Growth and metabolism of adult polar cod (*Boreogadus saida*) in response to dietary crude oil

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ABSTRACT

The increasing human presence in the Arctic shelf seas, with the expansion of oil and gas industries and maritime shipping, poses a risk for Arctic marine organisms such as the key species polar cod (*Boreogadus saida*). The impact of dietary crude oil on growth and metabolism of polar cod was investigated in the early spring (March-April) when individuals are expected to be in a vulnerable physiological state with poor energy stores. Adult polar cod were exposed dietarily to three doses of Kobbe crude oil during an 8-weeks period and followed by 2 weeks of depuration. Significant dose-responses in exposure biomarkers (hepatic ethoxyresorufine-O-deethylase [EROD] activity and polycyclic aromatic hydrocarbons [PAH] metabolites in bile) indicated that PAHs were bioavailable. Condition indices (i.e. Fulton's condition factor, hepatosomatic index), growth, whole body respiration, and total lipid content in the liver were monitored over the course of the experiment. The majority of females were immature, while a few had spawned during the season and showed low hepatic lipid content during the experiment. In contrast, males were all, except for one immature individual, in a post-spawning stage and had larger hepatic energy stores than females. Most specimens, independent of sex, showed a loss in weight, that was exacerbated by exposure to crude oil and low hepatic liver lipids. Furthermore, females exposed to crude oil showed a significant elevation of oxygen consumption compared to controls, although not dose-dependent. This study highlights the importance of the energy status of individuals for their response to a crude oil exposure.

KEYWORD

Polar cod, crude oil, growth, metabolic rate, reproductive status, dietary exposure.

1. INTRODUCTION

Climate variability and global warming have changed and will continue to change the Arctic, most notably seen in the abrupt decline in Arctic sea ice extent and thickness (Barber et al. 2015). In parallel with these changes, anthropogenic activities including oil and gas exploration, maritime shipping, and tourism are all predicted to increase (Smith and Stephenson 2013), posing a risk to arctic marine organisms.

The sensitivity of polar cod (*Boreogadus saida*), a key fish species in the Arctic marine ecosystem (reviewed by Mueter et al. 2016), to petroleum related compounds, has been investigated intensively in the past two decades (Christiansen and George 1995, Nahrgang et al. 2010a,b,c, Geraudie et al. 2014, Andersen et al. 2015, Bender et al. 2016, Vieweg et al. 2018, Bender et al. 2018). Early life stages have shown a high sensitivity to very low levels of a crude oil water-soluble fraction (WSF) (Nahrgang et al. 2016), while adult specimens are considered more robust when exposed to low environmentally relevant concentrations of dietary crude oil (Bender et al. 2016, Vieweg et al. 2018). Thus far, only few studies have investigated the effects of crude oil on energy homeostasis and associated physiological processes in polar cod (Christiansen et al. 2010, Vieweg et al. 2018, Bender et al. 2018). Crude oil and related contaminants, such as polycyclic aromatic hydrocarbons (PAHs) have been shown to affect growth (e.g. Gravato and Guilhermino 2009, Kerambrun et al. 2012, Claireaux et al. 2013, Sandrini-Neto et al. 2016) and metabolism (Claireaux and Davoodi 2010, Christiansen et al. 2010, Klinger et al. 2015) in fish. The mechanisms behind these effects can be multiple, including an increased energy costs from detoxification metabolism, and toxicity (Klinger et al. 2015), behavioral changes leading to reduced nutrient assimilation (Moles and Rice 1983, Christiansen and George 1995), and toxicant induced alterations in nutrient assimilation (Saborido-Rey et al. 2007). Adult polar cod have previously been shown to exhibit altered growth performance when exposed to dietary crude oil (Christiansen and George 1995) and a depression in routine metabolism following an exposure to the WSF of crude oil (Christiansen et al. 2010).

The Arctic is characterized by a strong seasonality in light availability, profoundly affecting biological activity and basic physiological processes in arctic marine ecosystems (Berge et al. 2015). The vast majority of experimental studies on adult polar cod have dealt with specimens

during the late summer/fall concurrent with gonadal maturation (Hop et al. 1995, Hop and Graham 1995, Christiansen and George 1995, Nahrgang et al. 2010b,c, Christiansen et al. 2010, Bender et al. 2016, accepted). Therefore a marked need exists to determine the physiological trade-offs and sensitivity to contaminant exposure during the late winter/spring season, which also coincides to a post-spawning stage for mature individuals. Polar cod invest important amounts of energy into reproduction (Hop et al. 1995) and may be highly susceptible to post-spawning mortality. Exposure to crude oil related compounds may have consequences for post-spawning survival and be directly relevant to population level effects. The present study aimed therefore at investigating the effects of dietary crude oil exposure on growth, lipid class composition, and routine metabolic rate in adult specimens in the early spring. At this time, energy levels in polar cod are expected to be significantly reduced after reproduction and following a period of low food availability during the dark winter months. The primary hypothesis of the present study was that the exposure to crude oil might lead to a reallocation of energy from somatic growth towards detoxification, and lead to an increase in oxygen consumption. The dietary route of exposure was chosen as it allows for accurate control of the dose of contaminant given to the test organism. Furthermore, although it is in general less studied for petroleum products, it may constitute an important pathway for long-term toxicity (Agersted et al. 2018).

2. MATERIALS AND METHODS

2.1. Sampling and acclimation period

Polar cod were caught in Rijpfjorden (Svalbard) with a Campelen bottom trawl attached to a fish-lift (McDonald et al 2000), onboard R/V *Helmer Hanssen* and were transferred to the experimental facilities at the University Centre in Svalbard (Norway) in mid January 2012. Upon arrival, polar cod were kept in acclimation until early March in two 700L tanks under running 25 µm filtered seawater and constant darkness. Temperature loggers (HOBO onset) recorded continuously (19/01/2012 - 25/05-2012) air temperature (4.1 ± 0.2°C), water temperature (6.9 ± 1.0°C), and salinity (28.6 ± 1.3 psu) in the acclimation tanks. During acclimation, polar cod were fed every 3 days with aquaculture feed AgloNorse TROFI AS, Tromsø, Norway (protein 59%; fat 18-20%; ash 10%; fibres 1%; moisture 8-9%; PUFA n-3 2.4%; PUFA n-6 2.6%). During acclimation, feeding was done by giving food in excess to the tanks. One week before exposure start, fishes (n=36 per treatment) were randomly transferred

to exposure tanks (200L) placed in the same room as the holding tanks and containing 5 µm filtrated seawater. During transfer, each fish was anesthetized with metacain (1 mg/ L seawater), tagged (Floy Fish Dangler Tags), and total length and body weight were recorded to the nearest 0.1 mm and 0.1 g.

2.2. Preparation of the food

The treatments consisted of aquaculture feed pellets hydrated with 0.77 g water per g dry pellets, and blended with 0.1, 1, and 5 mg Kobbe crude oil per gram food wet weight, for the low, medium, and high treatments, respectively. For the control group, the feed pellets were hydrated but crude oil was not added. Individually tagged syringes were prepared in advance with food mixture corresponding to 4% body wet weight of each specific fish and stored at -80°C. The choice of the crude oil doses was selected based on literature review of similar experiments that employed dietary crude oil exposure or PAH mixture exposure and that showed alterations at physiological levels. In particular the study by Christiansen and George (1995) showed alterations in growth performances. Our levels correspond to a range one order of magnitude lower to one order of magnitude higher than those used in Christiansen and George (1995).

2.3. Experimental design

The experiment started in March and consisted of four treatments (4 tanks, n=34 per tank) with fish exposed once a week to crude oil contaminated feed (control, low, medium, and high doses) during eight weeks and followed by two weeks (one feeding) of recovery. During the recovery, all specimens received the same uncontaminated feed as that of the control group during the exposure period. Once a week, on the day of feeding, a batch of syringes were thawed and fish were force fed 4 % body weight using 1 ml Luer-lokk syringes (BD Plastipak™). The feeding took maximum 20 sec per fish. Force feeding was chosen to control crude oil dose and avoid confounding effects of differential feeding behavior on growth response (Christian and George 1995, Saborido-Rey et al. 2007). Upon force-feeding, fish were transferred to new tanks containing fresh seawater (5 µm filtered) that had been equilibrating to room temperature during 24 hours. In addition, 80% of the water of the experimental tanks was changed every second day. Water temperature (5.9 ± 0.7 °C) and pH

(7.9 ± 0.1) in the semi-static experimental tanks were monitored daily over the course of the experiment using a handheld WTW multimeter.

Polar cod were sampled at exposure start (holding tanks, n=14), and after one and after eight weeks of exposure (experimental tanks, n=12 per treatment per timepoint). A final sampling point for recovery consisted in 10 additional specimens sampled per treatment (ten weeks). Total length, total weight (TW), gonad weight (GW) and liver weight (LW) were recorded. The liver samples were snap frozen in liquid nitrogen, and stored at -80°C for further analyses. At the start (week zero) and end (week eight) of the exposure period, a portion of gonad tissue was fixed in 4% neutral buffered formaldehyde for histological analysis. Otoliths were collected for age analysis.

Hepato- and gonadosomatic indices (HSI and GSI, respectively) were determined using the following equations:

$$GSI(\%) = \frac{GW}{(\mathrm{TW} - (\mathrm{GW} + \mathrm{LW}))} \times 100$$

$$HSI(\%) = \frac{LW}{(\mathrm{TW} - (\mathrm{GW} + \mathrm{LW}))} \times 100$$

The specific growth rate (SGR, % per d) was based on records of initial (i, at tagging) and final (f, at sampling) TW records, using the following equation:

$$SGR\ (\%\ per\ d) = \frac{(\ln \mathrm{TW_f} - \ln \mathrm{TW_i})}{\text{Time in days}} \times 100$$

2.4. Respirometry

Whole body respiration was measured on polar cod after two, four, six, eight, and ten weeks (n=8 per treatments) using an automated intermittent flow through respirometer equipped with eight chambers (volume of 573 ml) (Loligo® Systems, Denmark). The oxygen consumption was measured using a polymer optical fiber dipping probe. Measurement were always performed on the day prior to feeding, i.e. six days after the previous feeding, in order to limit the effects of specific dynamic action (SDA). The chambers were placed in individual tanks containing filtered seawater equilibrated to room temperature (5.9 ± 0.7 °C). When possible, the same individuals, identified by tags, were used each time; however, due to some

mortality, different fish were used at the end of the experiment. Fish were weighed and placed in individual chambers. The automated respiration consisted in seven-minute cycles of closed respirometry and flushing. Prior to the experiment, eight fish from the holding tank were placed in the chambers and oxygen consumption was recorded during 24 hours to evaluate the time necessary to reach the routine metabolism (Fig. S1). The oxygen consumption decreased typically exponentially over the course of the first three hours and the average oxygen consumption between 2.5 hours and 3 hours was used for the data analysis for the experimental fish.

2.5. Age estimation

Polar cod age (years) was based on otolith readings: for small transparent otoliths, white winter rings were counted in sub-surface light with a Leica M205 C stereo microscope and a Planapo 1.0× objective lens (Gjøsæter and Ajiad 1994); for all larger otoliths, cross sectioning with a scalpel blade and counting the rings under polarised light was necessary.

2.6. EROD activity

Liver samples were homogenized in a phosphate buffer (0.1M, pH 7.4) using a precellys bead-beater and centrifuged 9000g during 30 min (S9 fraction). EROD activity was measured according to Eggens and Galgani (1992). The reaction mix consisted of 10 µl microsomal fraction in 100 mM of Tris-phosphate buffer (pH 7.4), ethoxyresorufin 46 µM as substrate in a final volume of 230 µl. Reaction started by adding 0.25 mM NADPH in the microwells. The resorufin production was measured in four replicates during 20 min at room temperature with a Biosynergy H1 plate reader at 544/584 nm excitation/emission wavelengths, respectively. A resorufin standard curve (0–2 µM) was used for determination of the reaction rates in pmol of resorufin produced $min^{-1}mg^{-1}$ of total protein (S9 fraction).

2.7. Histology

The fixed gonad samples were routinely processed by dehydration and embedded in paraffin wax in a Shandon Citadel 1000 (Micron AS, Moss, Norway). Embedded tissues were sectioned at 5 µm thickness in a Leitz RM 2255 microtome, stained with hematoxylin/eosin, and examined under a Leica Wild M10 dissecting scope with a Leica DFC295 camera for maturity status and indications of previous spawning. For each fish, six replicate slices were

prepared and viewed under 40× and 80× magnification. Characterization of the gonadal development was based on Brown-Peterson et al. (2011) with 5 categories (immature, developing, spawning capable, regressing, regenerating) for females (N=27), and for males (N=34). The presence of late vitellogenic (Vtg3) atretic residual oocytes was interpreted as evidence that specimens had spawned in the present season.

2.8. Lipid analysis

Lipids composition was analyzed on liver of 10 individuals per treatment after eight weeks of exposure. The lipids of the liver samples were extracted and the different lipid classes were separated by Solid Phase Extraction (SPE) prior to analysis of fatty acids by gas chromatography. The total lipids of liver samples were extracted by a modified Folch method with chloroform/methanol (2:1 v/v) (Folch et al. 1957).

The lipid extract was separated into major lipid classes by a SPE procedure adapted from the Kaluzny et al. (1985), using aminopropyl bonded phase columns to separate lipid mixtures into individual classes. Briefly, 0.5 ml of lipid extract (approximately 8 mg lipid) was loaded in a 500 mg aminopropyl modified silica minicolumn (Macherey-nagel gmbh & co. Germany), which had been previously activated with 4 ml of hexane. Neutral lipid (Triacylglycerol's and cholesterol, NL), free fatty acid (FFA), and phosphatidylcholine/ phosphatidylethanolamine (PC/PE) were sequentially eluted with 7 ml of chloroform/isopropanol (2:1 v/v), 5 ml of 2 % acetic acid in diethyl ether, and 10 ml of methanol. The eluates were collected in 15 ml thick-walled glass tubes with Teflon lined screw caps, which contained nonadecanoic acid (19:0) as internal standard. The phosphatidylserine/phosphatidylinositol (PS/PI) fraction was obtained by opening the column and collecting all of the stationary phase directly to the test tubes. All the eluates were dried by nitrogen gas and the fatty acids were analyzed by gas chromatography with a flame ionization detector (GC-FID). Prior to analysis on GC-FID, all SPE fractions from the liver samples were methylated with 2.5 M dry HCl in methanol (HPLC-grade, Merck in Oslo, Norway) to obtain fatty acid methyl esters (FAME) that was analyzed on gas chromatograph according to Meier et al. (2006). Analyses of PAHs in the fish feed were carried out by Akvaplan-niva (accredited for the methods). Three replicate feed samples per dose were analyzed. Each sample was thoroughly grounded and homogenized prior to analyses. Samples were weighed and a potassium hydroxide-methanol solution and an internal standard-mix of deuterated PAHs were added. The solution was boiled with reflux for 4 h (saponification),

before filtration and extraction with pentane. Samples were purified using gel permeation chromatography (GPC), with dichloromethane as a mobile phase. Samples were filtrated and further purified by solid phase extraction (SPE). Analyses were performed using a GC-MSD (Agilent 7890 GC with split/splitless injector, Aglient 7683 and Agilent 5975C, mass spectrometer with EI ion source). Blind samples were run in parallel to all samples, and proficiency test samples (Quasimeme, Netherlands) were used as control samples. The limit of detection (LOD) was determined from analyses of a series of blank samples, processed along with real samples, and calculated as: LOD = (blank average) + 3 × (blank standard deviation). For the calculation of sum PAHs, values below detection limit were not considered.

2.9. Biliary 1-OH phenanthrene metabolite

1-OH phenanthrene was analyzed according to Nechev et al. (unpublished) on bile samples from the experimental fish collected at week zero, one and eight of exposure. Briefly, 1-OH phenanthrene was extracted from bile samples through enzymatic hydrolysis. Bile samples were freeze dried overnight and 40 µl of water was added to each sample. Samples were incubated for one hour at 37 °C with β-Glucuronidase/aryl sulfatase (5 µL) and an internal standard (5µL triphenylamin in methanol, 160 ng/ml) was added. After incubation, 750 µl of methanol were added and centrifuged for ten min at 13000 g and supernatants were collected. Extracts were analyzed using a HPLC Agilent 1200 Series equipped with a fluorescence detector FLD Agilent 1200 Series G1321A. Separation of the compounds was performed in a C18 column (Eclipse XDB-C18, 150 x 4.6 mm; 5 µm particle size; Agilent, USA) heated to 35 °C. The injected volume was 25 µl. The initial composition of the mobile phase was 40:60 acetonitrile:water (vv) and a linear gradient to 100% acetonitrile was programmed in 30 min, with a final hold of 5 min. Initial conditions were reached in one min and maintained for two min before the next run. The total run time was 38 min with a flow rate of 1 ml/min. 1-OH phenanthrene was detected at its optimal excitation/emission wavelength pair 256/378 nm (1-OH-phenanthrene). Samples of bile in the medium group at eight weeks exposure were lost during extraction and are thus not analyzed.

2.10. Statistical analyses

All statistical analyses were conducted with R 3.1.1 (R Core Team 2014). As our data fell

outside a normal distribution, non-parametric Kruskal-Wallis tests by ranks were employed to investigate differences between the sexes and crude oil treatments at each time point on the continuous factors of age, morphometrics, EROD activity, PAH bile metabolite concentrations, SGR, and oxygen consumption. Length and sex were tested as covariates of response variables to account for initial difference between treatment groups at tagging and inherent differences in physiology between sexes. When significant results were encountered, a post hoc pairwise Dunn´s test using rank sums was performed between the control and crude oil treatments. These tests were done in conjunction with a Benjamini-Hochberg adjustment on p-values to account for potential errors arising from multiple comparisons (Benjamini and Hochberg 1995). Correlation tests were performed using the Spearman method for PAH levels, HSI, SGR, and liver lipid levels. Linear models were used to explore the relationship between SGR and HSI in relation to crude oil treatment, sex, and length for fish after one week of exposure. Comparisons were considered significantly different than the control when $p \leq 0.05$ level. Values are reported as mean ± standard deviation (SD).

3. RESULTS

3.1. Dietary doses of PAHs, levels of 1-OH phenanthrene in the bile and EROD activity.

Polar cod from the low, medium, and high treatment were exposed weekly to 4, 40, and 200 µg crude oil/g fish, respectively (Table 1). This weekly dietary dose corresponded to a sum 26 PAHs (Σ26PAHs) in the feed of 0.004, 0.06, 0.4, and 2.4 µg 26 PAHs /g fish/ week in the control, low, medium, and high treatments, respectively. The levels of PAHs measured in the feed were significantly correlated to the nominal crude oil doses (R^2=0.97, $p<0.001$), indicating that the crude oil was homogenously mixed in the feed. Typical for crude oil, the most abundant PAHs in the feed were the low molecular weight naphthalenes with predominance of substituted compounds in the order C3>C2>C1, followed by substituted phenanthrenes (Table 2). All 26 PAHs analyzed in the feed were above detection limit in the highest exposed feed, except for indeno(1,2,3cd)pyrene (Table 2).

Levels of 1-OH-phenanthrene in polar cod bile (Fig. 1A), and EROD activity (Fig. 1B) increased in a dose-dependent manner after one week of exposure and remained at similar levels after eight weeks of exposure regardless of fish sex or length.

3.2. Fish morphometrics, gonadal maturation stages and mortality during the exposure

Although polar cod were collected and randomly distributed to the experimental tanks, at tagging the average fork length and total weight from the fish in the "low" group were significantly higher compared to medium and control groups ($p=0.03$ and 0.05 for length and weight, respectively) (Table S1). At subsequent sampling times, however, the fork length and total weight were no longer significantly different among treatments and sexes. Overall, the sex ratio was biased towards males in the medium and high treatments, unfortunately affecting the sex balance in the high treatment at eight weeks exposure (n = 1 female). Sex and length were included as covariate in response analysis to account for potential bias by the initial conditions. Specimens were between two and four years old.

The sexual maturity status of polar cod varied between gender and sampling time, but not with oil treatment. Female specimens showed low GSI (1-3 %) throughout the experiment, and the majority (n= 19 out of 27 analyzed) were immature, i.e. they had never spawned (Fig. 2 and Table S2). Specimens categorized as "regressing" (n=6) showed late vitellogenic (Vtg3) atretic residual oocytes that suggested spawning during the season. Two of these regressing females showed residual oocytes at more advanced atretic stages, a potential indication of spawning that occurred during the previous season. Thus, these two specimens may have belonged to the regenerating category. Finally, two females were in a regenerating stage with late-stage atretic residual oocytes (i.e. had skipped the present reproductive cycle). Regressing females were not significantly different in length or total weight compared to immature or regenerating females, however and although not significant, they showed a slightly reduced HSI ($p=0.15$) and reduced lipid concentration in the liver ($p=0.09$, Table S2).

Males showed significantly higher GSI (11-17 %) than females at week zero ($p=0.01$) and week one ($p<0.001$) and compared to GSI in males sampled after eight and ten weeks ($p<0.01$). Histological analysis at week zero indicated specimens in an early post-spawning stage (regressing) at exposure start (Fig. 2) with discontinuous germinal epithelium

throughout the testis, no active spermatogenesis, and residual spermatozoa in lobule lumens and sperm ducts. After eight weeks of exposure, the GSI of males had decreased to levels similar to that of females (below 2 %, Table S2). Except for one immature specimen in the medium treatment, all males were in a late regressing or regenerating stage (Fig. 2).

During the experiment, some mortality occurred in the low (n=2 at three weeks) and high (n=1 after three days, and n=4 at three weeks) treatments. Furthermore, one individual was removed from the high treatment in the sixth week due to the appearance of finrot (disintegration of caudal fin). Mortality occurred only in males. Except for two specimens of the high treatment (death at three weeks), all mortalities occurred among the specimens used in respirometry experiments, although several days after the respirometry handling. The number of polar cod left for the last sampling time (respiration individuals at ten weeks) was reduced to eight, nine and five in the low, medium, and high treatments, respectively.

3.3. Lipid class composition in the liver

Total liver lipid content and lipid class composition was studied on ten of the twelve specimens sampled from each treatment after 8 weeks of exposure to dietary crude oil. The total lipid content of the liver was correlated to the HSI ($R^2 = 0.62$, $p<0.001$). Furthermore, males had significantly higher liver lipid content (329 ± 17 mg/g liver wwt) than females (212 ± 32 mg/g liver wwt, p=0.002, Table 3) with little variation correlated with length ($p= 0.52$). For both sexes, the neutral lipids (NL) accounted usually for more than 90 % of the lipid classes. The NL were totally dominated by storage lipids, triacylglycerols. The polar membrane lipids contributed with less than 7 % (PC/PE [3-5 %], PS/PI [2 %]) of the total lipids, and FFA with 1.5 % of the total lipids. In females, there was generally a high variability in liver lipid content, with some specimens (n=3) showing extreme low values (41 - 61 mg/g liver wwt). In general, females in a regressing stage showed lower lipid content than specimens in an immature or regenerating stage (Table S2). Unfortunately, two specimens with extreme low values were represented in the control group, which resulted in lower average lipid levels in the control group (average of 120 ± 40 mg/g liver wet weight) compared to the other groups (>243 ± 54 mg/g liver wet weight), and thus erroneously suggesting an increasing trend in lipid content with crude oil dose. This also led to differences in lipid classes distribution (e.g. average of 70 % NL) in the control group compared to the

other treatments (>90 % NL). In males, there were no significant differences between treatments.

3.4. Specific growth rates (SGR)

Males and female polar cod showed no significant differences in SGR over the course of the experiment and with regard to dose. Following one week of exposure, also corresponding to the first dietary dose, a significant decrease in SGR was observed with increasing oil exposure (Kruskal-Wallis, p=0.03). Interestingly, SGR was lowest in individuals that presented a reduced HSI (Fig. 3). This relationship increased in strength with increasing dose (R^2=0.2, p=0.23 in the low treatment and increased to R^2=0.63, p<0.001 in the high treatment). The negative interaction between crude oil treatment and HSI on SGR was strongest in the high oil treatment group (p=0.017) regardless of fish sex (p=0.81) or length (p=0.38).

After eight weeks of exposure, the SGR was no longer dose-dependent (Fig. 4). Specimens sampled after ten weeks i.e. eight weeks of exposure and two weeks of recovery, showed a negative SGR, and females also exhibited a tendency to a dose-dependent reduction in SGR, although not significant (p=0.44). These specimens were also those used for respirometry every second week, thus subjected to additional handling stress over the course of the experiment.

3.5. Whole body oxygen consumption

Oxygen consumption was, in general, elevated in oil-exposed females (min-max range 72.5-202.3 mg O_2/kg fish/hr) compared to controls (min-max range 61.8-102.9 mg O_2/kg fish/hr) after four weeks of exposure (Fig. S2). This increased oxygen consumption was however not dose-dependent. In males, oxygen consumption was elevated in the medium group (min-max range 112.5-226.2 mg O_2/kg fish/hr) compared to the other treatments (min-max range 43.1-131.5 mg O_2/kg fish/hr). Although not significant, this group was characterized by the smallest average total weight, condition factor, and GSI.

4. DISCUSSION

4.1. Uptake and bioavailability of dietary crude oil

The determination of 1-OH-phenantrene metabolites in the bile and EROD activity were used as biomarkers of exposure to PAHs. As indicated by the presence of 1-OH-phenantrene metabolites in the bile and the levels of EROD activity, metabolism of PAHs was already taking place following a single dose of crude oil (first week of exposure) in both females and males. Furthermore, the dietary exposure remained dose-dependent throughout the exposure period. Dietary oil compounds ingested weekly during eight weeks in the present study were most likely bioavailable for the entire experimental period (ten weeks), including the last two weeks of depuration where polar cod were force-fed uncontaminated feed. For instance, Bakke et al. (2016) showed that a single dietary dose of phenanthrene and benzo(a)pyrene (0.40 ± 0.12 µg/g and 1.15 ± 0.36 µg /g fish for phenanthrene and benzo(a)pyrene, respectively) was retained in the tissues for at least 30 days, even for not covalently bound metabolites.

It is important to note that the responses observed in exposed polar cod of the present study cannot be solely attributed to the PAH fraction but rather a complex mixture of several thousands of unidentified petroleum compounds from the unresolved complex mixture (UCM). The UCM contains highly bioaccumulative and potentially toxic substances, and for which the toxicokinetics and toxicodynamics are largely unknown (Scarlett et al. 2007, Melbye et al. 2009, Petersen et al. 2017).

The crude oil doses used in the present study (4 – 200 µg crude oil /g fish/week) were in the same range as previous dietary studies on polar cod (George et al. 1995, Bender et al. 2016, Vieweg et al. 2018) that also showed the induction of hepatic EROD activity at their highest doses (George et al. 1995, Vieweg et al. 2018). In comparison with dietary studies on different fish species, our doses were similar to those of Bratberg et al. (2013) for cod (*Gadus morhua*) and were considered environmentally relevant. It is however important to highlight that the exposure method is not reflecting realistic environmental exposure, that would have required exposure of live feed to dispersed oil. The dietary crude oil taken-up by polar cod in this experiment was thus not represented by a realistic fraction composed of potential metabolites produced by living prey items. Furthermore, all compounds present in the whole crude oil were ingested by polar cod including fractions that may not have been bioavailable to the fish through exposure to live feed in the natural environment.

4.2. Baseline physiological status, SGR, and routine metabolic rate in control specimens

The study design suffered from an unexpected strong divergence in the physiological state of females and males that forced a sex-specific data analysis for certain parameters such as lipid composition. Accounting for these initial conditions by including physiological covariates such as sex and length allowed for a thorough investigation of possible effects of crude oil exposure on physiological endpoints. Even though some of the parameters did not show significant effects ($p>0.05$), pronounced trends are discussed hereafter.

The majority of the female polar cod in the present study were immature and had thus never spawned before. By contrast, the histological analysis of the male gonads suggested that males had been spawning capable and most likely spawned earlier in the season, and advanced from a post-spawning (regressing) stage at the exposure start towards a resting (regenerating) stage at the end of the ten week experimental period concurrent with a significant decrease in GSI. This was further supported by the GSI at the exposure start that were lower than values known for ripe males in January (>30 % in e.g. Hop et al. 1995, Nahrgang et al. 2014), and in the known spawning timeframe (January-March) of polar cod populations of the Barents Sea (Hop and Gjøsæter 2013). The disparity in maturity stage between sexes may be explained by males reaching sexual maturity at a younger age than females (Hop and Gjøsæter 2013, Nahrgang et al. 2014). However, this hypothesis was not verified based on the otolith readings in the present study. In general, the very low HSI (75^{th} percentile = 3.4 % all treatments combined) indicated that the specimens were in a weak physiological state. No previous studies have shown such low HSI levels in this species for any season (Nahrgang et al. 2010a, Nahrgang et al. 2014, Bender et al. 2017, Vieweg et al. 2017). The hepatic lipid levels in our post-spawning males were half those reported in males in the fall and early winter (Hop et al. 1995, 1997), suggesting an important allocation to reproduction. Females in the present study showed even lower levels of hepatic lipid content than males. In particular, the few females that were in a post-spawning stage had less than half the total lipid levels observed in males, and storage lipids (NL) represented as little as 20 % of the total lipid class composition.

Specific growth rates were in general lower than rates reported in the same species elsewhere (Hop et al. 1997, Laurel et al. 2016, 2017). Although comparison to other studies may be difficult due to different factors (e.g. feed type, age, size range, temperature, and handling stress), SGR in polar cod fed to satiation have been shown to range between 0.5 % and 1.5 % wwt/day depending on size (Hop et al. 1995, Laurel et al. 2016). In the present study, SGR

levels were negative in most individuals. Our weekly feed rations (4 % body wwt/week) were similar to maintenance levels reported by Hop et al. (1997). However, our study was conducted at higher temperatures than in Hop et al. (1997) (ca 6 °C instead of 0 °C), and our metabolic rates in control specimens (e.g. 92.0 ± 12.1 mg O_2/kg fish/hr for mean ± SE at ten weeks) were elevated compared to levels (51.03 ± 6.27 mg O_2/kg fish/hr) reported in Hop and Graham (1995). Given the elevated metabolic costs at increased temperatures, the rations given in the present study were insufficient to reach a positive growth in weight. The weight loss could be further rationalized by the particular weak physiological state of our specimens in early spring, as indicated by the reduced hepatic lipid levels. Finally, the weekly force-feeding most likely represented an additional handling stress that affected growth performance (e.g. McCormick et al. 1998, Barton 2002, Jentoft et al. 2005). The specimens in the present study were therefore under sub-optimal conditions for growth including elevated temperatures, reduced feed ration, and low energy reserves.

4.3. Effect of crude oil on total wet weight alterations, and routine metabolic rate

The deleterious effect of crude oil or petroleum related compounds on fish growth has been shown previously in polar cod (Christiansen and George 1995, Bender et al. 2018), as well as in other fish species (Al-Yakoob et al. 1996, Moles and Norcross 1998, Kerambrun et al. 2012, Claireaux et al. 2013, Sandrini-Neto et al. 2016). In sexually developing polar cod, Christiansen and George (1995) found a reduction in weight gain when exposed to crude oil contaminated feed at levels (ca 2.1 - 2.6 µg crude oil/g fish/day) in the lower range of this study (0.6 – 28 µg crude oil/g fish/day). In the present study, there was a seemingly rapid (following the first dietary dose) and dose-dependent loss in weight, especially in individuals with an initial low condition (see section 4.2., and Fig. 3), suggesting an increased energy trade-off between somatic growth, and potential detoxification metabolism in individuals with reduced energy stores. Handling stress from the force-feeding may as well have been an aggravating factor on growth performance (McCormick et al. 1998). Indeed, fish were fed by hand during the acclimation period, and the first force-feeding event corresponded to the start of the exposure, one week following transfer to experimental tanks and tagging.

Another hypothesis that cannot be ruled out is the alteration of feed assimilation and/or conversion, in crude oil exposed groups, leading to a decrease in energy intake. A reduction in digestive function from crude oil exposure was suggested for river otters (*Lontra canadensis*) (Ormseth and Ben-Davi 2000), and juvenile turbot (*Scophthalmus maximus*) (Saborido-Rey et

493 al. 2007). It is not possible to discriminate the mechanisms leading to an accelerated loss in
494 weight, but the effects in the high oil treatment are likely due to a combination of several
495 factors such as an increased energy demand due to handling stress and detoxification
496 metabolism, and alteration in digestive function.
497
498 The dose-dependent increase in weight loss after one week of exposure seemed to be offset
499 over the eight week exposure period in all oil treatments. Similarly, Bender et al. (2018)
500 found a transient depression in growth in polar cod acutely exposed to dispersed oil, followed
501 by a period of increased growth in exposed individuals compared to controls. While the
502 mechanisms could not be explained, a temporary reduction in feeding activity in exposed fish
503 or potentially compensatory mechanisms for growth were suggested. In the present study,
504 force feeding allowed for control of the feed intake by each individual. Thus, changes in
505 feeding regime or appetite (Christiansen and George 1995) could not explain the accelerated
506 weight loss in oil exposed individuals, nor the following reduction in weight loss. Ali et al.
507 (2003) suggested that behavioral adjustments (e.g. reduction in locomotion and metabolic
508 costs) and changes in growth efficiency may play a role in growth compensation. Also, an
509 habituation to the force-feeding over the following seven weeks may also have attenuated the
510 combined effects of the exposure and stress on growth observed during the first sampling
511 point (McCormick et al. 1998). Fish that were monitored for growth following the final two
512 weeks of depuration (tenth experimental week) had also been used in respirometry
513 measurements every second week during the entire experimental period, and had thus
514 undergone additional handling stress. The worsening effect of handling stress was again
515 marked on the health of these individuals with the increased incidence of mortality in males
516 from the oil treatments and the trend to a dose-dependent reduction in weight loss in females.
517
518 The consistency in oxygen consumption levels found from week to week in both sexes
519 suggested that these specimens had reached a steady state, and had adjusted their routine
520 metabolic rate within the first weeks of exposures. Females exposed to crude oil showed a
521 dose-independent, but elevated oxygen consumption, suggesting a threshold response to an
522 elevated energy demand. This elevated oxygen consumption may correspond to the so-called
523 “resistance” phase in the conceptual model of the general adaptation syndrome developed by
524 Selye (1973). At equal feed intake and considering the increased trend in weight loss with
525 dose, it can be hypothesized that females of the high treatment may have had a more
526 important energy trade-off compared to the low and medium treatment females. On the

contrary, Christiansen et al. (2010) showed a decrease in routine metabolic rate in polar cod exposed to the crude oil WSF both acutely and for the following four weeks. In this case, the depression in oxygen consumption from acutely exposed specimens was mostly attributed to an immediate response associated with behavioral changes (e.g. immobility). The depression of long-term (four weeks) exposed individuals could not be explained, but was suggested to be related to a crude oil induced alteration in digestion or assimilation by Klinger et al. (2015), resulting in decreased SDA and associated metabolic rates relative to controls.

Male polar cod showed in general no significant alterations of neither weight loss nor routine metabolic rate with dose. The elevated metabolic rate found in males from the medium group at all time points studied, could be explained by a lower body mass (mean 13 ± 2 g wwt) compared to the other groups (mean 14 ± 4 g wwt) (Table S1). Mass specific oxygen consumption increases with decreasing body weight in fish, thus suggesting that the increased routine metabolic rate in the medium group was a size artefact rather than the effect of the crude oil exposure. Although, males seemed more robust to the exposure than females, exposure may alter active metabolic rate and thus the metabolic scope for activity, even though their minimum energy demands were maintained. Such effects were shown in common sole (*Solea solea*) exposed to fuel oil (Davoodi and Claireaux 2007). Furthermore, mortality during the experiment was solely observed in male specimens thus suggesting a sex-specific sensitivity.

Post-spawning survival is believed to be linked to remaining energy reserves and the capability of polar cod to resume feeding (Hop et al. 1995). This feature is highly important, especially in females for which fecundity is limited by body size, and are thus dependent on growing larger and reproducing over several winters to maximize fecundity (Nahrgang et al. 2014). Polar cod shows a high-energy investment in reproduction, compared to other gadids, with a total body weight loss of 30-50 % through gonadal development and spawning (Hop et al. 1995). Post-spawning survival is thus dependent on optimal conditions to resume feeding and acquiring new energy stores. The additional stress from exposure to petroleum may thus divert already low energy reserves to detoxification metabolism and away from growth, potentially leading to a significant reduction in condition or even death. While previous studies have investigated the sensitivity of polar cod to low exposure doses during gonadal maturation and concluded with a certain robustness (Bender et al. 2016, 2018), the low-energy status of females in spring, potentially related to spawning, may be a more critical

time period during the year, where initial low conditions of these specimens could have important consequences on their capacity to cope with additional stress factors such as pollutants. Females were also suggested to be more at risk due to their indiscriminate feeding behavior when offered both contaminated and uncontaminated feed (Christiansen and George 1995). The present study, does not allow drawing firm conclusions on the true risk implied in this hypothesis, and requires future work.

The two weeks of recovery where fish were fed clean feed did not show any changes in SGR compared to specimens exposed during eight weeks. The two weeks window may have been too short to highlight any significant physiological changes in the organisms or the endpoints measured were simply not sensitive enough to highlight any recovery. For instance, restoration of baseline levels within two weeks following crude oil exposure has been previously found in the same species but for molecular and cellular biomarkers (Nahrgang et al. 2010c, Andersen et al. 2015).

5. CONCLUSION

The present study revealed a negative impact of crude oil exposure on growth performance on adult polar cod with low condition in the early spring. The differential physiological states of both sexes in terms of liver lipid content and maturity status, influenced their response to crude oil exposure, with females increasing their routine metabolic rate, and mortality only observed in males. The present study suggests that hepatic storage lipids are a critical factor for growth of adult polar cod, especially when exposed to additional stressors such as dietary crude oil. Dietary levels of crude oil as low as 4 µg crude oil per g fish per fish led to reduced SGR in specimens with low HSI and this effect increased with increasing crude oil dose. The present study further stresses the importance of investigating the sensitivity to oil exposure of specimens in a post-spawning state. It also calls for caution in study designs that involve a significant amount of animal handling, as this can have important consequences on data quality and conclusions drawn.

ETHICAL STATEMENT

All work was performed according to and within the regulations enforced by the Norwegian Animal welfare authorities. The R/V Helmer Hanssen is owned by the University of Tromsø, which has all the necessary authorization from the Norwegian Fisheries Directorate to use a

bottom trawl to collect fish for scientific purposes. Permission to carry out this experiment was granted by the Norwegian Animal Welfare Authority in 2012 (ID 4377).

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797

798 Figure Legend
799
800 Figure 1. (A) 1-OH phenanthrene metabolites (ng/g bile dw) in polar cod bile, and (B) EROD
801 activity (pmol/min/mg protein) in polar cod liver, at exposure start (zero weeks), and after one
802 and eight weeks of exposure. Bile metabolite samples in the medium group at eight weeks
803 were not available (NA). Plots show individual data points distinguished by shape and color
804 for each treatment group, treatment group means are represented with a dash (–). Different
805 letters (a, b, c) indicate significant differences (Kruskal Wallis test, $p<0.01$) among treatments
806 for each time point.
807
808 Figure 2. Sexual maturity of polar cod at exposure start (T0) and after eight weeks of
809 exposure, based on histological examination of gonads. Only three maturity stages were
810 identified (immature, regressing, and regenerating). Bars are representing counts.
811
812 Figure 3. The interaction of HSI (%) and treatment on the total weight specific growth rate (%
813 TW per day) of mixed sex fish in the first week of exposure. Results from linear models and
814 95% confidence intervals are plotted for each treatment group with data points representing
815 individual fish.
816
817 Figure 4. Specific growth rate (SGR, % TW per day) of mixed females and males between the
818 period from tagging to one, eight and ten weeks. Plots show individual data points
819 distinguished by shape and color for each treatment group, treatment group means are
820 represented with a dash (–).

Table

Tables

Table 1. Crude oil nominal concentrations, and measured concentrations of sum of 26 PAHs in the feed (µg/g feed wwt) and as weekly doses in the fish (µg/g fish/week or µg/fish /week). Data represent mean ± standard deviation.

Treatments	Crude oil nominal doses		Sum 26 PAHs		
	Feed mg/g wwt	Fish dose µg/g fish/week	Measured concentration in feed, µg/g wwt	Fish dose µg/g fish /week	Fish dose µg/fish /week
Control	0	0	0.08 ± 0.01	0.003	0.05 ± 0.01
Low	0.1	4	1.6 ± 0.04	0.06	1.1 ± 0.4
Medium	1	40	11.7 ± 0.5	0.5	7.0 ± 2.1
High	5	200	64.4 ± 3.8	2.6	41.4 ± 8.0

Table 2. Concentration of 26 PAHs (µg/kg wet weight) and their sum (µg/g wwt) in the diet fed polar cod (*Boreogadus saida*) in the control, low (0.1 mg crude oil/g feed), medium (1 mg crude oil/g feed) and high (5 mg crude oil /g feed) treatments. For the determination of the sum PAHs, values below the limit of detection (LOD) were not considered. Data represent mean ± standard deviation.

	Control	Low	Medium	High
Naphthalene	< 5.8	85.7 ± 4.0	674.2 ± 27.5	3673.3 ± 163.8
C1-Naphthalene	< 10	208.2 ± 12.1	1684.5 ± 92.2	9325.3 ± 723.3
C2-Naphthalene	< 13	295.6 ± 11.7	2428.9 ± 107.4	13367.7 ± 634.0
C3-Naphthalene	78.4 ±13.7	701.9 ± 20.7	4819.5 ± 214.9	26505.0 ± 1650.4
Acenaphthylene	0.8 ± 0.1	0.7 ± 0.01	1.3 ± 0.2	4.2 ± 0.5
Acenaphthene	< 1.1	3.1 ± 0.4	23.8 ± 1.7	135.6 ± 11.5
Fluorene	< 0.8	9.0 ± 1.0	67.5 ± 1.9	369.2 ± 31.1
Dibenzothiophene	< 0.5	3.6 ± 0.2	27.1 ± 1.1	151.4 ± 2.5
C1-Dibenzothiophene	< 1.5	8.6 ± 0.2	61.8 ± 5.7	361.0 ± 35.9
C2-dibenzothiophene	< 4.5	16.8 ± 0.6	125.6 ± 6.6	656.0 ± 39.3
C3-dibenzothiophene	< 4.1	17.5 ± 0.6	136.8 ± 5.6	716.2 ± 30.7
Antracene	< 0.3	0.4 ± 0.1	0.5 ± 0.1	2.9 ± 0.3
Phenanthrene	< 3.0	21.6 ± 0.5	183.1 ± 5.5	996.5 ± 28.5
C1-Anthr/Phenanthrene	< 8.6	45.5 ± 1.6	343.9 ± 25.6	1959.6 ± 85.7
C2-Anthr/Phenanthrene	< 7.4	73.4 ± 2.1	602.4 ± 19.2	3305.6 ± 178.8
C3-Anthr/Phenanthrene	< 4.7	62.1 ± 7.0	473.3 ± 26.8	2692.7 ± 282.2
FLuoranthene	< 2.0	< 2.0	3.2 ± 0.5	17.6 ± 9.1
Pyrene	< 3.3	< 3.3	6.7 ± 3.1	34.5 ± 9.5
Benzo(a)anthracene	< 0.5	< 0.5	2.3 ± 0.3	16.5 ± 5.2
Chrysene	< 0.6	0.8 ± 0.02	6.9 ± 0.2	44.1 ± 3.3
Benzo(b)fluoranthene	< 0.7	< 0.7	1.2 ± 0.4	10.4 ± 9.7
Benzo(k)fluoranthene	< 0.2	< 0.2	< 0.2	3.7 ± 3.1
Benzo(a)pyrene	< 0.3	< 0.3	0.6 ± 0.2	6.8 ± 6.1
Indeno(1,2,3-cd)pyrene	< 0.7	< 0.7	< 0.7	< 0.7
Benzo(ghi)perylene	< 0.6	< 0.6	< 0.6	5.7 ± 4.8
Dibenzo(a,h)anthracene	< 0.26	< 0.26	< 0.26	1.60 ± 1.5
SUM 26 PAHs, µg/g	0.08 ± 0.01	1.6 ± 0.04	11.7 ± 0.5	64.4 ± 3.8

Table 3. Lipid content (mg/g liver wet weight) and lipid class distribution (% distribution of the fatty acids in the different lipid classes) in polar cod liver following eight weeks of exposure. Out of the four control group females, two had atretic vitellogenic oocytes and extreme low levels (<61 mg/g liver wet weight) of liver lipids. Males and females showed significant differences in total lipid levels (Kruskal-Wallis test, p=0.002). NL; neutral lipid, PC/PE; phosphatidylcholine/phosphatidylethanolamine, PS/PI; phosphatidylserine/phosphatidylinositol, FFA; free fatty acid

	Control	Low	Medium	High
Females	(n=4)	(n=6)	(n=3)	(n=1)
Lipid (mg/g)	120 ± 40	244 ± 58	243 ± 54	292
Lipid class distribution (%)				
NL	70.2 ± 17.0	91.6 ± 2.5	92.1 ± 2.7	93.6
PC/PE	19.9 ± 11.3	5.2 ± 1.5	4.4 ± 2.0	3.8
PS/PI	7.1 ± 4.6	2.1 ± 0.8	1.9 ± 0.5	0.9
FFA	2.8 ± 1.2	1.2 ± 0.3	1.7 ± 0.3	1.6
Males	(n=5)	(n=4)	(n=7)	(n=9)
Lipid (mg/g)	363 ± 21	345 ± 78	322 ± 25	308 ± 28
Lipid class distribution (%)				
NL	95.7 ± 0.2	93.6 ± 2.0	94.9 ± 0.5	94.4 ± 0.5
PC/PE	2.3 ± 0.1	3.9 ± 1.5	2.4 ± 0.3	3.4 ± 0.4
PS/PI	0.5 ± 0.1	1.2 ± 0.5	1.1 ± 0.2	1.0 ± 0.2
FFA	1.5 ± 0.1	1.3 ± 0.2	1.6 ± 0.1	1.2 ± 0.2

Figure 1

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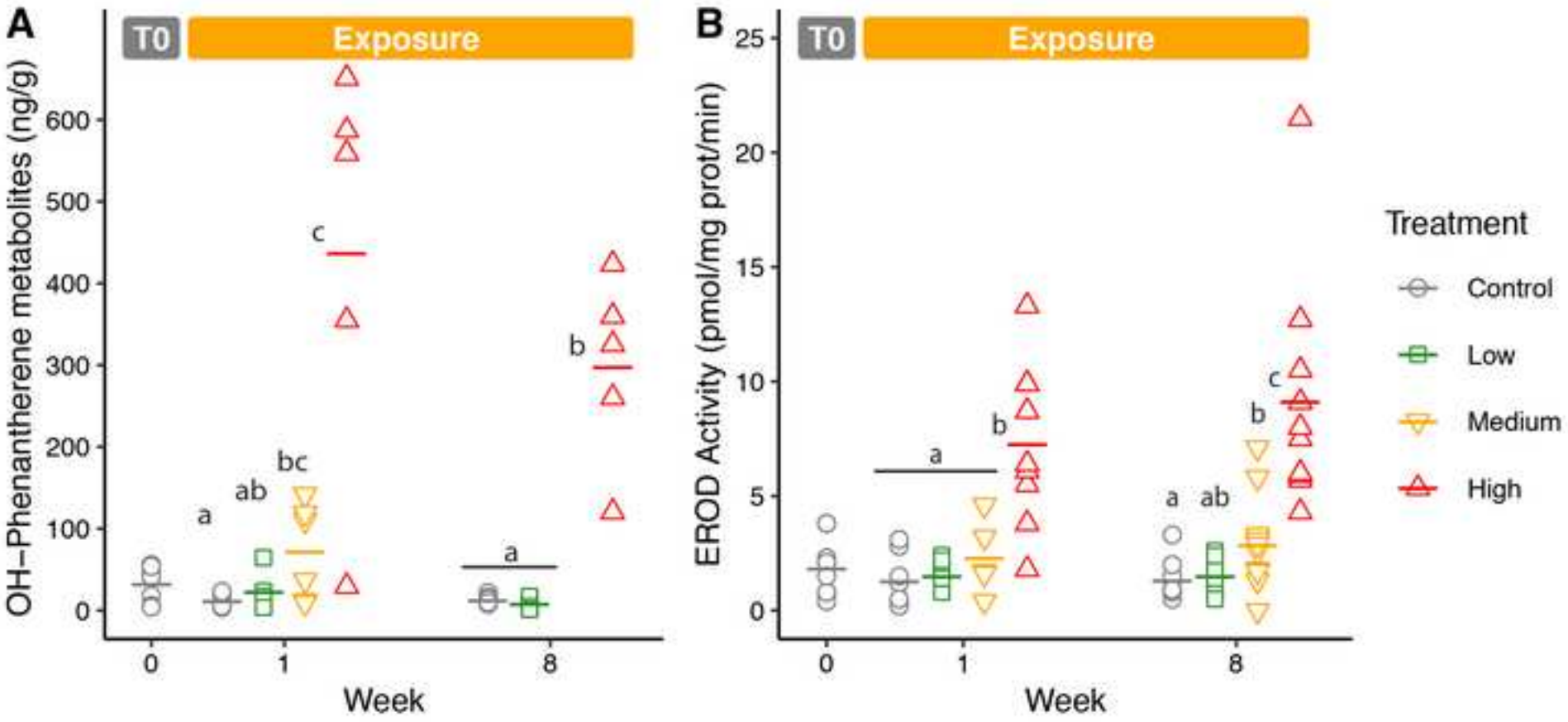

Figure 2

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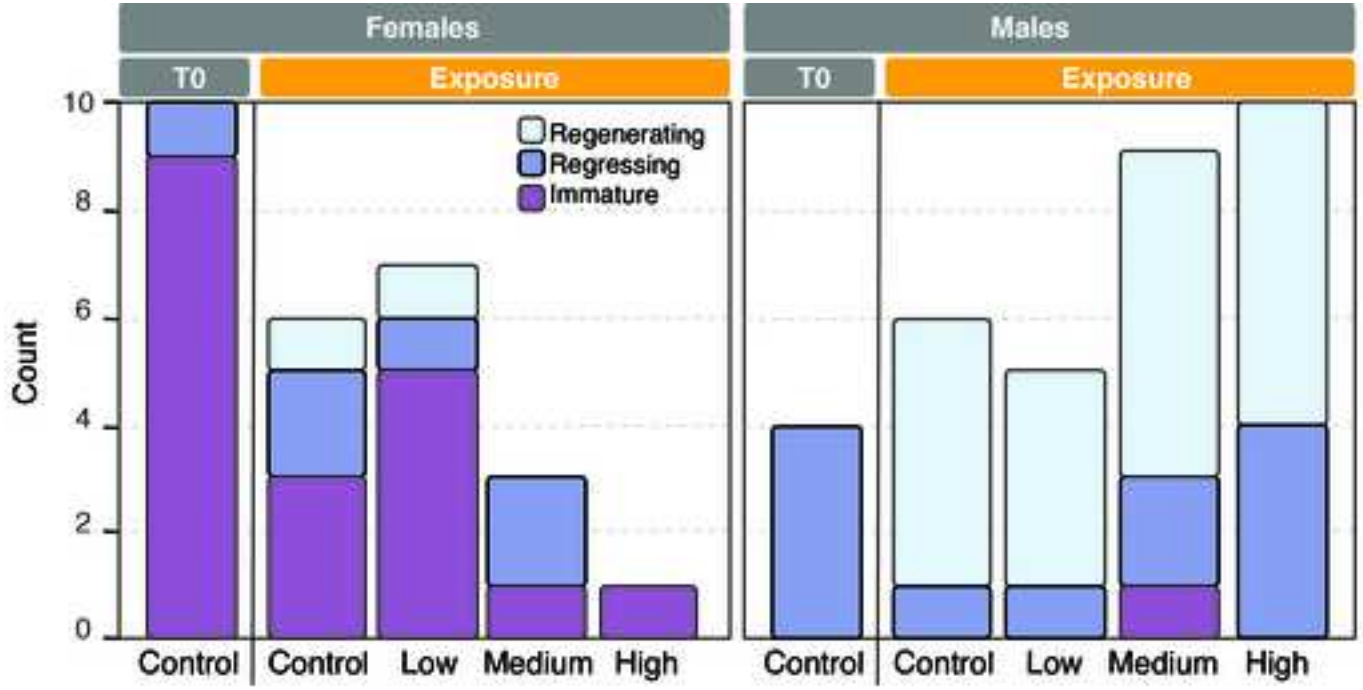

Figure 3
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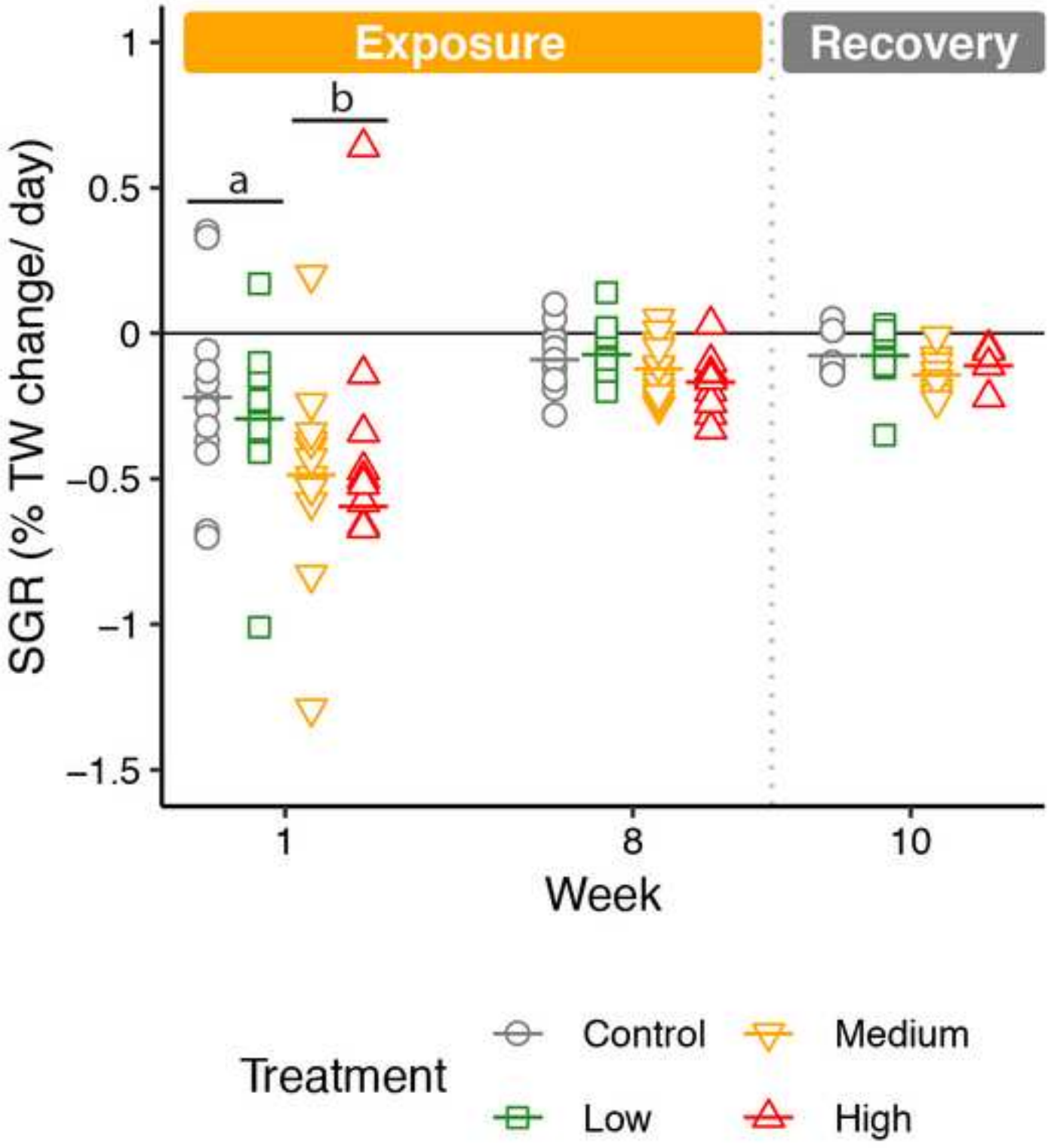

Figure 4

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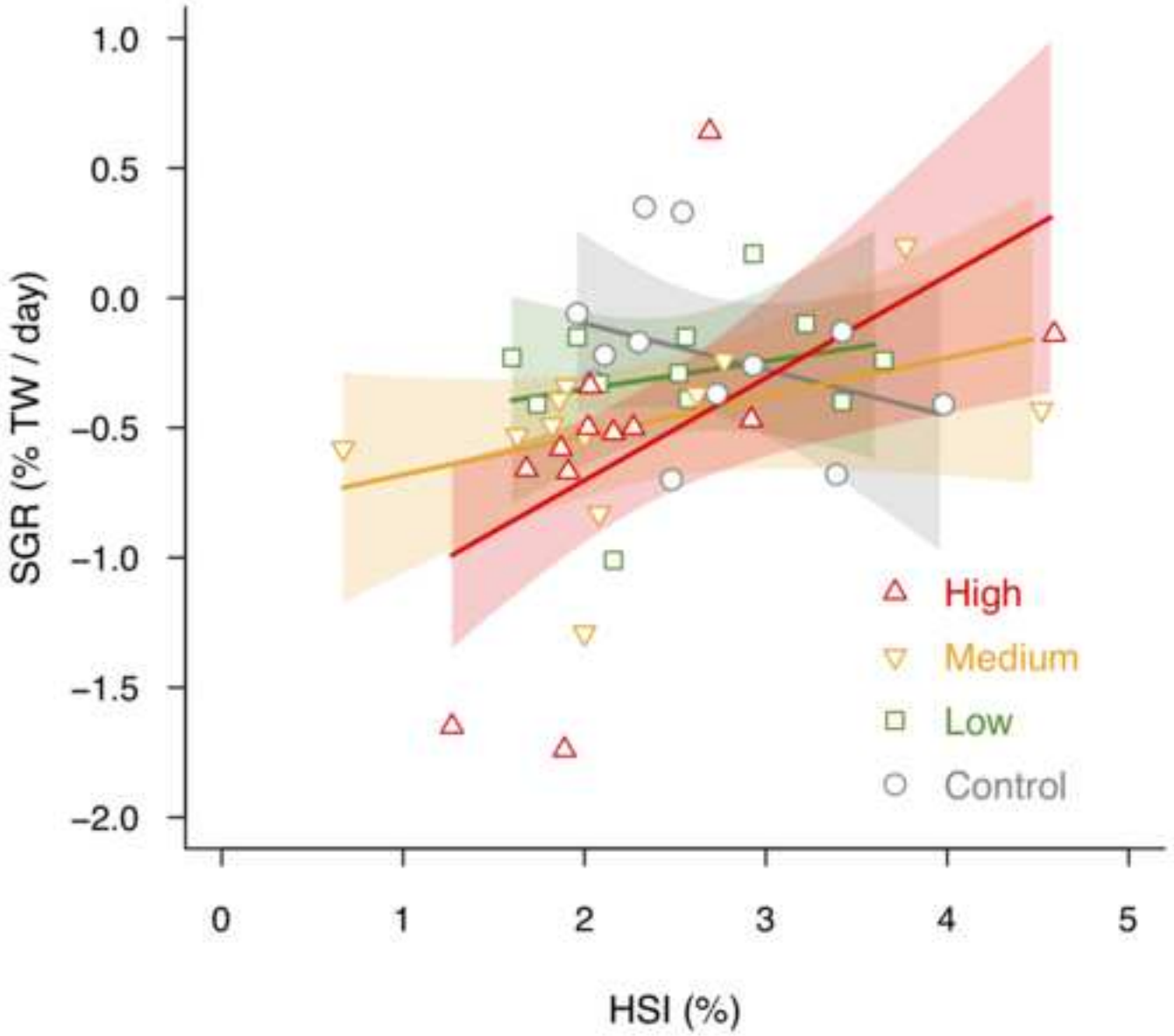

SUPPLEMENTARY INFO

Table S1. Overview of polar cod age (years, min-max range), fork length (mean ±SD, cm), total weight (mean ±SD, g), Fulton's condition factor K (mean ± SD, %), GSI (mean ± SD, %), and HSI (mean ± SD, %) per treatment (control, low, medium, and high) and for all organisms before exposure start (tagging) and at each sampling time (zero, one, eight and ten weeks). F for females and M for males. During tagging and transfer to the different treatments, sex was unknown. Letters show significant differences (Dunn's Test, $p<0.05$) between treatments for each sex and time point. Numbers in bold show significant differences (Kruskal Wallis Test, $p<0.05$) between sexes for each treatment and time point.

Time	Treatment	N	Age (years)	Fork length (cm)	Total weight (g)	K	GSI	HSI
Tagging	Control	34		13.7 ± 1.0[a]	15.0 ± 3.0[a,b]			
	Low	34		14.6 ± 1.4[b]	17.8 ± 5.7[b]			
	Medium*	33		13.7 ± 1.1[a]	14.5 ± 3.4[a]			
	High	34		14.0 ± 1.4[a,b]	16.1 ± 3.5[a,b]			

Time (weeks)		F	M	F	M	F	M	F	M	F	M	F	M	F	M
0	Control	10	4	2-3	2	14.9 ±1.8	14.3 ±2.1	16.2 ±6.7	17.1 ±8.4	0.45 ±0.02	0.47 ±0.07	**2.6 ±0.8**	**11.8 ±7.9**	2.2 ±0.9	1.8 ±0.8
1	Control	5	7	2-3	2-3	13.8 ±0.6	13.7 ±1.2	13.3 ±1.5	14.3 ±3.5	0.48 ±0.08	0.47 ±0.05	**2.1 ±0.6**	**14.3 ±4.5**	2.7 ±0.6	2.6 ±0.7[a]
	Low	6	6	2-4	2-3	15.2 ±0.8	15.1 ±1.2	17.2 ±9.2	20.0 ±5.4	0.44 ±0.02	0.48 ±0.03	**3.5 ±1.5**	**16.3 ±3.1**	2.5 ±0.8	2.6 ±0.6[a]
	Medium	6	6	2-4	2-3	14.3 ±1.0	13.9 ±1.4	13.6 ±2.4	14.9 ±5.3	0.45 ±0.07	0.45 ±0.04	**2.3 ±0.5**	**17.3 ±7.5**	**2.8 ±1.1**	**1.8 ±0.6[b]**
	High	4	8	2-4	2-4	15.6 ±2.7	14.3 ±0.5	18.1 ±6.8	15.5 ±2.2	0.45 ±0.08	0.46 ±0.04	**2.8 ±0.8**	**14.5 ±3.4**	2.7 ±1.4	2.1 ±0.3[a,b]
8	Control	6	6	2-3	2-3	14.5 ±1.0	14.1 ±0.8	15.2 ±2.8	15.0 ±2.0	0.48 ±0.03	0.51 ±0.02	1.7 ±0.3	1.2 ±0.7[a,b]	**2.0 ±0.4**	**3.5 ±0.7**
	Low	7	5	2-4	2-3	14.4 ±1.5	14.4 ±0.8	16.5 ±5.4	16.1 ±3.2	0.51 ±0.05	0.51 ±0.03	1.5 ±0.3	1.2 ±0.5[a,b]	2.7 ±0.7	3.4 ±1.2
	Medium	3	9	2-3	2-3	14.5 ±1.7	13.2 ±1.1	14.8 ±2.3	12.2 ±2.7	0.48 ±0.08	0.50 ±0.03	1.7 ±0.04	0.9 ±0.2[a]	2.7 ±0.3	3.2 ±0.9
	High	1	11	2-3	3	14.2	13.7 ±1.0	15.2	13.6 ±2.6	0.51	0.50 ±0.04	1.2	2.1 ±2.6[b]	3.3	3.5 ±0.8
10†	Control	4	6	2-3	2-3	15.0 ±1.4	13.4 ±1.1	15.5 ±3.8	13.0 ±3.6	0.48 ±0.04	0.51 ±0.05	**1.4 ±0.3**	**0.8 ±0.3**	3.0 ±0.9	2.6 ±1.0
	Low	3	5	3	2-3	15.1 ±0.9	14.5 ±1.4	18.9 ±3.5	16.0 ±4.9	0.52 ±0.02	0.49 ±0.04	**1.5 ±0.1**	**1.0 ±0.2**	3.2 ±1.4	2.2 ±1.3
	Medium	3	6	2	2	13.9 ±2.0	13.7 ±0.7	13.2 ±2.0	12.8 ±2.4	0.47 ±0.02	0.48 ±0.03	0.7 ±0.4	0.7 ±0.2	2.3 ±0.6	2.7 ±0.7
	High	2	3	n.a	2-3	13.9 ±0.1	13.4 ±0.4	14.0 ±0.7	13.0 ±2.2	0.50 ±0.007	0.51 ±0.05	2.0 ±1.6	1.3 ±0.7	2.6 ±0.4	3.9 ±0.9

* one fish had jumped out before experiment start.
† specimens at 10 weeks were individuals used in respirometry

Table S2. GSI (mean ± SD, %), HSI (mean ± SD, %), and lipid content (mean ± SD, mg/g liver wet weight) in female and male polar cod according to sexual maturation stages after eight weeks of exposure. Statistical significance (Kruskal-Wallis test, p-values<0.05) between reproductive stages are in bold.

	n	GSI (%)			n	HSI (%)			n	Lipid content (mg/g)		
Female												
Immature	10	1.53	±	0.26	10	2.73	±	0.58	8	252.5	±	113.2
Regressing	5	1.66	±	0.23	4	1.92	±	0.44	5	122.8	±	89.8
Regenerating	2	1.79	±	0.08	2	2.51	±	0.62	1		331	
Male												
Immature	1	**0.78**			1	3.28			1		376	
Regressing	8	**2.49**	**±**	**2.84**	8	**3.66**	**±**	**0.71**	8	336.9	±	96.1
Regenerating	21	**1.03**	**±**	**0.27**	21	**3.33**	**±**	**0.93**	17	326.6	±	85.2

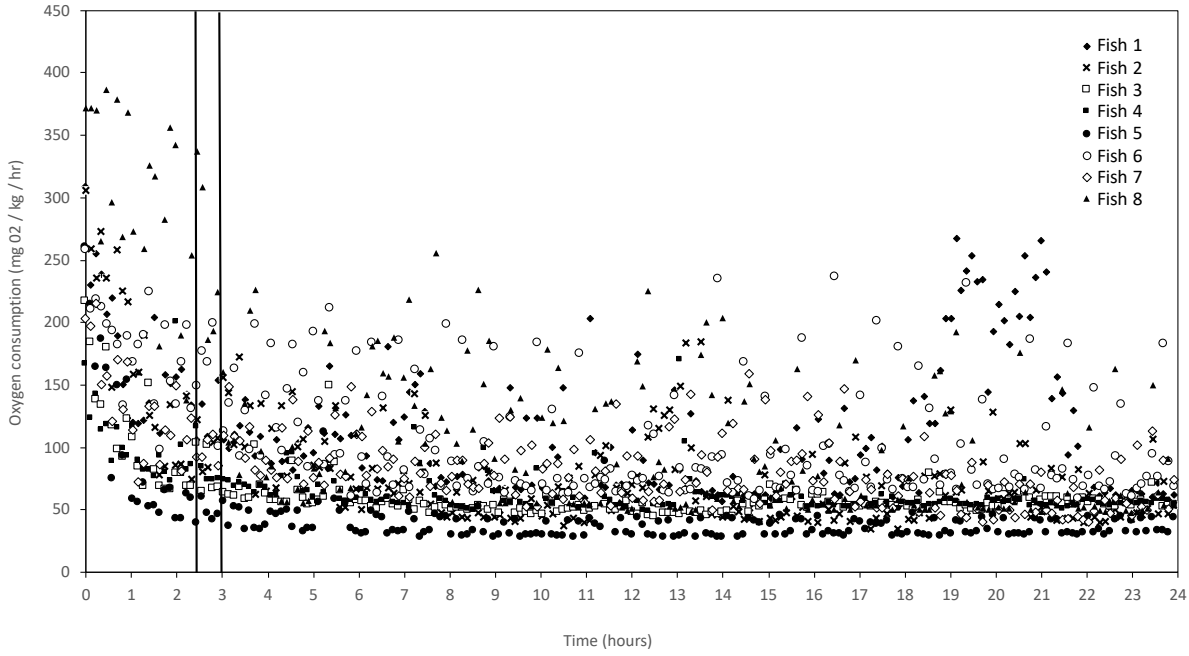

Figure S1. A 24 hour cycle of the whole body respiration of eight polar cod during the acclimation period. The elevated respiration in the first two hours is due to the stress of handling and the new environment in the respirometry chambers. Lines at 2.5 hours and 3 hours show the time in between which respiration data was used and averaged for experimental results.

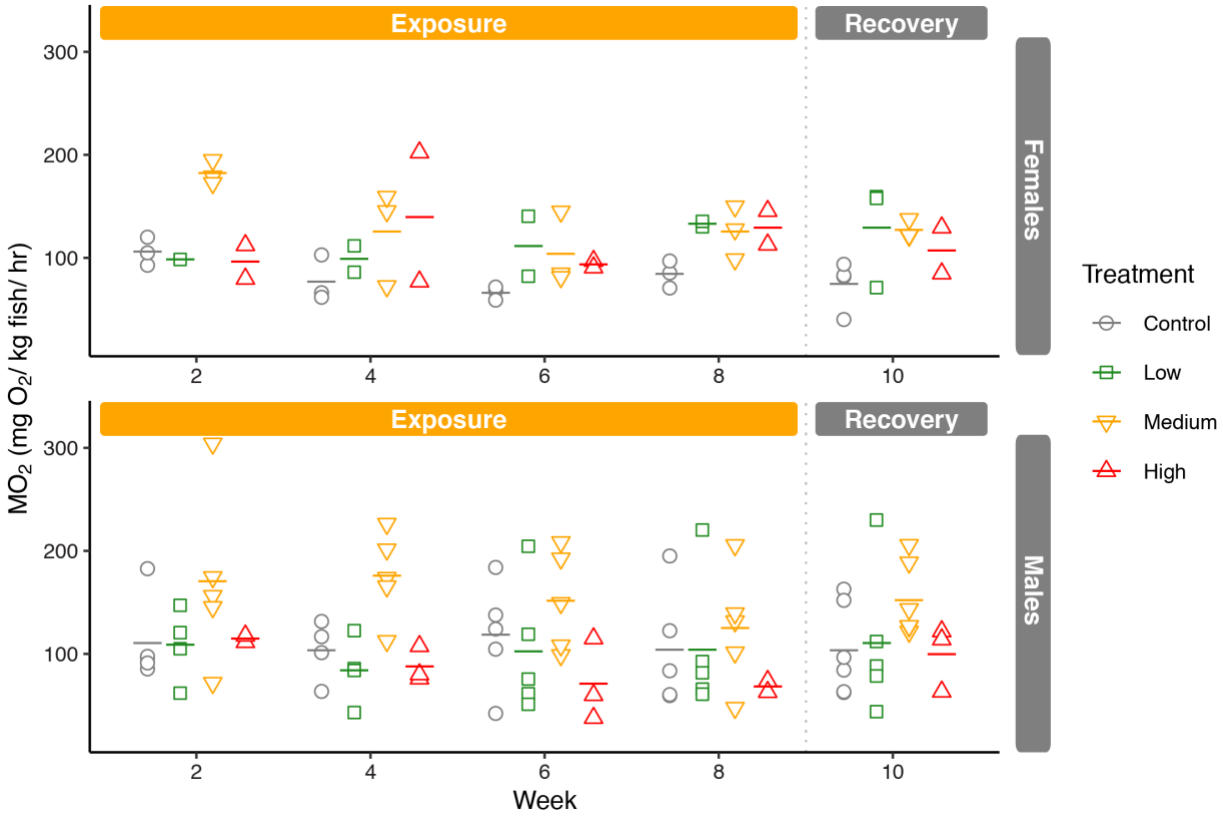

Figure S2. Mass specific oxygen consumption (mg O_2 per kg fish per hour) of female and male polar cod measured in the same specimens with two weeks intervals (11-17 days) over the course of the experiment, i.e. eight weeks of exposure to dietary crude oil (2-8 weeks) and following two weeks of recovery (10). Plots show individual data points with the average (rectangle).