Food quantity does not compensate for poor food quality in fitness of juvenile Chinook salmon (*Oncorhynchus tshawytscha*)

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Highlights

High concentrations of DHA, EPA and DHA/EPA ratios resulted in increased fish growth and condition

- Higher food intake does not compensate for low DHA, EPA and DHA/EPA
- Changes in zooplankton species composition as food source affect juvenile Chinook condition
- Climate change effects on zooplankton species composition can affect juvenile Chinook condition

Abstract

Changes in marine prey availability and nutritional quality can have effects on juvenile salmon fitness (i.e., growth, condition, and mortality) during the early marine phase. There is limited knowledge of the interplay between prey availability and prey quality, and the importance of food quality under food satiated conditions. Here, a four-phase and 11-week long feeding experiment measured the effects of nutritional quality (fatty acid composition and ratios) juvenile Chinook salmon (*Oncorhynchus tshawytscha*) fitness. Experimental diets were chosen based on the ratio of two essential fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). We tested the effects of three diets with different DHA/EPA ratios representing different naturally occurring prey species (DHA/EPA: Artemia = 0.05; Aquafeed = 0.79; Krill (*Euphasia pacifica*) = 0.99). The four experimental phases were: 1 - acclimation; 2 - weaning onto treatment diets; 3 - experimental feeding phase; 4 - Artemia-reared fish switching to commercial aquafeed. Fish were sampled weekly for all treatments and replicates, and growth rates, condition (RNA/DNA and Fulton K), fatty acid composition and mortality rates were measured. Fatty acids were incorporated into salmon muscle at varying rates but on average reflected dietary concentrations. High dietary concentrations of DHA, EPA and high DHA/EPA ratios resulted in increased fish growth and condition. In contrast, low concentrations of DHA and EPA and low DHA/EPA ratios in the diets were not compensated for by increased food quantity. This result highlights the importance of food quality being considered when assessing fish response to changing ocean conditions.

Key words: fatty acids, RNA/DNA, growth rates, mortality, energy mobilization, zooplankton

1. Introduction

Environmental variability in pelagic coastal marine ecosystems is a key determinant of timing, abundance, and species composition of zooplankton prey for foraging fish (Mackas et al., 2013; Mahara et al., 2018). This environmental variability occurs in space (e.g., bathymetry, tidal mixing) and time across a range of scales imposed by both natural cycles (e.g., seasonality, climate oscillations), and anthropogenic forcing (e.g., ocean warming, acidification). Dietary changes due to changes in prey species composition may play an important role in the feeding success, growth, and mortality of fish (Burke et al., 2013; Miller et al., 2014, 2013), and particularly during the vulnerable juvenile phase (Beamish et al., 2004; Pearcy and McKinnell, 2007). Understanding how prey composition impacts consumers has become a necessary step for estimating the survival of commercially important fish species. The quantity of prey is one facet of zooplankton composition that may directly contribute to a fish's condition, survival, and growth (Ahlgren et al., 2005; Daly et al., 2010; Olsen, 1999). An additional aspect of zooplankton prey relevant to consumers is their nutritional quality, reflected by the essential nutrients that they provide (Copeman and Laurel, 2010; Litz et al., 2017; Paulsen et al., 2014; Reitan et al., 1997).

Measures of zooplankton nutritional quality include energy density (e.g., lipid content) and biochemical composition (fatty acids and amino acids) (Litz et al., 2017). Lipids are organic compounds important for energy storage, cell membrane structure, gene expression, and biosynthesis (Arts and Kohler, 2009; Sargent et al., 1999; Tocher et al., 2003a). Fatty acids are major constituents of lipids and are individually important for organism fitness. Essential fatty acids (EFAs) cannot be

synthesized *de novo* by consumers in sufficient quantities to meet metabolic needs and must therefore be obtained from in their diet (Dalsgaard et al., 2003). Since EFA content is not necessarily correlated with higher lipid content (Litzow et al., 2006), evaluating diets based on their EFAs provides a more nuanced assessment of diet quality than lipids alone. Generally, it is believed that docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (Puttick et al., 2009) (EPA, 20:5n-3), and arachidonic acid (ARA, 20:4n-6) are the most important long chained polyunsaturated fatty acids (PUFA) in fish and mammals. As reviewed in Parrish (2009), these EFAs play a significant role in an organism's growth, neural tissue development, hormonal regulations, and reproduction (Bell et al., 1995; Takeuchi, 2014; Watanabe, 1993). The relative proportion of DHA and EPA to other FAs and to each other are important when the physiological roles of these two FAs are considered (Tocher et al., 2003b). Neural tissue phospholipids of vertebrates are rich in DHA, which play a critical role in visual and learning processes (Tocher et al., 2003b). Thus, a diet lacking DHA or with a low DHA/EPA ratio, can lead to visual maldevelopment, and consequently reduced hunting efficiency and growth of marine fish (Watanabe et al., 1989). The ratio of DHA to EPA has thus become a commonly used metric for determining the quality of prey as both are required in an adequate balance for successful rearing of marine fish (Copeman et al., 2002; Sargent et al., 1999; Tocher, 2015). The PUFAs alpha-linolenic acid (ALA, C18:3n-3) and linoleic acid (LN, C18:2n3), while appearing to have having no physiological function (Trushenski and Rombenso, 2020), are also considered as EFAs if a species is capable of using them as precursors for *de novo* synthesis of DHA, EPA, and ARA. In the case of juvenile Chinook, *de novo* synthesis of DHA and EPA from ALA has been shown to occur under starvation scenarios (Litz et al., 2017).

Pacific salmon have been in long-term decline, especially in the south of their range in places like British Columbia, Canada (Grant et al., 2019). Evidence suggests that these declines have been due to decreased survival during the early marine phase (Beamish and Mahnken, 2001; Tomaro et al., 2012). Food quality is one of the factors that may have contributed to this decreased survival. Zooplankton time series from coastal British Columbia have demonstrated long term fluctuations in abundance and species composition coinciding with variations in the Pacific Decadal Oscillation index (PDO), North Pacific Gyre Oscillation (NPGO), sea surface temperature, and ocean circulation (Hooff and Peterson, 2006; Miller et al., 2017; Peterson and Schwing, 2003). For example, zooplankton data collected since 1990 show that during a negative PDO the summer copepod community is dominated by large-bodied, lipid-rich, subarctic boreal species and in years with a positive PDO, the copepod community shifts to a predominance of smaller, subtropical, lipid-poor species (Mackas et al., 2013, 2007). Longer term data from juvenile sockeye salmon diet time series in the Strait of Georgia have demonstrated a shift from a more copepod rich diet in the 1960s to one more dominated by amphipods and decapods in the 2000s (Preikshot et al., 2010). Such changes would have been accompanied by corresponding shifts in prey fatty acid composition, potentially affecting juvenile salmon fitness (Figure 1). Ongoing warming and ocean acidification are expected to further impact the composition of zooplankton prey, favouring small, lipid-poor prey species (Richardson, 2008) and altered DHA and EPA proportions (Deschutter et al., 2019; Garzke et al., 2017).

The response of consumers to diet quality can be measured in a variety of ways. These include direct measurement of fatty acid profiles, insulin-like growth factor 1 (IGF-1) levels, otolith growth, and the ratio of RNA to DNA in tissues (Clemmesen, 1994; Clemmesen and Doan, 1996; Ferriss et al., 2014). The RNA/DNA ratio integrates feeding history over a period of 3-5 days (Clemmesen and Doan, 1996), which makes it useful for assessing fish response to rapid spatial changes in dynamic coastal migration habitat, and multi week experiments. This approach uses the ratio of RNA to DNA in white muscle tissues, with the underlying principle that the concentration of DNA per cell is constant, whereas the RNA concentration per cell varies with its anabolic activity. Thus, a well-fed, active, and growing individual should have a relatively high RNA to DNA ratio compared to an underfed, low activity, and low growth individual.

In this study, we set out to investigate the effect of prey fatty acid composition and DHA/EPA ratios on juvenile Chinook salmon (*Oncorhynchus tshawytscha*) fitness measured with respect to RNA/DNA ratio, length-weight relationships, growth rates, and overall fatty acid composition. A literature compilation of Chinook diets summarized the major zooplankton prey of this species (Higgs et al., 2010; Hiltunen et al., 2021). We subsequently extracted typical (average) DHA/EPA ratios of prey species from the literature and used these as the basis for selecting experimental diets. The experimental diets were chosen to represent the DHA/EPA content of fish-dominated (commercial aquafeed with fishmeal and oil), krill-dominated (*Euphausia pacifica*), and crab larvae-dominated (*Artemia* sp.) diets (Fig. 1). We conducted a 11-week feeding study where fish were reared on the three diets. We hypothesized that low food quality, in terms of fatty acid composition, specifically essential fatty acids

and DHA/EPA ratio, would negatively affect growth and increase mortality. We discuss our results in the context of previous studies on juvenile salmonid fitness, and the implications of changes in food quality in response to changing ocean conditions.

2.1 Materials & Methods

2.1 Ethics Statement

All animals and procedures used in this experiment were approved by DFO Pacific Region - Animal Care Committee (AUP # 2017-008).

2.2 Experimental design

This study was conducted on the premises of Fisheries and Oceans Canada, at the Pacific Science Enterprise Centre, in West Vancouver, British Columbia, Canada. Juvenile Chinook salmon (ocean type, Harrison River strain) from the brood year 2018 (4.14 g ± 0.72 body weight; 73.6 mm ± 4.67 standard length) were obtained from Capilano River Hatchery (49.35º N 123.11º W) and transported to the research facilities. The juvenile fish were acclimated in freshwater tanks for one week before transitioning to saltwater, during which time only one individual died. The remaining 517 fish were used in the experiment (Fig. 2)

Prior to starting the trial, the fish were fed a commercial salmon feed (Bio-Oregon, BioVita, Skretting Canada). All treatments were not fed on the day of transfer. The rearing system consisted of nine 200 L oval, fiberglass tanks each supplied with flow through seawater (drawn from Burrard Inlet and passed through a sand filter) at 9

L/min and provided with supplemental air through an air stone. Lighting was supplied from overhead lights and photoperiod followed natural daylength. At the beginning of the trial, the fish were randomly assigned to the tanks, and each tank assigned to three diet treatments (n=3, Fig. 2). The commercial aquafeed pellets, *Artemia* and Krill were stored frozen and thawed prior feeding. All diets were offered four times daily (7:30 - 8:00 am; 11:00 - 11:30 am; 12:30 - 1:00 pm; 2:00 - 2:30 pm) until the fish stopped actively feeding (*satiation*) depending on the individual needs of the treatment fish (Yu and Sinnhuber, 1979). The tanks were cleaned three times a week after the last feeding by stopping the water flow and brushing the tank walls and bottom while the drain was plugged with a stopper. The sediment was then siphoned cleaned. After the cleaning, the water flow was restarted, and the drain unplugged.

Fish were maintained in the experimental set-up for a total of 11 weeks. The experimental phase was split into four individual Phases (Fig. 2): (1) the acclimation phase when the fish were transferred to the experimental tanks (Phase 1); (2) weaning fish from aquafeed diets to their respective treatment diets (1 week; Phase 2); (3) feeding phase on only experimental diets (4 weeks; Phase 3); after high mortality occurred in the three designated *Artemia* treatment tanks during Phase 3 it was decided to switch the diets of these fish leading to (4) fish fed with *Artemia* switched to the commercial aquafeed while the other treatments were maintained unchanged (5 weeks; Phase 4). Over the experimental period the fish were held on an average of 11.6ºC (± 0.61ºC).

Throughout the experiment, each week four fish were randomly sampled from each tank. Fish were removed from the tanks using nets, and then euthanized in TMS™ (0.4 g L^{-1}). All fish were weighed to the nearest 0.1 g (Mettler Toledo EXCELLENCE Plus balance, model XP16001L) and fork length (FL) was measured (mm). Individual fish were placed into separate Whirlpak™ bags, labelled with a unique identifier, and frozen at -80 ºC. The time that elapsed between the mortality of the fish and the fish being placed in the -80 ºC freezer was less than two minutes.

2.3 Feed composition

The three diet treatments for the study were a commercial aquafeed (Bio-Oregon BioVita, Skretting Canada), frozen *Euphausia pacifica* (Krill; Krill Canada), and frozen *Artemia* (*Artemia*; San Fransisco Bat Brand® Sally's Frozen Spirulina Brine Shrimp™, Table S1). The commercial aquafeed had the lowest moisture content of the three diets (avg. = 5.79% ± 0.007), while Krill and *Artemia* had much higher moisture content (Krill: avg. = 82.09% ± 0.24; *Artemia*: 91.63% ± 0.32). The diets were initially chosen to be well suited representatives of naturally occurring prey species based on their DHA/EPA content (Table 1, Fig. 1).

Table 1. Fatty acid composition of chosen feed species

	Aquafeed	Krill	*Artemia*
TFA	143.24 mg FA g^{-1} DW ± 26.56	113.79 mg FA g^{-1}DW ± 7.60	84.16 mg FA g^{-1}DW ± 13.11
SFA	31.66 % ± 1.25 %	29.69 % ± 1.28 %	22.30 % ± 1.12 %
MUFA	29.08 % ± 1.23	25.15 % ± 0.48 %	29.68 % ± 1.23 %
PUFA	39.26 % ± 1.78 %	45.14 % ± 1.74	48.01 % ± 0.73 %

EFA	32.78 % ± 1.68 %	37.47 % ± 0.75 %	41.72 % ± 1.76 %
DHA	11.16 % ± 0.46 %	15.18 % ± 0.41 %	0.11 % ± 0.37 %
EPA	14.22 % ± 0.29 %	15.26 % ± 0.39 %	2.39 % ± 0.29 %
ALA	1.36 % ± 0.06 %	2.94 % ± 0.34 %	30.90 % ± 0.74 %
DHA/EPA	0.79 ± 0.02	0.99 ± 0.05	0.05 ± 0.15

2.4 Laboratory Analysis

Tissue was sampled from each fish (n=336) for analysis of RNA/DNA ratios and fatty acids using sterilized dissection tools. Prior to dissection, the dissection area was cleaned first with 95% ethanol and then with EZ-Zyme (Integra™ Miltex™ EZ-Zyme™ All-Purpose Enzyme Cleaner, Fisher Scientific, Cat No 12-460-427). White muscle tissue was collected from close to the dorsal fin for RNA/DNA and fatty acid analysis, and 400-600 mg of muscle tissue from close proximity to the dorsal fin for fatty acid analysis. Tissue samples were then returned to a -80°C freezer until further analysis.

2.5 RNA/DNA analysis

The ratio of RNA and DNA of juvenile Chinook was determined according to Garzke et al. (2020) using the cyanine base fluorescence dye RiboGreen® (Thermo Fisher Scientific) Nucleic acids were extracted from freeze dried white muscle tissues, which had been homogenized in 1%N-lauroylsarcosine extraction buffer (VWR, Cat. No. VWRV0719-500G), the supernatant was diluted to match the RiboGreen saturation range (1:10, 1:20, 1:30, 1:50, 1:100). In short, we used a DNA standard (ranging from 0-3 µg mL^{-1}; calf thymus, VWR, Supp. No. MB-102-01100) and RNA

(ranging from 0-3 µg L^{-1}; *Eschericia coli* 16S and 23S rRNA, Quant-iT RiboGreen RNA assay Kit Thermo Fisher Scientific Cat. No. R11490). Triplicate technical replicates from the diluted sample were added to a black, flat-bottom 96-well microtiter plate. RiboGreen® reagent was added to the homogenized muscle tissue samples and incubated in the dark for 5min; initial fluorescence (F_1) readings were taken using a VarioSkan Flash Microplate Reader (ThermoFisher Scientific). RNase A (bovine pancreas, Alpha Aesar, Cat. No. CAAAJ62232-EX3) was added to each well and incubated at 37 ºC for 30min in the dark followed by a second fluorescence reading of the DNA only (F_2). Finally, we calculated the RNA concentration using F_1 - F_2. RNA and DNA concentrations were calculated based on the standard curves, followed by calculations of RNA/DNA.

2.6 Fulton K, growth rates, and mortality rates

For each sampling date and each diet treatment we calculated the average body condition factor as Fulton's K:

$$K = \frac{W}{L^3} \times 10^5 \quad (1)$$

where *W* is the wet body mass (g), *L* is length (mm) (Fulton, 1904). Specific growth rates (SGR) for weight (% W d^{-1}) were measured for experimental Phase 3:

$$SGR = \left(\frac{\left(ln(x_f) - ln(x_i)\right) \times 100}{t}\right) \quad (2)$$

where x_f is the weight of the last sampling day of Phase C, x_i is the weight of the first sampling day of Phase 3, and *t* is the time (days) between observations.

We calculated the instantaneous mortality rate (*Z*) by comparing the numbers of fish from one sampling day to the next for the duration of the experiment using (Sundby et al., 1989):

$$Z = \left(\frac{ln(N_0) - ln(N_t)}{t} \times 100\right) \quad (3)$$

where N_0 is the initial number of fish and N_t is the number of remaining fishes between sampling days, and *t* is the number of days between samplings.

2.7 Fatty Acid analysis

Fatty acids were analysed using a modified protocol following Puttick et al. (2009) in a one-step fatty acid methyl ester (FAME) method. White muscle tissue samples were weighed (wet weight), freeze-dried, and the respective dry weights measured for calculations of moisture content. FAMEs were obtained by extraction with 3 N HCl in CH_3OH (Sigma-Aldrich cat. #90964-500ML). Prior to extraction, the internal standard nonadecanoic acid (C19:0) was added to each sample. After extraction, the FAMEs were analysed with a gas chromatograph (Scion 436-GC, Scion Instruments Canada, Edmonton, Alberta, Canada) using a flame ionization detector (FID). Peaks were identified against external standards (GLC 455 and GLC 37 Nu-chek Prep, Inc., Elysian, Minnesota, USA), and measured before and after each sample measurement session.

2.8 Statistical analysis

Linear mixed models were used to examine the effects of the three diet treatments on fish fitness (RNA/DNA, Fulton's K) and individual fatty acid composition in fish muscle tissues (using RStudio Version 1.1.456, lme4 R package (Bolker et al., 2009)).

Non-parametric PERMANOVA was used to test for differences in fish fatty acid composition between the three diet treatments. Subsequently, an analysis of similarity percentages (SIMPER) was used to identify the FA species that contributed most to the Bray-Curtis dissimilarities among the diet treatments. All multivariate tests were performed using the R package vegan (Oksanen et al., 2017). Kolmogorov-Smirnov analysis was performed to test for normal distribution (α = 0.05). Analysis of Variance (ANOVA) was performed to identify differences between SGR between diet treatment, followed by a Tukey honest significance difference post hoc test. For non-normally distributed data a Kruskal-Wallis test (Kassambara, 2020), followed by analysing the effect size (H^2) and a pairwise comparison using the Dunn's test were used to identify differences in instantaneous mortality rate (Z) and diet fatty acid data. Error rate for all tests were held at 5% probability. All statistical tests were run at significance threshold of α=0.05.

3. Results

The experiment consisted of four experimental Phases: Phase 1 was an acclimation Phase of one week where all fish were transferred to their designated experimental tanks. Phase 2 was when fish were weaned onto the experimental diets. Treatments fed with Krill and *Artemia* were slowly acclimated to their respective diets over the course of six days by slowly increasing the proportion of their experimental diet relative to the initial aquafeed. Over the whole experimental time, higher quantities of Artemia were voluntarily eaten by fish compared to aquafeed and Krill (*Artemia*: 1.97% BW d^{-1} (of dry matter) ± 0.75 (range: 1.93% BW d^{-1} – 2.86% BW d^{-1}): aquafeed: 1.42% BW d^{-1} (of dry matter) ± 0.55% BW (range: 0.99% BW d^{-1} – 2.35% BW d^{-1}); Krill: 1.68% BW d^{-1} (of dry matter) ± 0.66% (range: 1.04%BW d^{-1} – 2.50%

BW d^{-1}), Fig S1). Fish consuming *Artemia* during Phase 3 had an average mortality of 1.96 ind d^{-1} (± 0.59) which was significantly higher than the instantaneous mortality rates of fish consuming Krill (1.21 ind d^{-1} ± 0.05; p = 0.04) and fish consuming aquafeed (1.23 ind d^{-1} ± 0.07; p = 0.04). High mortalities in the three designated *Artemia* treatment tanks occurred during Phase 3 leading to the decision to switch the diets of these fish. In Phase 4, *Artemia* treatments were switched back to aquafeed and this Phase lasted for 36 days.

3.1 Fish fitness

Chinook salmon RNA/DNA ratios did not differ significantly among the three diet treatments in experimental Phase 1 (acclimation Phase) or Phase 2 (weaning Phase) (Fig 3A, Table 2, Table S2). When fish moved from Phase 2 into Phase 3 (feeding study), within the first week RNA/DNA of Krill and aquafeed treatments increased by 7.47% and 21.21%, respectively, and *Artemia* treatments RNA/DNA decreased by 47.38% (Fig 3A). Over the course of Phase 3, *Artemia* treatments had a significantly lower RNA/DNA ratio (2.68 ± 1.1) than Krill treatments (4.43 ± 0.94) and aquafeed treatments (4.78 ± 1.11). In fact, the RNA/DNA of the *Artemia* treatment declined over the course of Phase 3. The Krill and aquafeed treatments had the highest RNA/DNA ratios and were not significantly different from each other (Table 2, Table S2). When *Artemia* treatments were switched to aquafeed in Phase 4, a significant RNA/DNA increase of 54.82% was measured within the first two weeks after the diet switch, whereas Krill and aquafeed treatments showed lower RNA/DNA in Phase 4 than by the end of Phase 3 (Krill: -35.25%; aquafeed: -37.83%).

Table 2. Analysis of deviance of linear mixed models based on full models testing effects of diet treatments over time with tank as random effects (full linear mixed model results see Table S2. TFA = Total fatty acids, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, EFA = essential fatty acids, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, ALA = alpha-linolenic acid.

	Predictor	Chi^2	Df	P value
RNA/DNA	Treatment	77.194	2	<0.001
	Sampling date	10.989	1	<0.001
Fulton K	Treatment	16.959	2	<0.001
	Sampling date	24.902	1	<0.001
TFA	Treatment	5.168	2	0.075
	Sampling date	14.318	1	<0.001
SFA	Treatment	37.997	2	<0.001
	Sampling date	84.812	1	<0.001
MUFA	Treatment	15.099	2	<0.001
	Sampling date	0.642	1	0.422
PUFA	Treatment	28.114	2	<0.001

	Sampling date	15.279	1	<0.001
EFA	Treatment	30.242	2	<0.001
	Sampling date	18.258	1	<0.001
DHA	Treatment	53.184	2	<0.001
	Sampling date	23.060	1	<0.001
EPA	Treatment	29.836	2	<0.001
	Sampling date	58.886	1	<0.001
DHA/EPA	Treatment	37.184	2	<0.001
	Sampling date	40.481	1	<0.001
ALA	Treatment	244.25	2	<0.001
	Sampling date	2.84	1	0.0919

Fulton's K body condition index increased significantly after Phase 2 (Table 2, Table S2). However, this increase was dependent on the diet treatment. Fulton's K during Phase 1 and 2 did not differ within or among diet treatments (Fig 3B). When the fish transitioned from Phase 1 into Phase 2 only small changes in Fulton K were measured (*Artemia*: -1.86%; Krill: +1.88%; aquafeed: -3.6%). After the transition to Phase 3, after the first week Fulton's K increased by 5.61% and 2.78%) in the krill and aquafeed treatments, respectively. Over the course of Phase 3, Fulton's K

increased significantly in the Krill and aquafeed treatments (start: aquafeed 1.11 ± 0.04, Krill 1.12 ± 0.04; end: aquafeed 1.15 ± 0.05, Krill 1.14 ± 0.05). Fulton's K of *Artemia* treatments were significantly lower than Krill and aquafeed treatments. Two weeks after switching the diet of the *Artemia* treatment to aquafeed (Phase 4), Fulton's K increased significantly by 6.19% and was comparable to fish in the Krill treatment.

Specific growth rates across Phase 3 were not significantly different among the three diet treatments (Table 3). The average weight increase of fish from the end of Phase 2 and the end of Phase 3 was 1.29% d^{-1} ± 0.40 for *Artemia*, 1.35% d^{-1} ± 0.29 for aquafeed, and 1.91% d^{-1} ± 0.58 for Krill (Fig 4A). Instantaneous mortality rate (Z) did not differ between treatments within experimental feeding Phase 3 ($p = 0.57$) but was highest for *Artemia* treatments (~1.7 vs. ~1.2 for Krill and aquafeed treatments; Fig 4B). The cumulative mortality was higher in Artemia treatments compared to Krill and aquafeed treatments (Fig. S2)

Table 3. Analysis of Variance (ANOVA) results of specific growth rates (SGR) of fish from last day Phase 2 to last day of Phase 3. Df = degrees of freedom, Sum Sq = Sum of Squares, Mean Sq = Mean squares).

		df	Sum Sq	Mean Sq	F value	p
SGR weight	Treatment	2	0.8914	0.4457	3.202	0.113

	Residuals	6	0.351	0.1392		

3.2 Fatty Acids (FA)

The PERMANOVA showed that fish FA profiles were significantly affected by diet during experimental Phase 3 ($p < 0.001$; Table S3). DHA contributed the most (>20%) to dissimilarity between all treatment pairs and was greatest for Krill vs Aquafeed (32%; Table S4). The proportion of ALA in *Artemia* differed by 18% and 18.8% from Krill and aquafeed, respectively, while oleic acid (C18:1n-9) differed by >12% between Krill and aquafeed. C16:0 was also detected as a contributor to the dissimilarity between aquafeed and Krill and *Artemia*. We excluded this from our further analysis as C16:0 is a typical trophic marker for fish meal which was a main ingredient in the aquafeed (94). Total fatty acid concentration in fish did not differ significantly between the three diet treatments (Fig 5B, Table 2, Table S2).

Fish SFA percentages were different among the three tested diets (Table 2, Table S2). SFA percentages were significantly higher in Krill and aquafeed treatments compared to *Artemia* treatments (Fig 5D, Table 2, Table S2). SFA percentage increased significantly from the first day of Phase 3 to the end of Phase 3 in Krill and aquafeed fed fish (Krill: start 26.75% ± 0.92, end 26.82% ± 0.78; aquafeed: start 26.39% ± 0.95, end 27.46% ± 0.77 Fig 5D; Table 2, Table S2). The SFA percentage of *Artemia* fed fish decreased in the same Phase from 26.02% (± 0.81) to 25.47% (± 0.36) (Fig 5D). No significant difference was detected in the MUFA percentage between the different diet treatments (Fig 5F, Table 2, Table S2). PUFA contribution was significantly lower in aquafeed fed fish (49.79% ± 2.11) compared to *Artemia*

(51.37% ± 2.52) and Krill fed fish (50.67% ± 3.24; Table 3). PUFA composition remained constant in *Artemia* and Krill fed fish during Phase 3 (Krill: start - 50.19% ± 3.03, end - 50.16% ± 3.08; *Artemia*: start - 50.92 ± 1.85, end - 51.50% ± 3.00; Fig 5H). PUFAs in aquafeed fed fish decreased slightly from the start to end of Phase 3 (49.61% ± 2.01 - 48.60% ± 2.62). No significant difference was detected in EFA percentages between the different treatments, or over time, or between the different Phases (Table 2, Table S2, Fig 6B).

DHA was significantly higher in Krill treatments compared to aquafeed and *Artemia* treatments (Table 2, Table S2), whereas DHA in aquafeed and *Artemia* treatments were similar (Fig 6D). All fish had a comparable inter-treatment DHA percentage in Phase 2 (*Artemia*: 30.68% ± 2.59; aquafeed: 29.25% ± 3.24; Krill: 30.27% ± 1.87) before the fish were switched to their experimental diets. One week after the diet switch all treatments slightly increased in their DHA (*Artemia*: +0.74%; aquafeed: 0.94%; Krill: +1.59%). Over the experimental Phase 3, DHA slightly increased in the Krill treatment (start 31.86% ± 4.07, end32.42% ± 4.14), whereas DHA in aquafeed and *Artemia* treatments slightly decreased over time (aquafeed: start 30.19% ± 2.24, end 29.37% ± 3.91; *Artemia*: start 31.42% ± 2.97, end 27.95%± 4.4, Fig 6D). In Phase 4, a slight decrease in DHA was observed in *Artemia* during the first week of switching *Artemia* treatments back to aquafeed, and in aquafeed treatments (*Artemia*: -1.76%; aquafeed: -3.37%), whereas Krill treatments remained a similar DHA percentage. EPA percentages were significantly higher in Krill and aquafeed treatments compared to *Artemia* treatments (Table 2, Table S2, Fig 6F). In Phase 2 (weaning on diets), EPA percentages in fish were comparable among all treatments (*Artemia*: 6.70% ± 0.67; aquafeed: 7.12% ± 0.76; Krill: 6.87% ± 0.39). After the full

switch to their respective diets, EPA decreased in *Artemia* treatments (0.81%), aquafeed treatments (0.18%), and in Krill treatments (0.47%) within one week. The EPA percentage in *Artemia* treatments decreased slightly over Phase 3, from 5.89% ± 0.31 to 4.93% ± 0.30, whereas in the Krill and aquafeed treatments EPA was constant (Krill: start 6.40% ± 0.51, end 6.64% ±0.63; aquafeed: start 6.94% ±0.60, end 7.10% ±0.61). After transitioning *Artemia* treatments to aquafeed (Phase 4), EPA increased from 4.93% ± 0.31 to 7.77% ± 0.75 but aquafeed and Krill treatments remained constant. ALA percentage was significantly higher in the *Artemia* treatment compared to Krill and aquafeed treatments (Table 2, Table S2). During Phase 2, ALA in all treatments were similar (*Artemia*: 1.27% ± 0.07; aquafeed: 0.99% ± 0.15; Krill: 0.99% ± 0.07). ALA increased in *Artemia* after the full diet switch at the beginning of Phase 3 (2.27%) but Krill and aquafeed treatments remained relatively constant. ALA percentage increased over time during Phase 3 in *Artemia* treatments (start 2.27% ± 0.65, end 6.72% ± 1.46). In Krill treatments, ALA percentage increased slightly over time (start 1.03% ± 0.10, end 1.25% ± 0.15), and in aquafeed treatments ALA percentage remained constant over time (start 0.91% ± 0.81, end 0.91% ± 0.11, Fig 6H). After the diet switch of the *Artemia* treatments to aquafeed (Phase 4) ALA decreased within one week by 3.73% (6.72% ± 1.42 – 2.99% ± 0.51). DHA/EPA ratios were significantly affected by time (Table 2, Table S2). During Phase 2, DHA/EPA ratios were comparable among treatments (*Artemia*: 4.64 ± 0.74; aquafeed: 4.18 ± 0.83; Krill: 4.42 ± 0.41). After transitioning into Phase 3, DHA/EPA ratios increased in *Artemia* (+0.7) and Krill treatments (+0.61) (Fig 7B). The DHA/EPA ratio in the *Artemia* and Krill treatments increased slightly over the course of experimental Phase 3 (Fig 7B). DHA/EPA ratios in the aquafeed treatment were significantly lower than *Artemia* and Krill and decreased slightly over Phase 3 (4.37 ±

0.47 - 4.19 ± 0.85). DHA/EPA ratios were highest in *Artemia*-fed Chinook in Phase 3 and slightly increased over time (start 5.34 ± 0.61, end 5.72 ± 1.21, Fig 7B). Krill treatments had a slightly lower DHA/EPA ratio compared to the *Artemia* treatment (start 5.04 ± 0.98, end 4.96 ± 1.01, Fig 6H). After switching *Artemia* treatments to aquafeed in Phase 4, DHA/EPA ratios decreased within one week by 2.32 (5.72 to 3.40) to a similar level of aquafeed (3.47) and Krill treatments remained high (5.01; Fig 7B).

4. Discussion

Previous research has highlighted the potential effects of food quantity on juvenile salmon growth, however, few if any studies have investigated the effect of food quality on juvenile salmon fitness. This study examined the effects of food quality with respect to essential fatty acid composition and DHA/EPA ratios, on short-term juvenile Chinook growth and condition. High dietary concentrations of DHA and EPA and high DHA/EPA ratios resulted in increased growth and condition. Furthermore, low concentrations of DHA and EPA and low DHA/EPA ratios were not compensated for by increased food quantity. This indicated that food quality needs to be considered when assessing fish response to changing ocean conditions. Below we evaluate in detail the response of juvenile Chinook salmon to individual fatty composition as measured by condition factor, RNA/DNA ratio and growth rate. Finally, we assess the implications of this for the response of fish to climate driven changes in zooplankton communities.

We used RNA/DNA ratios and the Fulton K index as measures of fish condition and both are commonly used in fish ecology (Clemmesen and Doan, 1996; Duguid et al.,

2018). After the weaning Phase (Phase 2), the RNA/DNA ratio and Fulton's K of fish in the *Artemia* treatment indicated reduced anabolic activity within the first week. Numerous studies have correlated differences in fish RNA/DNA ratio with food limitation (Clemmesen, 1994; Malloy and Targett, 1994; Richard et al., 1991), however, this can be excluded in the present study as fish were fed to satiation. The *Artemia* and Krill treatments differed from the aquafeed treatment in being a wet diet. However, the RNA/DNA ratio and K were not negatively affected by the wet diet in the case of the Krill treatment. This points to food quality as the factor behind the reduced RNA/DNA and K in the *Artemia* treatment. The response of the fish occurred within the first week, which was in line with studies experimentally testing the rates of RNA/DNA ratio response to starvation, finding that RNA/DNA ratios responded within two days in studies of European lobster larvae (Schoo et al., 2014), rainbow trout (Boersma et al., 2009), herring (Boersma et al., 2008), clams (Fathallah et al., 2010), and copepods (Malzahn and Boersma, 2012).

The requirement of EFAs for Chinook salmon are unknown but is known to be ~1% of body weight for Chum, Pink, Cherry and Atlantic salmon (Tocher, 2010). Dietary DHA/EPA ratios >2 are generally considered optimal for larval and juvenile marine fish (Bell et al., 1995; Watanabe, 1993), however, ratios of ≈1 have been shown to be sufficient in many species (Copeman and Laurel, 2010; Rodriguez et al., 1997). In our study, there were detectable effects of diet DHA/EPA on salmon condition (Fulton K and RNA/DNA) and growth rates (SGR) for a DHA/EPA range of 0-1. The DHA/EPA ratio of Krill and aquafeed diet was 0.75-1, which showed a positive effect on growth, RNA/DNA, and Fulton K, while the low DHA/EPA ratio in the *Artemia* diet led to a lower growth and significantly reduced RNA/DNA and Fulton K in the fish,

indicating that DHA/EPA were sufficient in aquafeed and Krill and insufficient in *Artemia*. Although all treatments were fed to satiation, we observed a higher feed intake in *Artemia* treatments with low EPA and DHA (Fig S1, S3).

Based on data for Chum, Coho, and Atlantic salmon, the proportion of SFA and MUFA to TFA in diets should both be approximately 33% (Turchini et al., 2009). However, all diets used in the present study were below 33% in SFA and MUFA, respectively. Additionally, EFAs (DHA, EPA, ALA, LN) should contribute >1-2% of the diets' dry weight (Tocher, 2010). In the present study, the contribution of EFA was highest in *Artemia* and lowest in Krill and the commercial aquafeed, but the individual FA composition varied significantly among feeds. *Artemia* had the highest concentration of ALA which contributed to the significantly higher amount of EFAs compared to Krill and aquafeed with lower ALA concentration. In particular, SFA was significantly lower in *Artemia* than in both Krill and aquafeed diets. These differences were related to specific growth and FA composition responses in the experimental fish. The fastest metabolic pathway synthesizing adenosine triphosphate (ATP) is from SFA, followed by MUFA, and PUFA by β-oxidation in the peroxisome (Turchini and Francis, 2009). In salmon, EPA is extensively used for β-oxidation when supplied in dietary surplus (Codabaccus et al., 2011; Stubhaug et al., 2005) and DHA is more conserved, irrespective of its dietary concentration (Tocher, 2010). Chinook SFAs in the *Artemia* treatments decreased over time suggesting that this was related to the low dietary SFA requiring ATP synthesis from stored SFA. Conversely, SFAs in Krill and aquafeed treatments increased over the same experimental phase, indicating a sufficient supply of ATP for growth.

Although we detected no differences in the broader FA groups MUFA and PUFA among the diet treatments, we identified changes within the EFAs mainly in DHA, EPA and ALA. Generally, EFAs were highest in *Artemia* and *Artemia*-fed fish (except during Phase 4, when Krill-fed fish had the highest EFA percentage) but the main driver was the contribution of ALA in *Artemia* whereas DHA and EPA remained very low in diets and fish. We observed that EPA percentages decreased after the weaning phase in *Artemia* treatments indicating - similarly to SFA - that EPA was metabolized to ATP but DHA remained constant. Contrary to other studies, the FAs indicate that the fish in the *Artemia* treatments were not able to synthesize EPA from ALA because the ALA proportion increased over time while the EPA proportion decreased (Litz et al., 2017). These changes in EPA were reflected in the increase of the DHA/EPA ratio within the fish, meaning DHA was retained, and EPA was metabolized. Furthermore, based on our observation that DHA/EPA in the Krill treatments were significantly higher than in the *Artemia* and aquafeed treatments, even though both Krill and aquafeed diets had similar DHA/EPA, we hypothesize that there may be a threshold DHA/EPA ratio of ≥ 1, above which DHA assimilation by the fish becomes more efficient than with a dietary DHA/EPA <1. Although it is suggested that salmon, as an anadromous fish, are able to convert ALA into DHA, and subsequently EPA by two desaturation (Δ6 and Δ5 desaturation) and two elongation steps (Vagner and Santigosa, 2011), desaturase activity decreases in seawater (Bell et al., 1997; Zheng et al., 2005). We conclude that DHA and EPA were rather directly used from the diets.

The fatty acid changes and the need to metabolize energy from FAs in *Artemia* treatments can also be found in the measured fish condition proxies RNA/DNA ratio,

Fulton's K and SGRs. Low amounts of dietary EFAs and low DHA/EPA ratios in diets led to decreased RNA/DNA by slowing down the individual's metabolic activity, reduced growth, and slightly increased instantaneous mortality. Although feed intake was higher for *Artemia* treatments (Fig S1) and was accompanied by higher intake in total energy (kJ, Fig S3), fish appeared unable to compensate for the low EPA and DHA consumption with higher food quantity intake (Fig S1, Fig S3, Fig S4), and that cellular energy for maintaining basic anabolic processes was too low and needed to be synthesized from storage lipids. It is possible that differences in diet type — whole organisms (*Artemia* and Krill) versus manufactured fish feed pellets — and moisture content affected assimilation or that satiation was reached before fish obtained the required quantities of fatty acids and other essential nutrients. However, the fact that there were no significant differences in RNA/DNA and growth rates between Krill and aquafeed treatments suggests that this was not a factor, but rather that the quality of the *Artemia* diet was critical. We have to note that besides the differences in fatty acid composition between the three diets other biomolecules e.g., protein content, lipid content, or amino acid content may have played a role in growth and fitness responses as crude protein was lower in Artemia (3%) compared to Krill and aquafeed (50% and 58% respectively) but energy content was comparable (Table S1).

Additionally, we were able to show the importance of SFA, DHA, EPA and DHA/EPA for fish condition by switching the diets from *Artemia* to aquafeed in the second half of the experiment (Phase 4). After this switch, both RNA/DNA and Fulton K increased to almost the same level as the Krill treatments within two weeks. Furthermore, SFA and EPA contribution increased after the diet switch and the

increase in EPA decreased the DHA/EPA ratio in the white muscle tissue. Many studies have shown that fish are able to recover from 1-2 weeks of food deprivation, or even starvation, and are able to regain a comparable weight to non-food deprived fish after 2 - 4 weeks, depending on the species (Ali et al., 2003). Fish experience faster growth during recovery from starvation or food deprivation than they do during phases of continuous food availability (Jobling, 1996; Wilson and Osbourn, 1960). This response is called compensatory, or catch-up growth. Fish can either overcompensate (become larger or heavier after recovery than continuous-fed fish), full-compensate (regain the same size or weight), or partial compensate (remain smaller and lighter) (Jobling, 1996). Full-compensatory growth appears to typical in salmonids after phases of low or zero food quantity (Atlantic salmon (Metcalfe and Thorpe, 1992; Morgan and Metcalfe, 2001; Skilbrei, 1990); rainbow trout (Kindschi, 1988; Weatherley and Gill, 1981), Coho salmon (Damsgird and Dill, 1998), Sockeye salmon (Bilton and Robins, 2011). Although the present study did not test food quantity, the growth and condition response of the fish to the different treatments indicates that low food quality is in fact an indirect form of starvation (Fig S5).

The effects of food quality on fish growth demonstrated here and in other studies (Brett and Müller-Navarra, 1997; Malzahn et al., 2007), highlight the potential important role of prey quality in fish fitness. In the case of zooplanktivorous fish, the quality of zooplankton prey can be determined either by species composition of the zooplankton themselves or by shifts in the phytoplankton food web base that supports them. Fatty acid composition of zooplankton in the Salish Sea varies substantially among species (Costalago et al., 2020; Hiltunen et al., 2021). The relative proportion of these prey taxa can vary with ocean conditions, including whole

community shifts from lipid-rich boreal species during cold conditions to lipid-poor species, including more gelatinous plankton, during warm conditions (El-Sabaawi et al., 2009; Mackas et al., 2013). Such environmentally driven shifts have been demonstrated to occur over decadal scales and in rapid response to recent marine heatwaves (Fisher et al., 2015; Miller et al., 2017; Peterson et al., 2017; Young et al., 2019). While distributional shifts in zooplankton are expected to be important, changes in their phytoplankton food base have also been demonstrated to be an important factor in their FA composition (El-Sabaawi et al., 2009; El-Sabaawi et al., 2009). EFAs are primarily synthesized by phytoplankton and transferred through the food web via zooplankton to higher trophic levels. Phytoplankton have taxon-specific FA profiles, for example, diatoms have higher proportions of EPA than DHA, while dinoflagellates have higher proportions of DHA than EPA (Dalsgaard et al., 2003). Fatty acid composition within species can also be affected by environmental conditions. For example experimental studies showed that nitrogen and phosphorus limitation can increase TFA, SFA and ω-3 and ω-6 FA concentrations in phytoplankton (Bi et al., 2018; Malzahn et al., 2007; Schoo et al., 2014); warming increased the SFA to PUFA ratio (Hixson and Arts, 2016; Jin et al., 2020); and ocean acidification leads to reduced concentrations of TFA and PUFA (Meyers et al., 2019), but also that changes are species-specific and change with community species composition (Dörner et al., 2020; Leu et al., 2013). Such changes in nutritional quality at the base of the food web have been demonstrated to transfer to higher trophic levels, affecting consumers, e.g., when phytoplankton has higher concentrations of DHA and EPA the same can be measured in zooplankton, and secondary consumers such as herring larvae, show increased nutritional condition (RNA/DNA) (Malzahn et al., 2007). The above examples identify two pathways by

which food quality experienced by zooplankton consumers may be affected: 1) changes in the species composition of the zooplankton; and 2) changes in the phytoplankton base driven by ocean conditions (either shifts in species composition or FA profiles within species). Such changes in food quality are potentially an important mechanism behind the response of zooplanktivorous fish to climate change.

5. Conclusions

This study found that high dietary concentrations of DHA and EPA and high DHA/EPA ratios promoted the growth and condition of juvenile Chinook salmon. Furthermore, poor food quality, characterised by low concentrations of DHA and EPA and low DHA/EPA ratios, was not compensated for by increased food quantity. This highlights the importance of food quality being considered when assessing fish response to changing ocean conditions. We recommend that future studies take food quality into account when assessing foraging conditions for juvenile salmon. Furthermore, species-specific data on zooplankton nutritional could be used to retrospectively qualify zooplankton time series to assess how foraging conditions have changed over time. Additionally, testing only various DHA/EPA ratios or concentrations with comparable base feed would be beneficial to understand the effect on juvenile Chinook growth and fitness.

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Author contributions

Conceptualization: J.G, I.F, D.C., B.P.V.H.; Methodology: J.G., C.G.; Software: J.G.; Validation: J.G.; Data curation: J.G., C.G.; Formal analysis: J.G.; Investigation: C.G.; Resources: I.F., B.V.P.H.; Writing – Original Draft: J.G.; Writing- Review & Editing J.G., I.F., C.G., D.C., B.P.V.H.; Visualization: J.G.; Supervision: B.P.V.H., I.F.; Project administration: B.P.V.H., I.F.; Funding acquisition: B.V.P.H., I.F.

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Figure 1 Summary of literature DHA/EPA ratios of known prey items of Chinook salmon in comparison to used diets during the experiment. (summarized from Higgs et al., 2010; Hiltunen et al., 2021))

Figure 2. Experimental design used in this study. Diets used were aquafeed (C); Artemia (A); and Krill (K). The experimental Phase was split into four individual Phases: (1) the acclimation Phase when the fish were transferred to the experimental tanks (Phase 1), (2) weaning fish from aquafeed diets to their respective treatment diets (1 week; Phase 2), (3) feeding Phase on only experimental diets (4 weeks; Phase 3), and (4) fish fed with Artemia switched to the commercial aquafeed (5 weeks; Phase 4). N = number of fish in each tank and Phase).

Figure 3. RNA / DNA and Fulton K for the experimental Phases 1 (acclimation), 2 (weaning on diets), 3 (experimental feeding Phase), 4 (after switch of Artemia treatments to aquafeed). A) Average RNA/DNA (n=4) (error bars are standard deviation) for each diet treatment. B) Average Fulton's K (n=4) (error bars are standard deviation).

Figure 4. Average (A) weight specific growth rate (SGR) and (B) instantaneous mortality over the 4 weeks during Phase 3 for each treatment (error bars denote mean ± standard deviation, n=12).

Figure 5. Fatty acid composition of diets and fish for the experimental Phases 1 (acclimation), 2 (weaning on diets), 3 (experimental feeding Phase), 4 (after switch of

Artemia treatments to aquafeed). A) average total fatty acid (TFA) concentration of diets (n=4 ± SD), B) total fatty acid (TFA) concentration in fish white muscle tissue (n=4 ± SD), C) percental composition of saturated fatty acids (SFA) to TFA in diets (n=4 ± SD), D) percental composition of saturated fatty acids (SFA) to TFA (n=4 ± SD) in fish white muscle tissue, E) percental composition of monounsaturated fatty acids (MUFA) to TFA in diets (n=4 ± SD), F) percental composition of monounsaturated fatty acids (MUFA) to TFA (n=4 ± SD) in fish white muscle tissue, G) percental composition of polyunsaturated fatty acids (PUFA) to TFA in diets (n=4 ± SD), and H) percental composition of polyunsaturated fatty acids (PUFA) to TFA (n=4 ± SD) in fish white muscle tissue.

Figure 6. Fatty acid composition of diets and fish for the experimental Phases 1 (acclimation), 2 (weaning on diets), 3 (experimental feeding Phase), 4 (after switch of Artemia treatments to aquafeed). A) percental composition of essential fatty acids (EFA) to TFA in diets (n=4 ± SD), B) percental composition of essential fatty acids (EFA) to TFA in fish white muscle tissue (n=4 ± SD), C) percental composition of DHA to TFA in diets (n=4 ± SD), D) percental composition of DHA to TFA (n=4 ± SD) in fish white muscle tissue, E) percental composition EPA to TFA in diets (n=4 ± SD), F) percental composition of EPA to TFA (n=4 ± SD) in fish white muscle tissue, G) percental composition of ALA to EFA in diets (n=4 ± SD), and H) percental composition of ALA to TFA (n=4 ± SD) in fish white muscle tissue.

Figure. 7 DHA / EPA ratios of diets and fish. A) DHA/EPA ratio in diets (n=4 ± SD), and B) DHA/EPA ratio for the experimental Phases 1 (acclimation), 2 (weaning on

diets), 3 (experimental feeding Phase), 4 (after switch of Artemia treatments to aquafeed) in fish white muscle tissue (n=4 ± SD).

Fig. 1

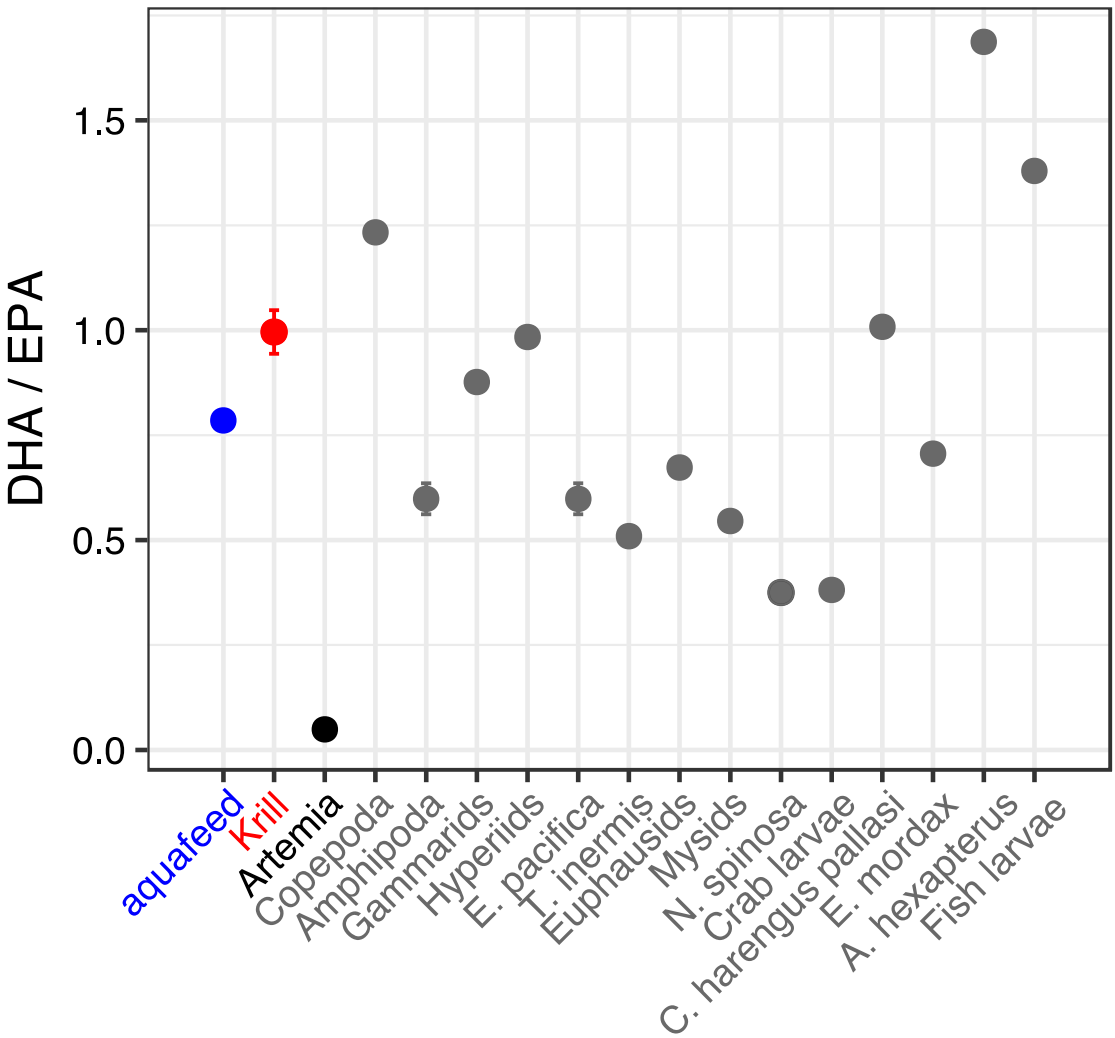

DHA / EPA
1.5
1.0
0.5
0.0
aquafeed
Krill
Artemia
Copepoda
Amphipoda
Gammarids
Hyperiids
E. pacifica
T. inermis
Euphausids
Mysids
N. spinosa
Crab larvae
C. harengus pallasi
E. mordax
A. hexapterus
Fish larvae

Fig. 2

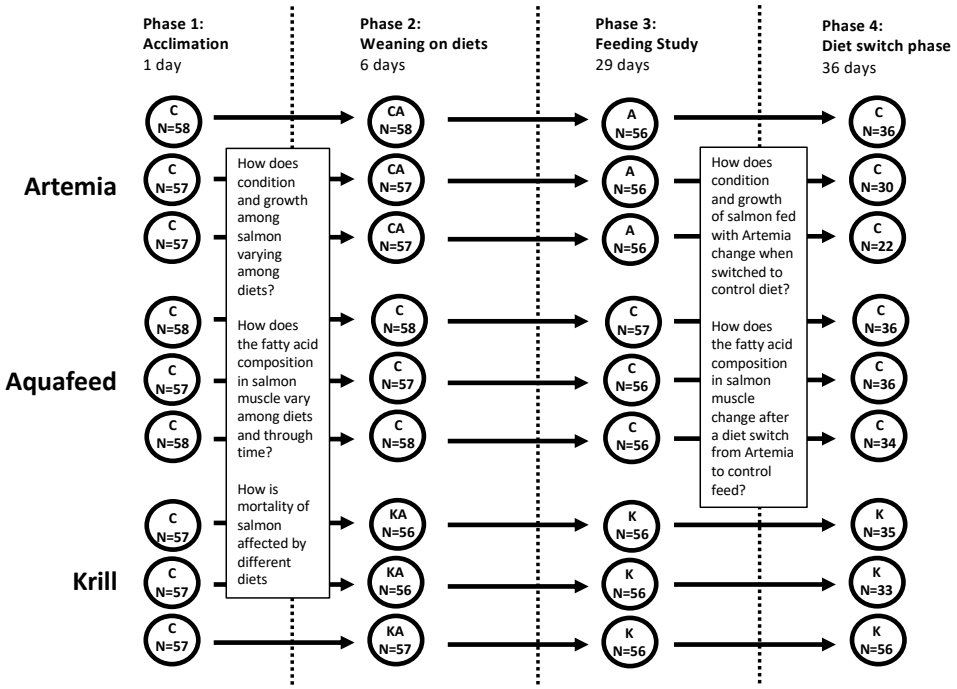

Phase 1:
Acclimation
1 day
Phase 2:
Weaning on diets
6 days
Phase 3:
Feeding Study
29 days
Phase 4:
Diet switch phase
36 days
Artemia
Aquafeed
Krill
C N=58
C N=57
C N=57
C N=58
C N=57
C N=58
C N=57
C N=57
C N=57
How does condition and growth among salmon varying among diets?
How does the fatty acid composition in salmon muscle vary among diets and through time?
How is mortality of salmon affected by different diets
CA N=58
CA N=57
CA N=57
C N=58
C N=57
C N=58
KA N=56
KA N=56
KA N=57
A N=56
A N=56
A N=56
C N=57
C N=56
C N=56
K N=56
K N=56
K N=56
How does condition and growth of salmon fed with Artemia change when switched to control diet?
How does the fatty acid composition in salmon muscle change after a diet switch from Artemia to control feed?
C N=36
C N=30
C N=22
C N=36
C N=36
C N=34
K N=35
K N=33
K N=56

Fig. 3

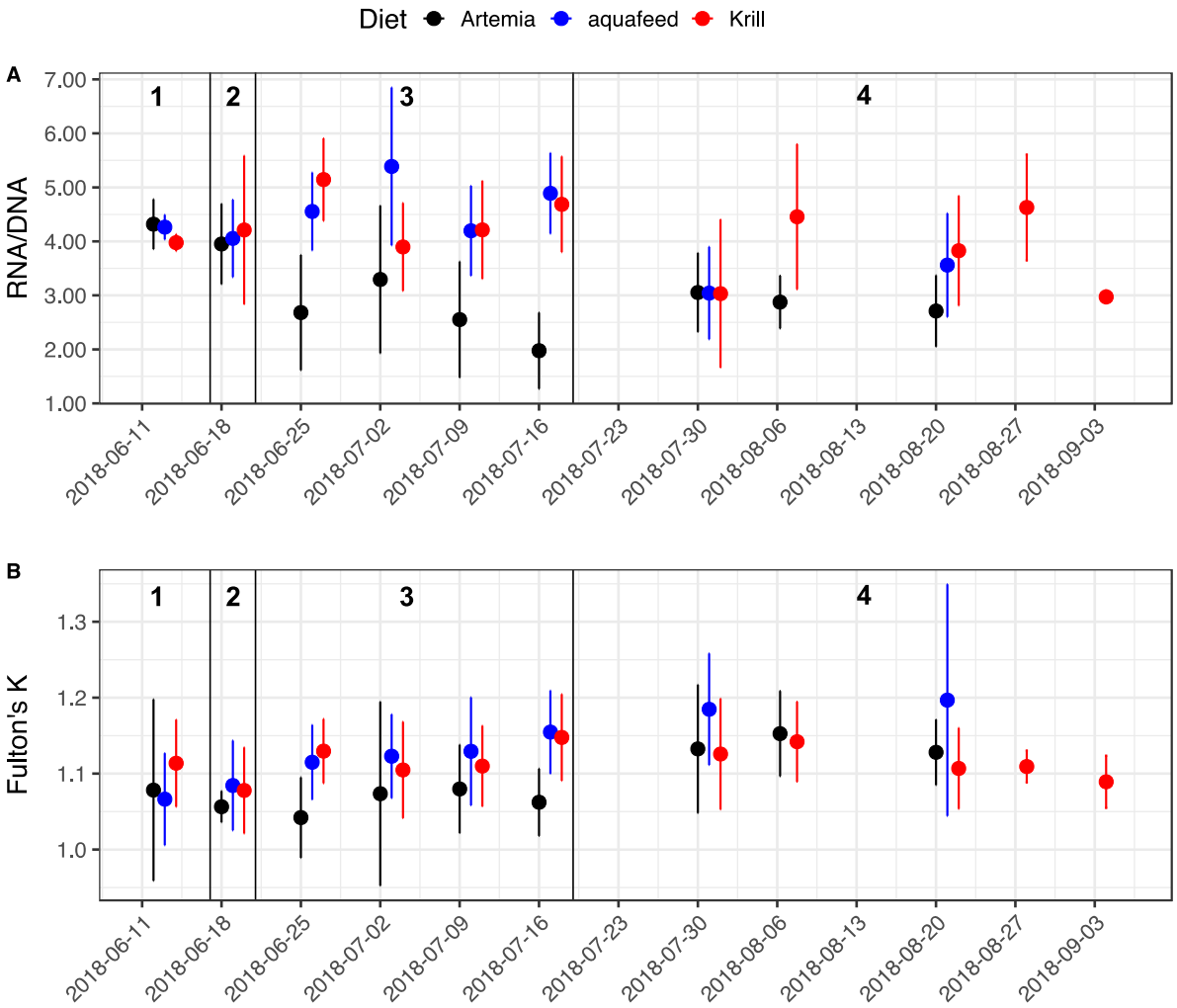

Diet
Artemia
aquafeed
Krill
A
RNA/DNA
7.00
6.00
5.00
4.00
3.00
2.00
1.00
1
2
3
4
2018-06-11
2018-06-18
2018-06-25
2018-07-02
2018-07-09
2018-07-16
2018-07-23
2018-07-30
2018-08-06
2018-08-13
2018-08-20
2018-08-27
2018-09-03
B
Fulton's K
1.3
1.2
1.1
1.0

Fig. 4

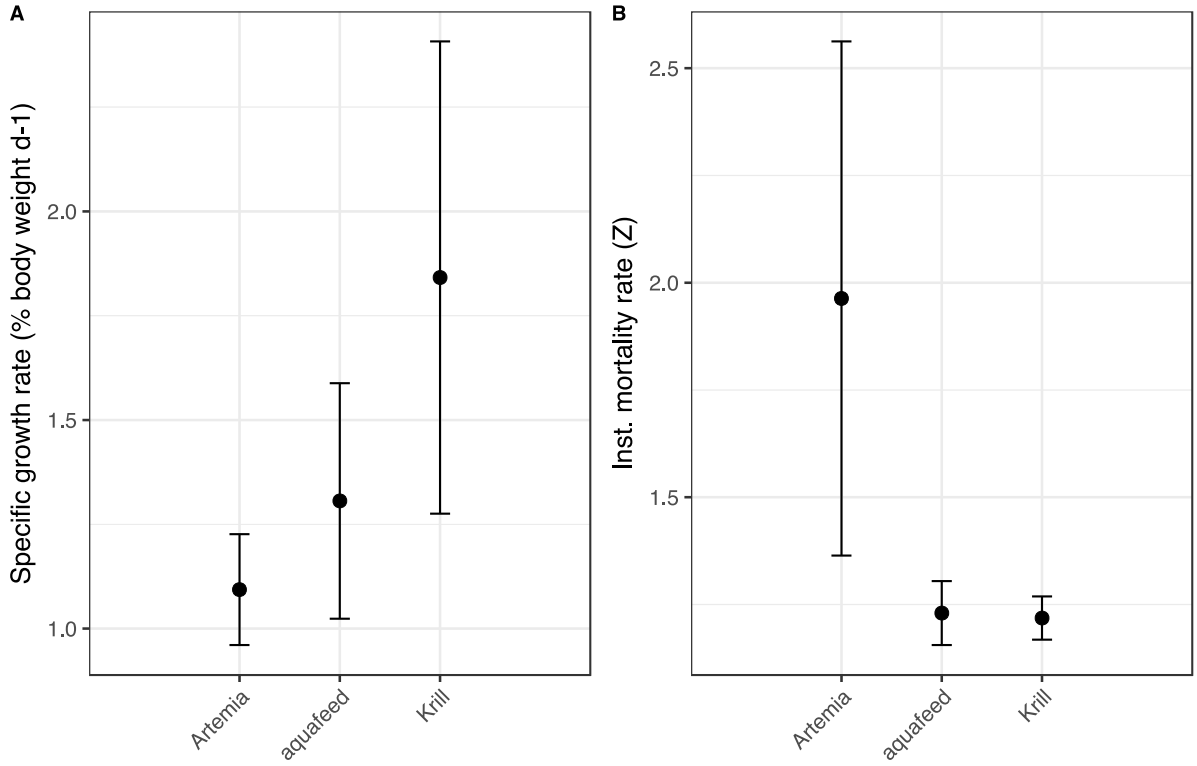
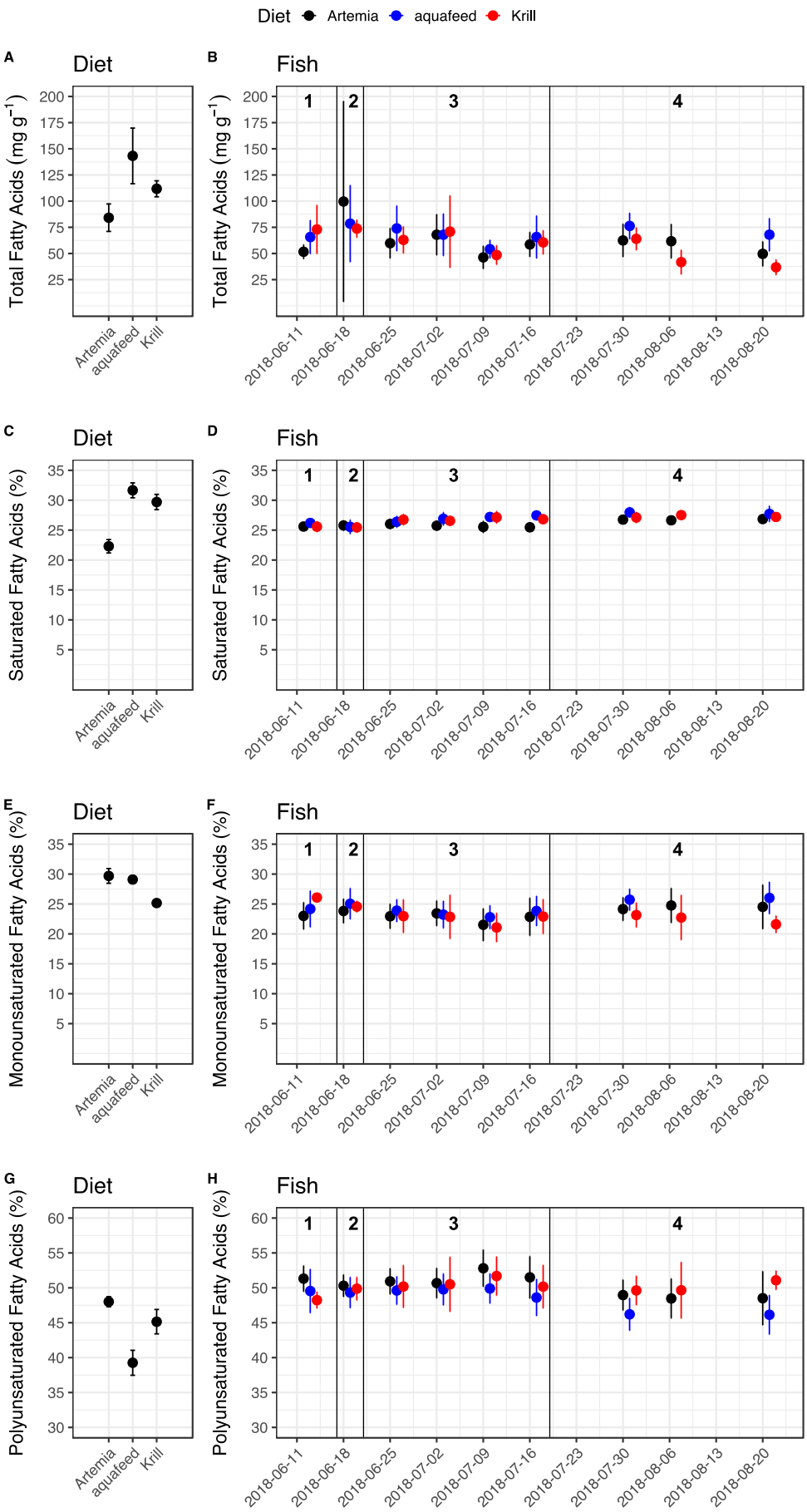
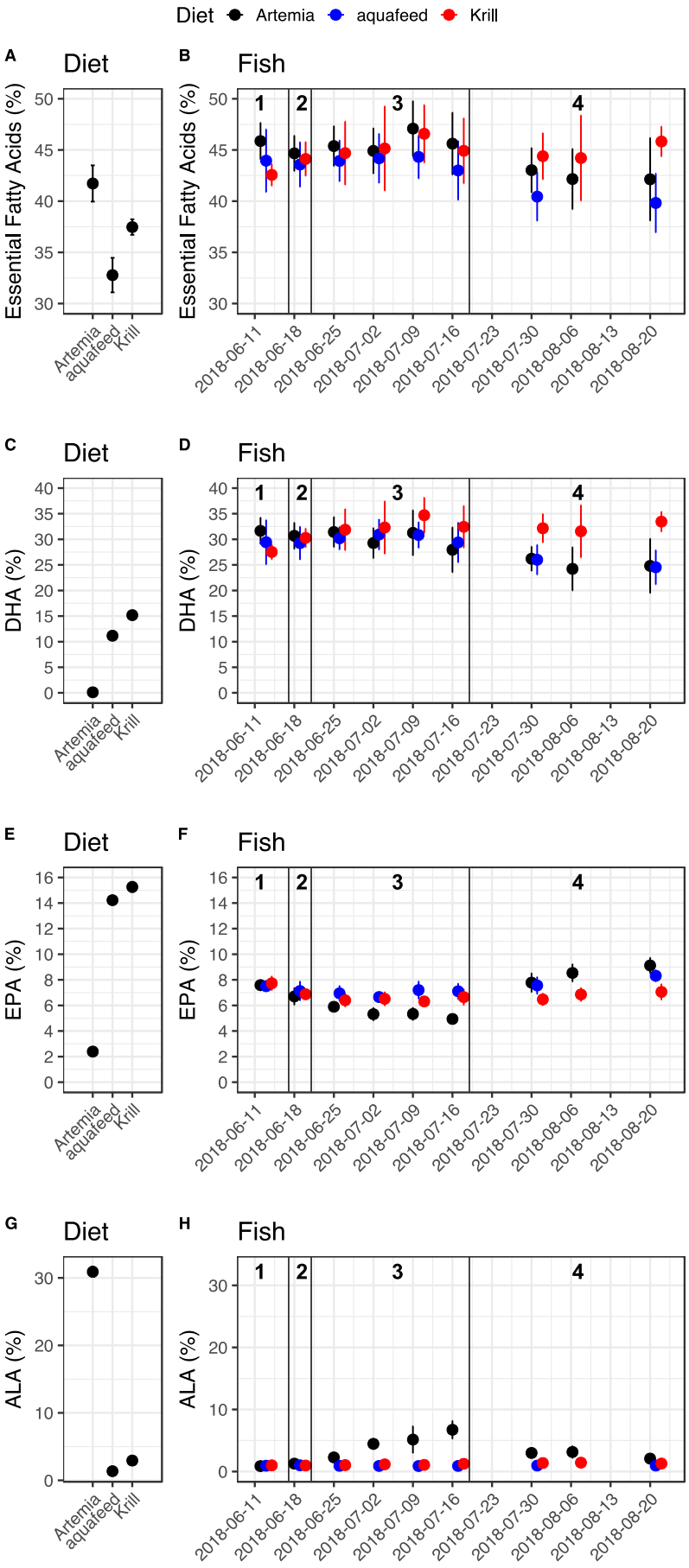

A
Specific growth rate (% body weight d-1)
2.0
1.5
1.0
Artemia
aquafeed
Krill
B
Inst. mortality rate (Z)
2.5
2.0
1.5
Artemia
aquafeed
Krill

Fig. 5


Diet
Artemia
aquafeed
Krill
A
Diet
Total Fatty Acids (mg g^{-1})
B
Fish
C
Saturated Fatty Acids (%)
D
E
Monounsaturated Fatty Acids (%)
F
G
Polyunsaturated Fatty Acids (%)
H
1
2
3
4
2018-06-11
2018-06-18
2018-06-25
2018-07-02
2018-07-09
2018-07-16
2018-07-23
2018-07-30
2018-08-06
2018-08-13
2018-08-20

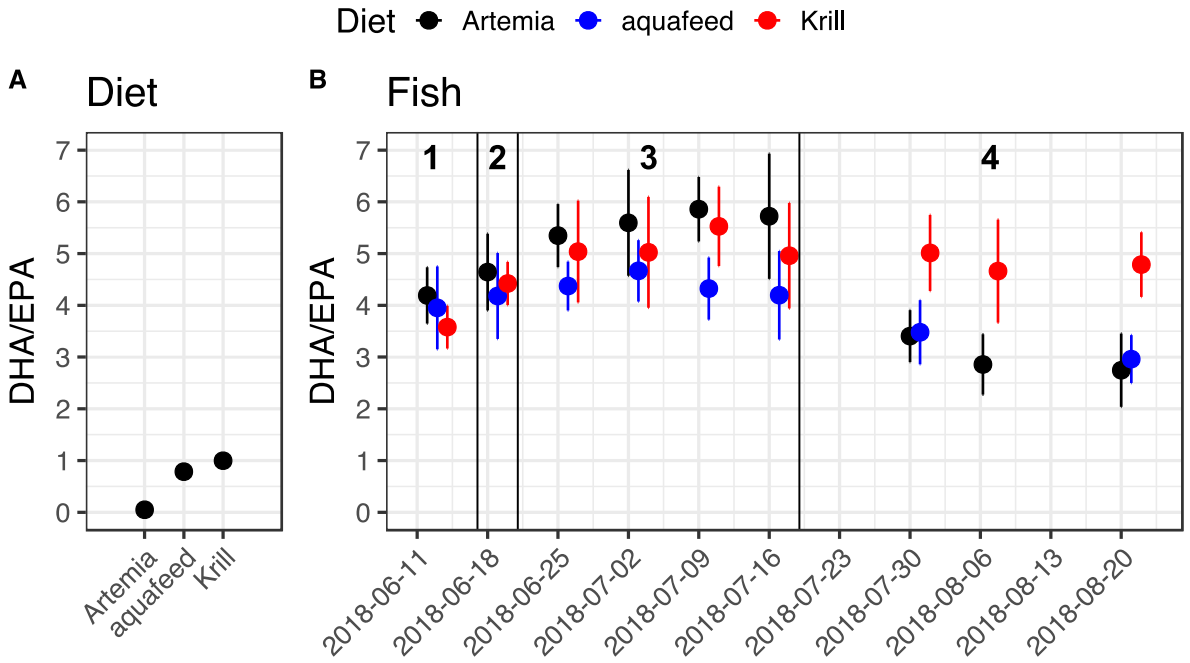
1064

Fig. 6


Diet
Artemia
aquafeed
Krill
A
Diet
Essential Fatty Acids (%)
Artemia
aquafeed
Krill
B
Fish
Essential Fatty Acids (%)
1
2
3
4
2018-06-11
2018-06-18
2018-06-25
2018-07-02
2018-07-09
2018-07-16
2018-07-23
2018-07-30
2018-08-06
2018-08-13
2018-08-20
C
Diet
DHA (%)
D
Fish
DHA (%)
E
Diet
EPA (%)
F
Fish
EPA (%)
G
Diet
ALA (%)
H
Fish
ALA (%)

1066

Fig. 7


Diet
Artemia
aquafeed
Krill
A
Diet
B
Fish
DHA/EPA
0
1
2
3
4
5
6
7
Artemia
aquafeed
Krill
1
2
3
4
2018-06-11
2018-06-18
2018-06-25
2018-07-02
2018-07-09
2018-07-16
2018-07-23
2018-07-30
2018-08-06
2018-08-13
2018-08-20

Supplementary material

Table S1. Feed composition (producer)

	Ingredients	Guaranteed Analysis from producer		Our analysis
Artemia	Brine shrimp, spirulina, carrageenan	Crude Protein (min) Crude Fat (min) Crude Fiber (max) Moisture (max) Ash (max) Phosphorus (min)	3.7% 1.2% 0.2% 94.8% 1.6% 0.1%	91.63 % water 20.440 kJ/g 4.881 kcal/g
Bio-Oregon BioVita	Fish meal, fish oil, vitamins, minerals, natural astaxanthin, beta-glucans	Protein (min) Oil (min) Fiber (max) Moisture (max) Ash (max) DE (MJ/kg)	50% 22% 1.0% 8.5% 13% 19.6	5.79 % water 23.676 kJ/g 5.654 kcal/g
Krill Canada	Krill (*E. superba*)	Protein (min) Fat (min) Crude Fiber (max) Moisture (max) Ash (max) KJ Calories	58% (55.06 g/100g) 18% (13.6 g/100g) 6% (3.1g/100g) 10% (7.6 g/100g) 13% (8.5 g/100g) 1692 (per 100g) 404 (per 100g) 15.6 (g/100g)	82% water 19.557 kJ/g 4.671 kcal/g

		Carbohydrates		

Table S2 Full statistical analysis of linear mixed models. TFA = Total fatty acids, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, EFA = essential fatty acids, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, ALA = alpha-linolenic acid

		Predictor				Random Effects			N	Marginal R^2
		Intercept	Aquafeed	Krill	Sampling date	σ^2	t_{Tank}	N_{Tank}		
RNA/DNA	Estimates	261.77	1.27	1.21	-0.01	1.19	0.00	9	254	0.268
	CI	145.08 – 378.46	0.86 – 1.68	0.79 – 1.62	-0.02 – -0.01					
	p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**					
	df	249.21	5.99	5.72	249.22					
Fulton K	Estimates	-19.06	0.05	0.03	0.00	0.00	0.00	9	204	0.168
	CI	-27.08 – -11.03	0.02 – 0.08	0.00 – 0.06	0.00 – 0.00					
	p-value	**<0.001**	**0.007**	**0.041**	**<0.001**					
	df	200.00	5.98	5.09	200.00					
TFA	Estimates	5483.10	5.63	-4.07	-0.31	731.01	2.26	9	2.64	0.072
	CI	2652.09 – 8314.10	-4.86 – 16.11	-14.57 – 6.44	-0.47 – -0.15					
	p-value	**<0.001**	0.238	0.376	**<0.001**					
	df	258.58	6.10	5.64	258.59					
SFA	Estimates	-414.27	0.97	0.65	0.02	0.81	0.01	9	264	0.340
	CI	-508.71 – -319.82	0.58 – 1.36	0.26 – 1.05	0.02 – 0.03					
	p-value	**<0.001**	**0.001**	**0.007**	**<0.001**					
	df	257.96	6.09	5.74	257.97					

MUFA	Estimates	-86.86	0.96	-0.55	0.01	6.76	0.00	9	264	0.056
	CI	-359.05 – 185.27	-0.00 – 1.93	-1.52 – 0.41	-0.01 – 0.02					
	p-value	0.530	0.051	0.208	0.426					
	df	258.80	6.11	5.60	258.81					
PUFA	Estimates	605.93	-1.92	-0.08	-0.03	7.21	0.00	9	264	0.139
	CI	324.95 – 886.92	-2.92 – -0.92	-1.08 – 0.92	-0.05 – -0.02					
	p-value	**<0.001**	**0.003**	0.856	**<0.001**					
	df	258.80	6.11	5.60	258.81					
EFA	Estimates	685.73	-1.82	0.41	-0.04	803	0.00	9	264	0.152
	CI	389.06 – 982.40	-2.87 – -0.77	-0.64 – 1.46	-0.05 – -0.02					
	p-value	**<0.001**	**0.005**	0.375	**<0.001**					
	df	258.80	6.11	5.60	258.81					
DHA	Estimates	1004.45	0.09	3.67	-0.06	14.73	0.00	9	264	0.220
	CI	602.68 – 1406.21	-1.33 – 1.52	2.24 – 5.10	-0.08 – -0.03					
	p-value	**<0.001**	0.880	**0.001**	**<0.001**					
	df	258.80	6.11	5.60	258.81					
EPA	Estimates	-375.81	0.71	0.05	0.02	0.89	0.00	9	2.64	0.248
	CI	-474.33 – -277.29	0.36 – 1.05	-0.30 – 0.40	0.02 – 0.03					
	p-value	**<0.001**	**0.003**	0.715	**<0.001**					
	df	258.80	6.11	5.60	258.81					

DHA/EPA	Estimates	341.14	-0.62	0.29	-0.02	1.00	0.00	9	264	0.222
	CI	236.57 – 445.71	-0.99 – -0.25	-0.08 – 0.66	-0.02 – -0.01					
	p-value	**<0.001**	**0.006**	0.104	**<0.001**					
	df	258.80	6.11	5.60	258.81					
ALA	Estimates	-102.60	-2.54	-2.29	0.01	1.41	0.00	9	264	0.484
	CI	-227.03 – 21.84	-2.98 – -2.09	-2.74 – -1.85	-0.00 – 0.01					
	p-value	0.106	**<0.001**	**<0.001**	0.094					
	df	258.80	6.11	5.60	258.81					

Table S3. PERMANOVA of fatty acids of Phase 3 (experimental feeding Phase) of fish permutations = 999, method = Bray Curtis

	Df	**Sum of Sqs**	**R2**	**F**	**p-value**
Treatment	2	0.0765	0.4333	45.112	0.001
Residual	118	0.1001	0.5667		
Total	120	0.1766	1.0000		

Table S4. SIMPER Cumulative contributions of most influential species

Artemia vs Krill	C22:6-n3 (DHA)	C18:3-n3 (ALA)	C16:0	C18:1-n9	C16:1	
Cumulative %	0.2785	0.4579	0.5521	0.6456	0.7072	
% dissimilarity	27.85%	17.94%	10.12%	9.35%	6.16%	
Artemia vs. aquafeed	C22:6-n3 (DHA)	C18:3-n3 (ALA)	C16:1	C16:0	C18:1-n9	C20:5-n3 (EPA)
	0.2019	0.3903	0.4876	0.5774	0.6649	0.7452
	20.19%	18.84%	9.73%	8.81%	8.75%	8.03%
Krill vs. aquafeed	C22:6-n3 (DHA)	C18:1-n9	C16:1	C16:0	C18:2-n6	
	0.3224	0.4487	0.5712	0.6373	0.7005	
	32.24%	12.63%	12.25%	6.61%	6.32%	

Figure S1. Feeding rates of fish of the different diet treatments over time for *Artemia*, aquafeed (control) and Krill in each individual tank. Feed intake of dry matter (DM) in percent of body weight (BW) per day.

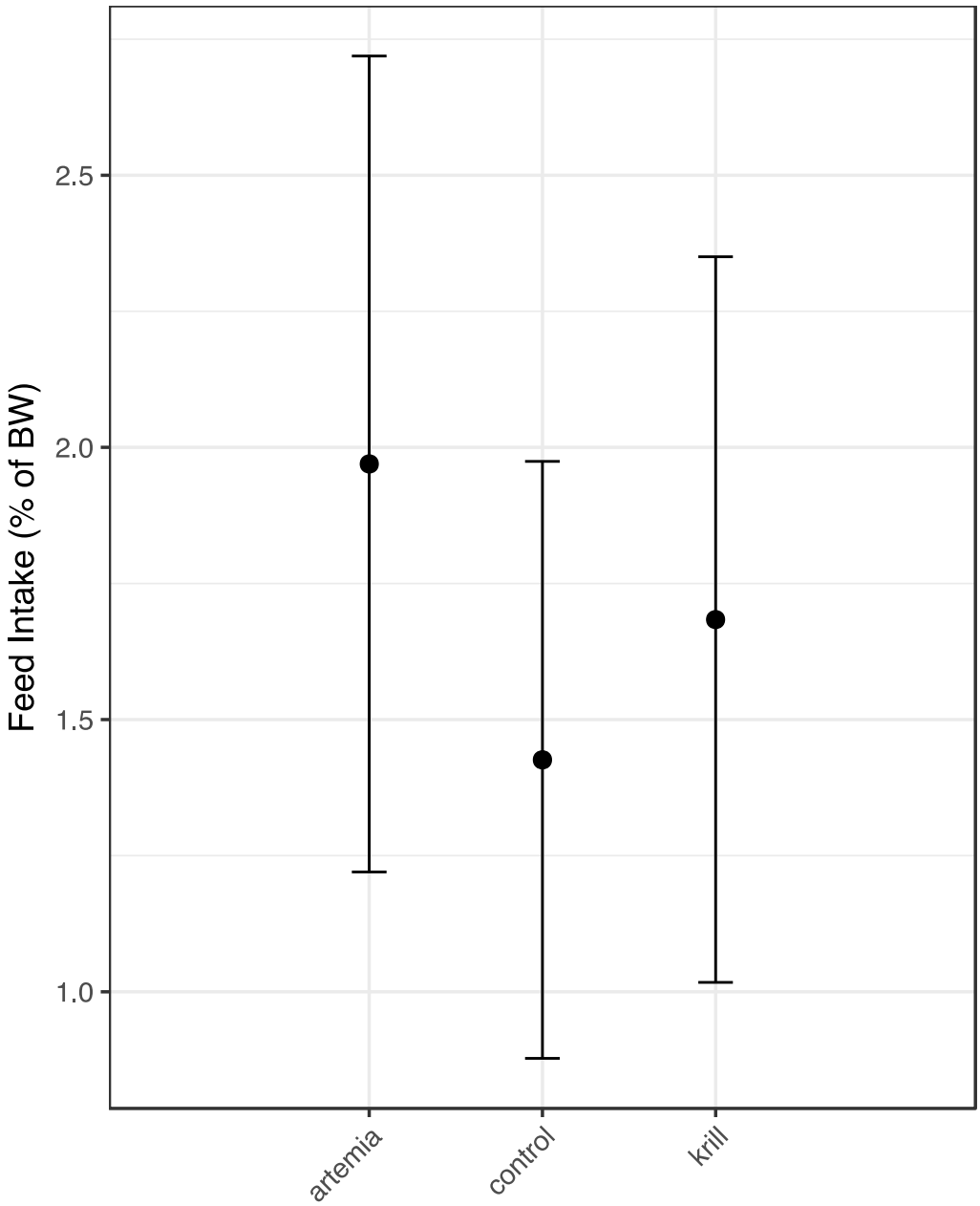


Figure S2. Cumulative mortality of fish in the different treatments (*Artemia*, control = aquafeed, Krill) over time.

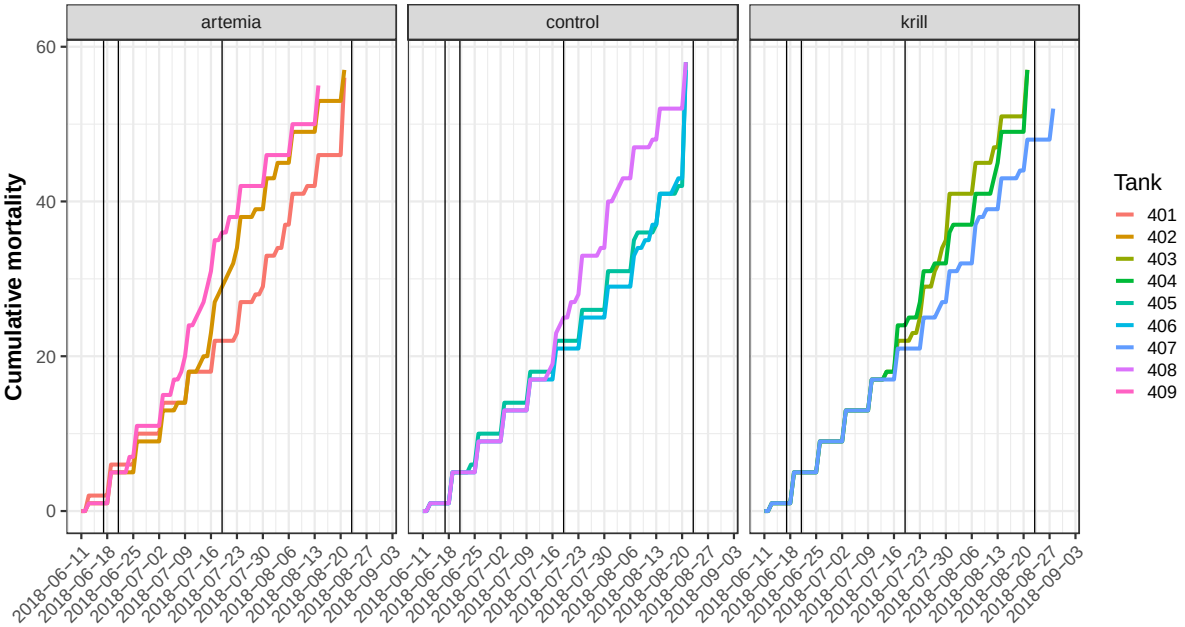


Figure S3. Energy intake (kJ per dry weight feed) based on amounts of feed given to the fish (Figure S1) for each treatment (*Artemia*, control = aquafeed, Krill) over time.

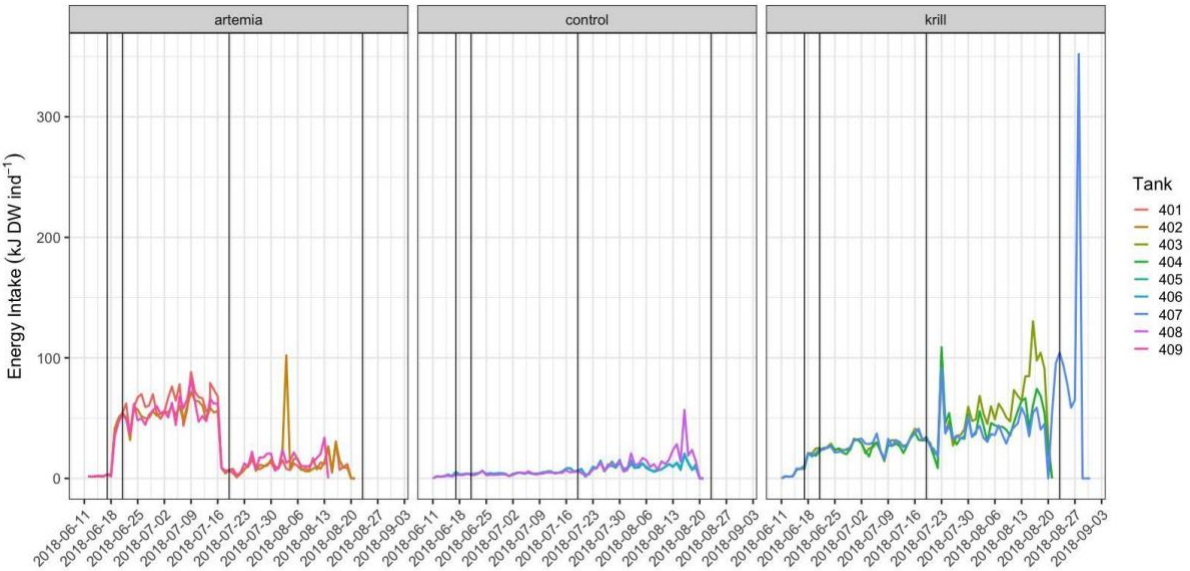
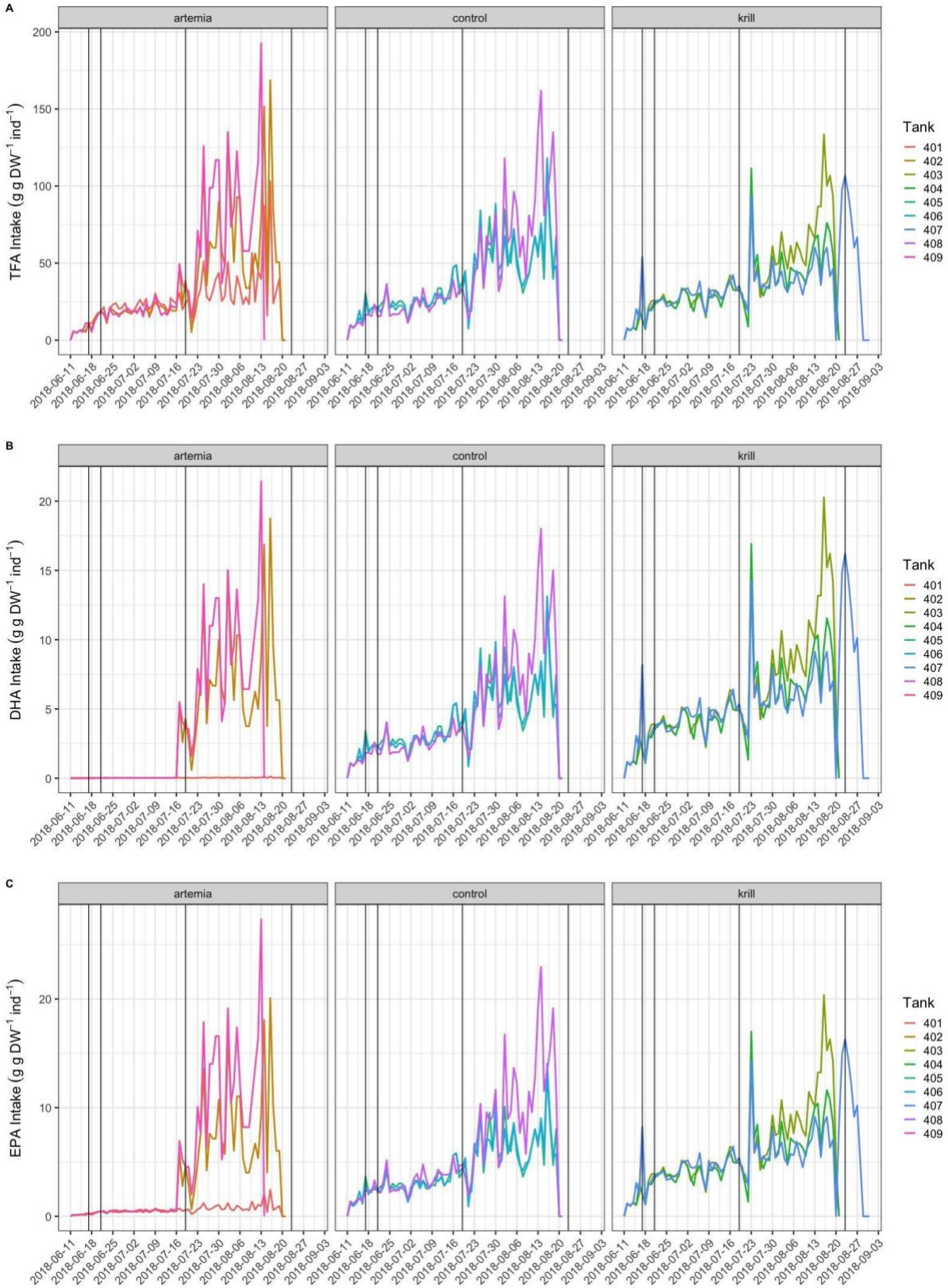


Figure S4. A) Total fatty acid intake (g TFA per g dry weight feed), B) DHA intake (g DHA per g dry weight feed), C) EPA intake (g EPA per g dry weight feed) based on amounts of feed given to the fish (Figure S1) for each treatment over time.



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