different shapes indicate parasite presence and absence in the fish and for GSI (B), different symbol shapes indicate different fish sex. The fitted lines are derived from the linear model describing the relationship between the somatic values and the predictors (details in section 2.4) and the shading indicates the 95% confidence interval on the fitted values. The crude oil treatments (control, low, medium, high) are indicated by different colors.

Figure 4. Differences in liver lipids (mg / g ww in liver tissue) at day 218 of (A) triacylglycerols; (B) free fatty acids; (C) phospholipids (summed masses of phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, lysophosphatidylcholine, sphingomyelin; (D) diacylglycerols for control and crude oil exposed treatments. Each fish is represented as an individual symbol, with treatments differentiated by color. Significant differences between treatment groups determined from the Dunn´s test are noted with letters above each box. Boxplots are plotted in background to distinguish median (bolded line), 2^{nd} and 3^{rd} quartiles (box), and 1^{st} and 4^{th} quartiles (whiskers).

Supplementary data

Table S1. Daily temperature measurements (°C) of inlet seawater that supplied the eight experimental tanks over the course of the experiment (June 06, 2014 – February 02, 2015). The temperature data is organized by day of the month (1. column) and month (2. – 10. column) of the measurement.

Figure S1. Time differences in hepatosomatic index (HSI, % body weight) of perished fish at day of death (i.e. day into the experiment) over the course of the experiment. Different shapes and colors indicate parasite presence and absence in the fish. The fitted lines are derived from the linear model describing the relationship between HSI and the predictors (day of death, parasite presence/ absence). The shading indicates the 95% confidence interval on the fitted values.

Figure S2. ANOVA simultaneous component analysis of 12 lipid classes (wax esters, triacylglyceride, free fatty acids, diacylglycerol, cholesterol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, lysophosphatidylcholine, and sphingomyelin) at day 218 for (A) liver tissue with crude oil treatment groups ($p=0.014$) and (B) gonadal tissue with sexes ($p=0.001$). Factor levels (crude oil dose [control, low, medium, high] and fish sex [female, male] for panels A and B, respectively) are distinguished by colour and symbol with mean values represented by larger bolded symbols.

Table S2. Total lipid content (mg/ g wet weight tissue) and lipid class composition (% of total lipid, ± SEM) in polar cod liver and gonad tissue of male and female fish at day 218 of the experiment. The values are presented as mean ± SEM for the different crude oil treatment groups (Control, Low, Medium, and High) and pooled for all treatment groups (All treatments). Abbreviations of lipid classes: triacylglyceride (TAG), free fatty acids (FFA), diacylglycerol (DG). The total phospholipid column represents the sum of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylcholine (PC), lysophosphatidylcholine (LPC), sphingomyelin (SM). Wax ester, glycolipid and pigmented material are combined in the column other lipid classes. Significant differences between sex and treatment are indicated by asterisk (*) and letters respectively (Kruskal Wallis test, *posthoc* Dunn`s test).

Figure S3. Time series of gonad lipid classes of unexposed polar cod during the period of reproductive development at day 0, day 121 and day 218) divided into male and female fish (A) and pooled by sex for each time point (B). Females and males included in the figures numbered 4 and 11; 7 and 9; 4 and 7, at each timepoint respectively. Abbreviations of lipid classes: Wax esters (Wax), triacylglyceride (TAG), free fatty acids (FFA), diacylglycerol (DG), cholesterol (Chol) phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylcholine (PC), lysophosphatidylcholine (LPC), and sphingomyelin (SM). Phospholipids represent the sum of PE, PG, PI, PS, PC, LPC, and SM.

Figure 1

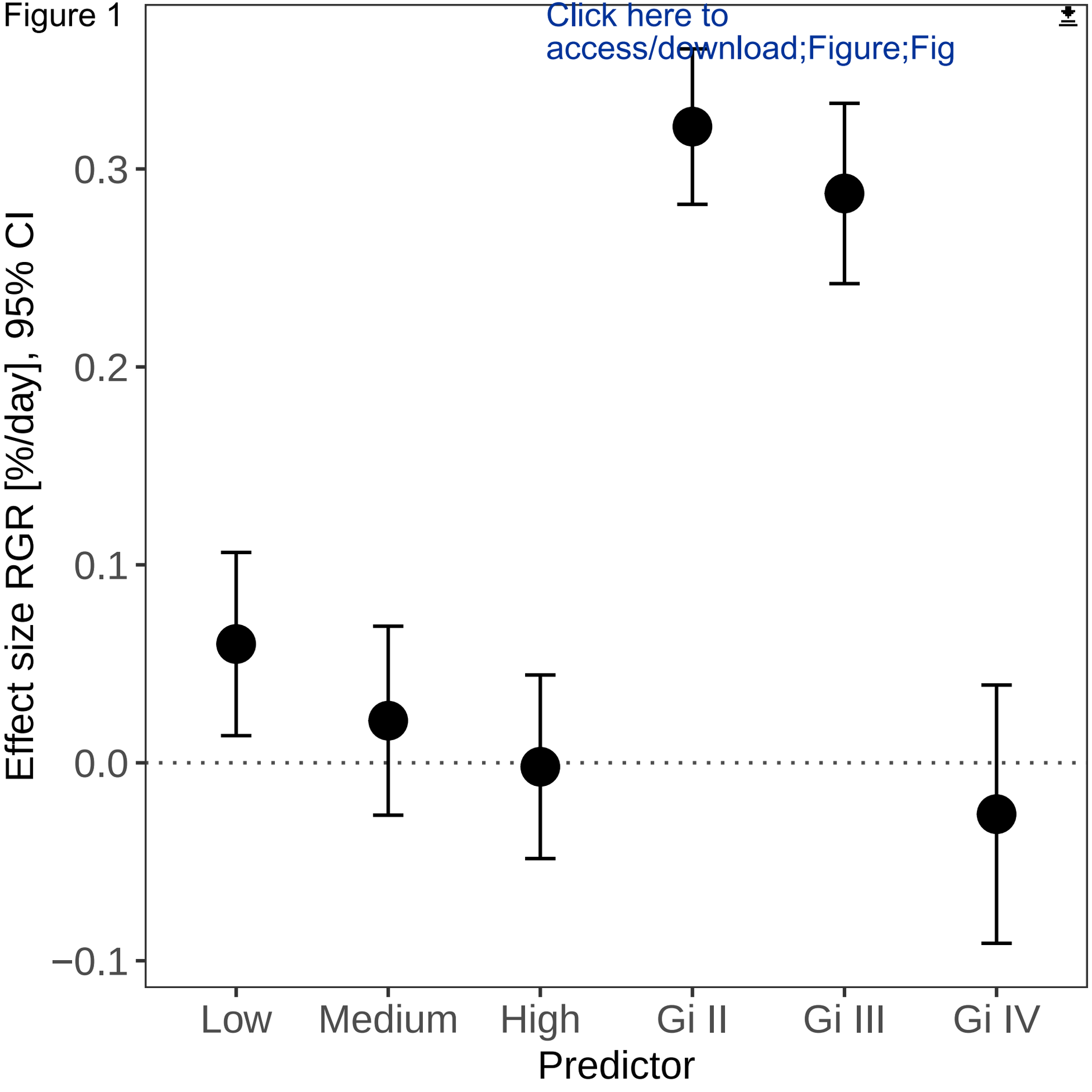


Figure 2

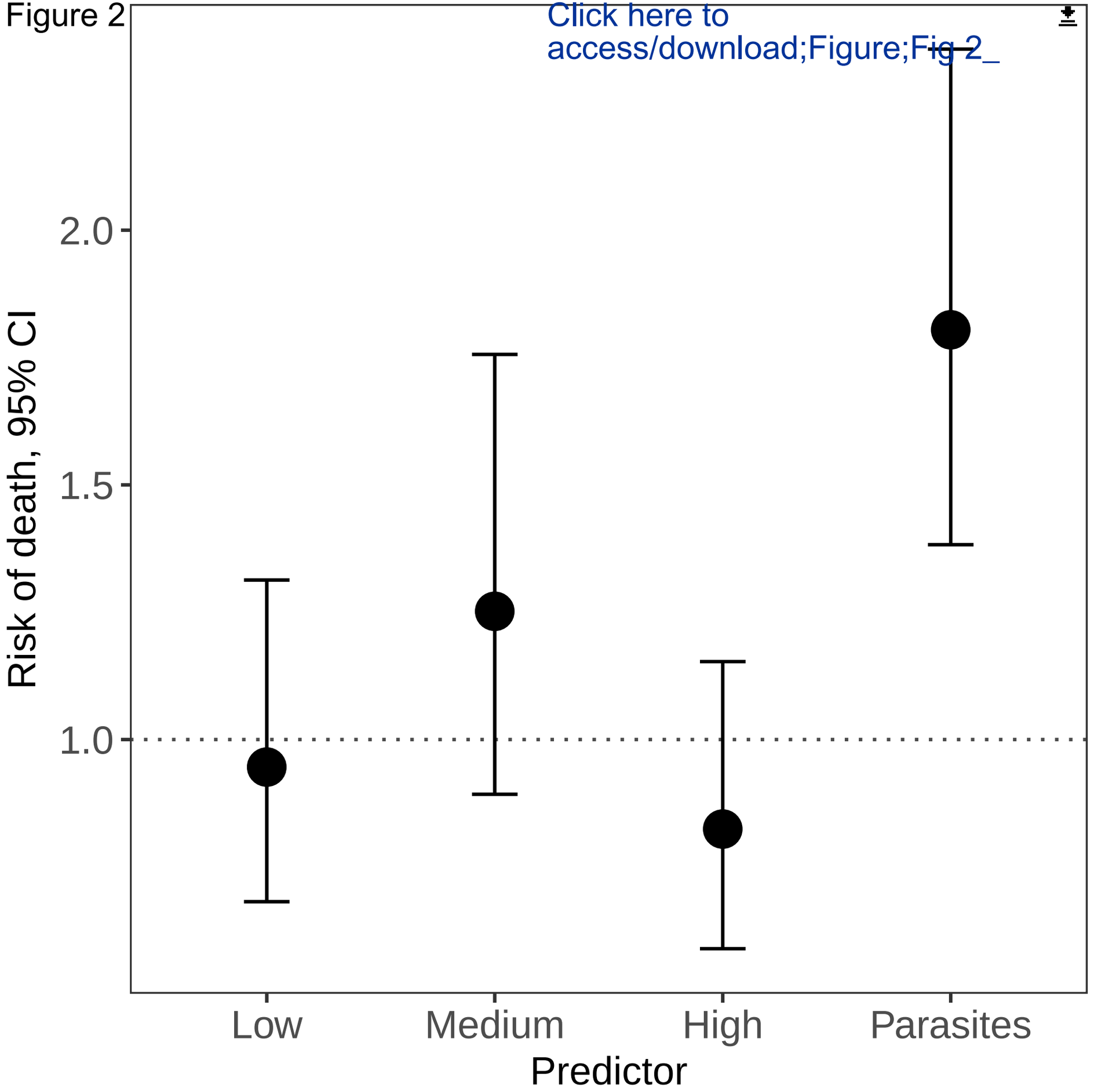


Figure 3

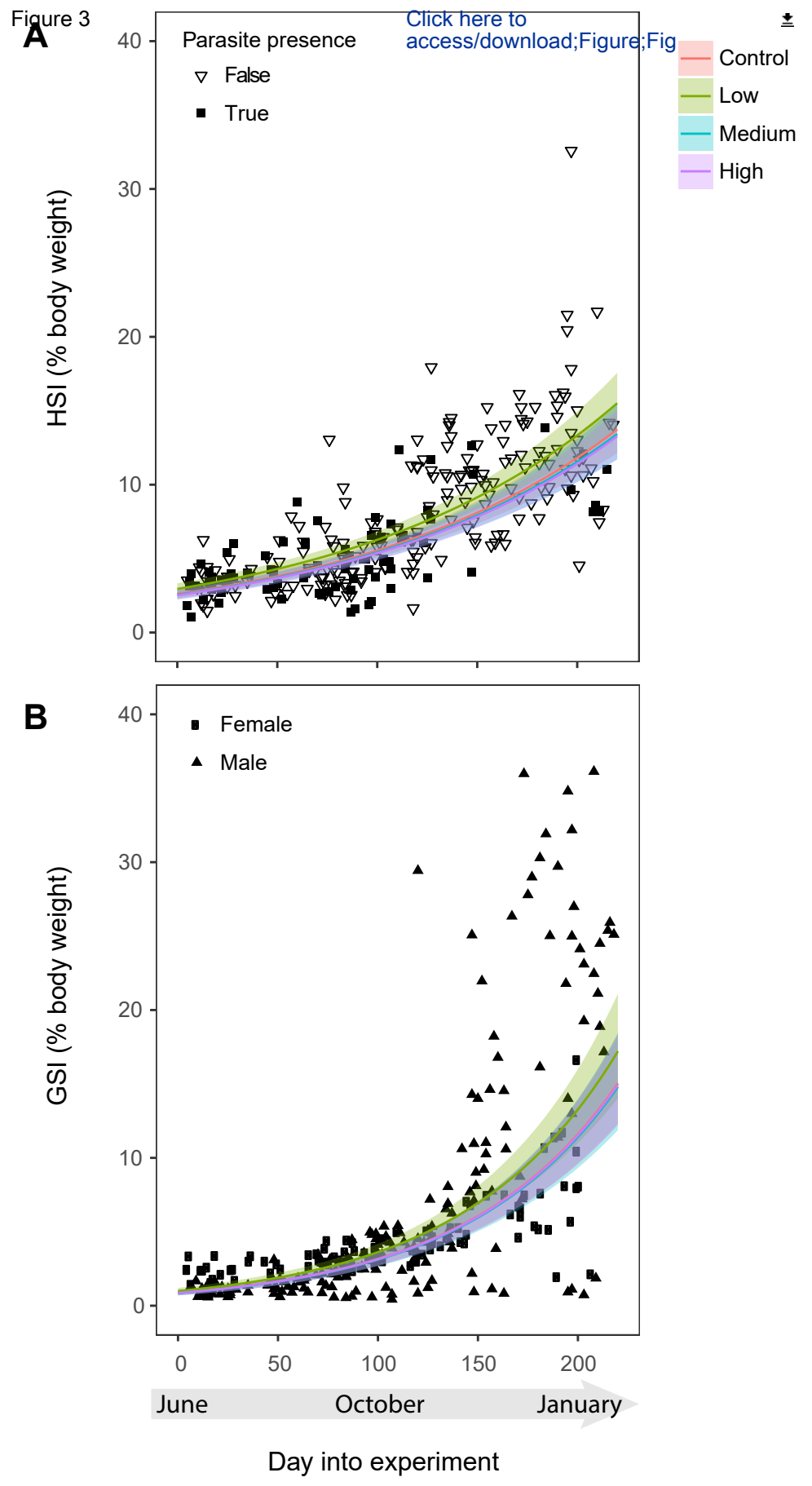
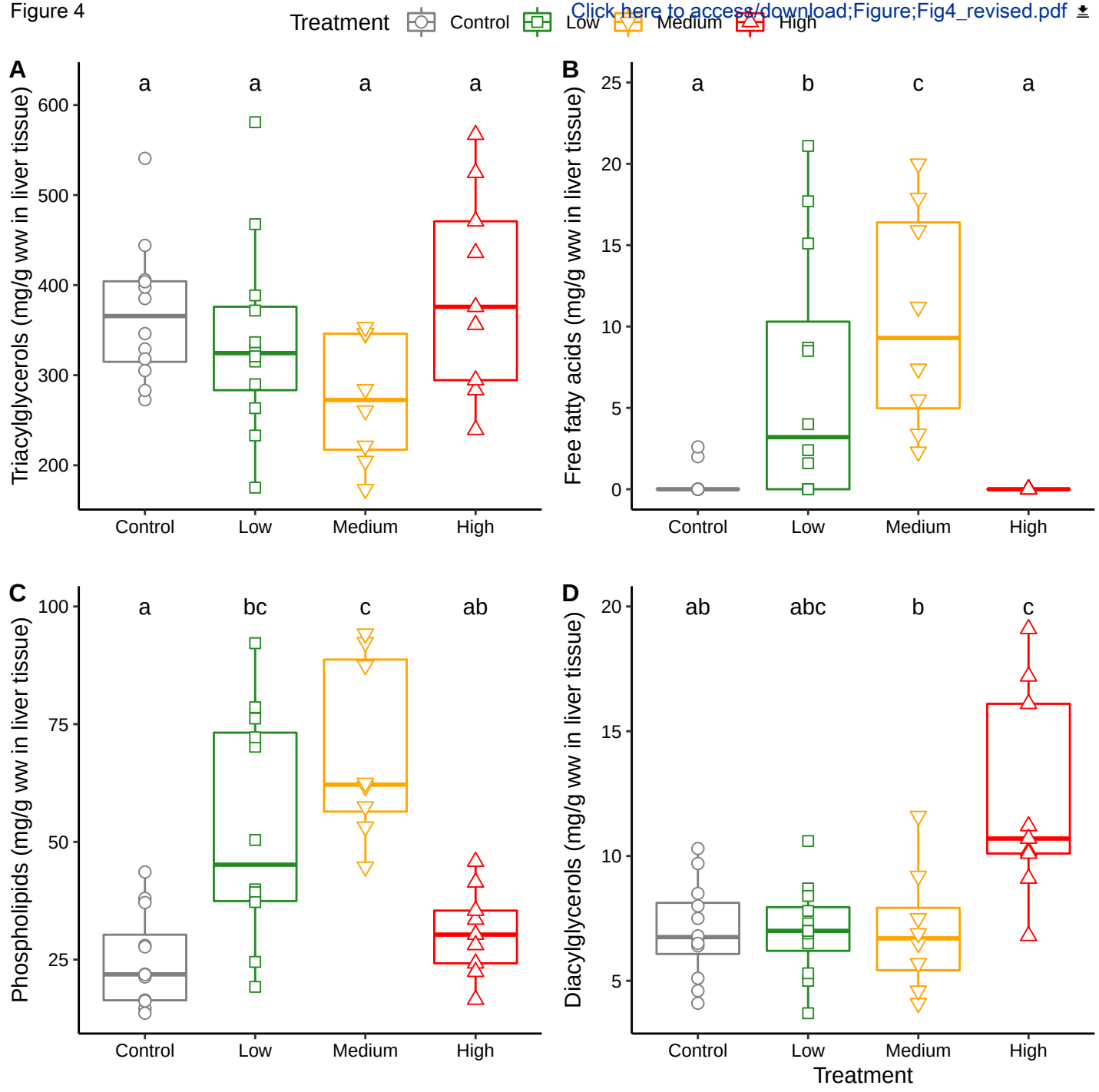

A
Parasite presence
False
True
Click here to access/download;Figure;Fig
Control
Low
Medium
High
HSI (% body weight)
40
30
20
10
0
B
Female
Male
GSI (% body weight)
40
30
20
10
0
0
50
100
150
200
June
October
January
Day into experiment

Figure 4



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author statement

Ireen Vieweg: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing – reviewing, editing, Project administration

Morgan Lizabeth Bender: Conceptualization, Methodology, Investigation, Formal analysis, Writing – reviewing, editing, Project administration

Philipp Robert Semenchuck: Formal analysis, Writing – reviewing, editing

Haakon Hop: Supervision, Writing – reviewing, editing

Jasmine Nahrgang: Funding acquisition, Conceptualization, Investigation, Supervision, Writing – reviewing, editing, Project administration

Table S1

Figure S1

Figure S2

Table S2

Figure S3