Aquaculture 308 (2010) 183–189

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

The effect of dietary fish oil and poultry fat replacement with canola oil on swimming performance and metabolic response to hypoxia in stream type spring Chinook salmon parr

Matthew D. Regan [a,*,1], Louise J. Kuchel [a,1], Susie S.Y. Huang [a,b], David A. Higgs [b], Joyce Wang [a], Patricia M. Schulte [a], Colin J. Brauner [a,*]

[a] Department of Zoology, University of British Columbia, 6270 University Blvd, Vancouver, B.C., Canada V6T 1Z4
[b] Department of Fisheries and Oceans/University of British Columbia, Centre for Aquaculture and Environmental Research (CAER), 4160 Marine Drive, West Vancouver, BC, Canada V7V 1N6

ARTICLE INFO

Article history:
Received 13 July 2009
Received in revised form 10 August 2010
Accepted 14 August 2010

Keywords:
Alternative dietary lipids
Aquaculture
Fatty acids
Oncorhynchus tshawytscha
Polar lipids

ABSTRACT

Swimming performance, metabolic rate, and the metabolic response to hypoxia were measured in stream-type spring Chinook salmon parr (*Oncorhynchus tshawytscha*) that had been reared for up to 40 weeks on diets where varying levels of refined canola oil (CO) replaced anchovy oil and poultry fat as the source of supplemental dietary lipids. Thus, CO comprised either 0% (dAPF), 25% (CO25), 49% (CO49), or 72% (CO72) of total dietary lipid. Aerobic swimming performance (repeat U_{crit} test) was unaffected by diet in freshwater or following 24 h exposure to 75% seawater. Resting oxygen consumption (MO_2) and metabolic response to hypoxia (as indicated by the critical oxygen tension, P_{crit}) were also unaffected by diet. Although dietary fatty acid (FA) composition clearly affected the FA composition of whole body total lipids in Chinook salmon parr, it had little effect on the FA composition and unsaturation index of whole body polar lipids. Chinook salmon parr maintained their polar lipid composition and unsaturation index, possibly through bioconversion of 18:3n−3 (linolenic acid; LNA) and 18:2n−6 (linoleic acid; LA) to their more highly unsaturated derivatives and/or by selective incorporation and retention of omega-3 FAs into the polar lipids. Because the polar lipids are largely found in membranes, this may indicate that membrane lipid composition was relatively constant across dietary groups and this, in turn, may largely explain the lack of physiological effects observed in this study. Taken together with a companion study on this same group of fish (Huang et al., 2008) where dietary treatment was not observed to adversely affect fish growth, feed intake and utilization, survival, and ionoregulatory development, there appears to be great potential for inclusion of CO in the diets of farmed pre-smolt Chinook salmon.

1. Introduction

Fatty acids (FAs) play vital roles in many physiological functions, from energy supply, to membrane integrity, to eicosanoid production (Tocher, 2003). In the production of fish aquaculture feeds, the provision of FAs traditionally comes from the incorporation of marine fish oils (e.g., anchovy oil). However, the increasing global demand and predicted supply shortfall of marine fish oils (MFO) from global fisheries (Barlow, 2000; Tacon, 2004; FAO-FD, 2006) has led to the exploration of suitable alternate sources of dietary lipids.

Terrestrial plant-derived oils are among the most economical and environmentally sustainable alternatives to MFO. However, they differ from MFOs in their FA profiles. Commercially available plant oils such as canola oil (CO) typically possess large amounts of unsaturated C18 polyunsaturated fatty acids (PUFA) and relatively low amounts of omega-3 (n−3) highly unsaturated fatty acids [n−3 HUFA; i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)]. Moreover, plant oils tend to have a low n−3:n−6 ratio (refer to Huang et al., 2008 for a detailed description of differences in the FA profiles of CO and MFO). It is well known that shifts in these dietary FA concentrations can dramatically influence a suite of physiological processes, from the survival, growth and health of fish (e.g., Bendiksen and Jobling, 2003; Tocher et al., 2000; Bell et al., 1997), to their metabolism and ionoregulation (Bell et al., 1985; McKenzie et al., 1998; McKenzie, 2001; Welker and Congleton, 2003; Tocher, 2003; Wagner et al., 2004).

In recent studies of juvenile Chinook salmon (*Oncorhynchus tshawytscha*; Huang et al., 2007; Grant et al., 2008), diets were altered by replacing different proportions of anchovy oil/poultry fat (traditional source of EFA) with refined canola oil (CO). Both studies demonstrated that these dietary substitutions resulted in no differences in growth,

* Corresponding authors. Regan is to be contacted at Tel.: +1 778 835 4622; fax: +1 604 822 2416. Brauner, Tel.: +1 604 822 3372; fax: +1 604 822 2416.
E-mail addresses: regan@zoology.ubc.ca (M.D. Regan), brauner@zoology.ubc.ca (C.J. Brauner).
[1] Contributed equally to this study.

0044-8486/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2010.08.014

feed intake, feed efficiency, protein, gross energy utilization, percent survival, body proximate constituents, ionoregulatory development (except for body chloride content), and seawater transfer ability over a 19-week (Grant et al., 2008) or 30 week (Huang et al., 2007) feeding trial. In the study by Grant et al. (2008), up to 54% of the total dietary lipid was comprised of CO, while in the study by Huang et al. (2008), up to 72% of the total dietary lipid was CO. Furthermore, dietary treatment did not affect the ability of these fish to survive a disease challenge (exposure to *Listonella anguillarum* Amirabbasi, unpublished). The basis for these results may lie in CO's FA profile and n−3:n−6 (2:1) ratio, unique among plant-derived oils but similar to the natural prey items of freshwater juvenile Chinook salmon (Higgs et al., 1995). However, all results discussed to this point were obtained on resting fish. Had the salmon be subjected to strenuous circumstances (e.g., exercise, hypoxia), it is possible the results may have been different.

Exercise involves the coordinated performance of a suite of physiological systems (Randall and Brauner, 1991) and is thus a useful tool for measuring the physiological limitations of an organism. Similarly, hypoxia tolerance (quantified by P_{crit}, the oxygen tension at which an aquatic organism converts from being an oxy-regulator to an oxy-conformer) can serve as a stressor to reveal physiological limitations. Because of the roles that n−3 and n−6 FAs play in the fluidity of cell membranes, the pacing of basal metabolism (Hulbert and Else, 1999, 2005), and the provision of metabolic energy in fish muscle tissue (Tocher, 2003), we hypothesized that an increase in dietary CO intake (and associated reduced levels of n−3 PUFA and HUFA, n−3:n−6 ratios, and attendant increases in other fatty acids such as LA) would negatively impact sustained swimming performance (as measured by U_{crit}), resting metabolic rate (as measured by MO_2), and hypoxia tolerance (as measured by P_{crit}).

The present study assessed the physiological consequences of increased use of CO in the diets of juvenile Chinook salmon under environmentally-relevant strenuous circumstances (i.e., exercise and hypoxia). Pre-smolt spring Chinook salmon from the study of Huang et al. (2008; where the specifics of the diets and rearing conditions have been reported) were fed one of four experimental diets for up to 30 weeks, with CO comprising either 0, 25, 49 or 72% of total dietary lipid content. Despite an absence of adverse effects of these diets on resting fish (Huang et al., 2008), we postulated that any physiological deficiencies resulting from these altered diets would be made apparent in Chinook salmon confronted with environmental challenges like sustained exercise and hypoxic exposure.

2. Materials and methods

2.1. Fish, fish diets and feeding

The specifics of fish acquisition, rearing conditions, dietary composition, and feeding husbandry have been reported by Huang et al. (2008). Since we employed fish from this study for the present investigation, this information is only briefly mentioned here. Stream-type spring Chinook salmon parr (born in the spring and destined to spend a full year in FW before migrating to SW) were kindly donated by Spius Creek Hatchery (Merritt, Canada), and in May 2005 were transported to and held at the Department of Fisheries and Oceans/University of British Columbia, Centre for Aquaculture and Environmental Research (CAER). Triplicate groups of salmon were fed one of four diets using a randomized complete block design to assign the diet treatments. From the swim-up stage through early development (total of 30 weeks), each group was fed their prescribed, steam-pelleted, dry diet that contained one of four concentrations of refined canola oil (CO). Feeding was conducted by hand 3–6 times daily, and to apparent satiation. The four experimental diets were isoenergetic (24.3 MJ/kg) and equivalent in protein (513 g/kg) and lipid (216 g/kg) content on a dry weight basis, but differed in lipid composition (see Huang et al., 2008, Table 1). The control diet's supplemental lipid source consisted of a 1:1 mixture of anchovy oil and poultry fat (APF). In the experimental feeds, CO was substituted progressively for APF so that it comprised 0, 33, 67 or 100% of the supplemental lipid, corresponding to 0% (control diet), 25%, 49% or 72% of the total dietary lipid content (dietary treatments designated respectively as dAPF, CO25, CO49 and CO72 from this point on). Considering their respective concentrations of LNA, LA, EPA and DHA, the essential fatty acid requirements of this species were met by all the dietary treatments (refer to Huang et al., 2008 for a detailed discussion of this point).

Table 1
Percentages of selected fatty acids (g respective fatty acid(s) (FA)/100 g total fatty acids), FA unsaturation index (total indentified unsaturated FAs/total indentified saturated FAs), and ratios of n−3:n−6 fatty acids in the lipids of the experimental diets. Refer to Huang et al. (2008) for complete information on the fatty acid compositions of the test diets.

Fatty acid	dAPF	CO25	CO49	CO72
∑n−6	13.7	15.2	17.2	18.9
∑n−3	16.3	14.5	12.4	11.0
AA	0.68	0.58	0.52	0.34
EPA	6.52	5.09	3.35	2.03
DHA	6.44	5.11	4.02	3.32
FA unsaturation index	2.31	3.07	4.32	6.36
n−3:n−6	1.19	0.96	0.72	0.58

Numerical values after CO refer to the percentage of total dietary lipid comprised of canola oil (CO). dAPF (control diet) was supplemented with only a 1:1 blend of anchovy oil and poultry fat.

A subset of 40 animals from each dietary group was transferred from CAER to the Department of Zoology at the University of British Columbia (UBC) at weeks 25 (mean fish weight, 6.82 ± 0.96 g) and 30 (mean fish weight, 11.25 ± 1.16 g) of the feeding trial for experimentation. Upon arrival at UBC, the fish were lightly anaesthetized with buffered MS-222 and the pectoral fin was injected with coloured elastomer dye according to the dietary treatment. Performance measurements were conducted on these individuals from October 14th to November 3rd 2005 (week 25 group), and November 29th to December 16th 2005 (week 30 group), where fish from all four dietary groups were swum simultaneously (n = 7 fish per diet; see below). Salmon from each diet were held at UBC in separate 350 L tanks at 8–10 °C in aerated (dissolved oxygen, 9.25–11.2 mg/L), flow-through, fresh water and fed their prescribed diet daily to satiation. Fish used for the metabolism and hypoxia measures (n = 8) were a subset of the week 30 group. They were fed to satiation on alternate days, but were not experimented on until they had been fed their respective diets for a total of 40 weeks.

2.2. Swimming performance in freshwater and following 24 h in 75% seawater

Juvenile Chinook salmon parr were held in freshwater at UBC for a minimum of three days to allow for adjustment to their new conditions, during which time they readily accepted feed. Feed was withheld from fish for 24 h prior to swim trials. Ramped, repeated maximum sustained swimming speed (U_{crit}) tests were conducted on salmon in both freshwater (FW) and 75% seawater (SW; 24 ppt; Brauner et al., 1994; Jain et al., 1997, 1998; Nendick et al., 2009), after a 24 h SW acclimation period. A concentration of 75% SW was chosen for the swim trials due to the high mortality rate of week 25 fish transferred to 100% SW, irrespective of diet (data not shown).

Four fish, one from each diet, were transferred to a 10 L Loligo swim tunnel (Loligo Systems APS) 10–14 h prior to the swim test. They were held overnight at a water temperature of 10–12 °C and flow of 0.5 body lengths per second (BL/s) to habituate to the swim tunnel. The upstream portion of the swim tunnel was covered by black plastic throughout the holding period and experiment to encourage the fish to reside in that part of the swim tunnel and minimize disturbance to the fish. At the commencement of the swim trial, water velocity was gradually increased to 60% of the estimated

U_{crit} over a 5 min period, and then maintained at that value for 20 min. Water flow was then increased by 0.5 BL/s every 20 min until the fish fatigued (U_{crit} #1). Fatigue was standardized as the point at which the fish were unable to remove themselves from the back grid of the swim tunnel after a series of gentle prods over a 10 s time period. The four fish were swum simultaneously, and as each fatigued, the speed and time at fatigue were recorded. Each fatigued fish was then swiftly removed from the swim tunnel so as to minimize disturbance to the other fish which continued swimming, and were held in aerated buckets of water at the same temperature and salinity as the swim tunnel until all fish had fatigued. After a minimum recovery period of one hour, they were reintroduced to the swim tunnel for a second U_{crit} trial (U_{crit} #2). U_{crit} calculations for each fish were done according to Brett (1964) and corrected for cross-sectional area of the fish as described by Beamish (1978). Recovery ratio was calculated as U_{crit} #2/U_{crit} #1.

2.3. Fish sampling

Each fish was euthanized by a blow to the head immediately following fatigue at U_{crit} #2, and total wet weight, fork length, body height and width were measured. Blood was sampled using a 30 μL heparinized capillary tube from the caudal artery after cutting through the tail. Blood samples were centrifuged at 5000 rpm for 3 min, after which haematocrit was measured and the plasma was frozen in liquid nitrogen for later analysis. A section of dorsal epaxial muscle was removed from the left side of the fish. This tissue was weighed, dried to constant weight in an oven at 80 °C for 48–72 h, and then re-weighed to obtain a measure of % muscle water content. Plasma samples were diluted 1000-fold and used to measure plasma sodium and chloride concentrations using a Corning flame photometer 410 and a mercuric thiocyanate/ferric nitrate chloride assay (Zall et al., 1956) assessed on a Spectramax 190 plate spectrophotometer, respectively.

2.4. Resting metabolic rate and response to hypoxia

Resting metabolic rate and the metabolic response to hypoxia were determined in juvenile Chinook salmon (mean weight, 17.3 ± 0.6 g) in February of 2006, 14 months post-hatch and after 40 weeks of feeding on the experimental diets. Both parameters were assessed via closed-system respirometry using a cylindrical chamber that was 270 ml in volume. The opaque chamber was submerged 3 cm below the surface of the flowing water to maintain a temperature of 12 °C. The partial pressure of oxygen (PO_2) in the water was monitored using a Loligo MINI-DO galvanic dissolved oxygen probe inserted into the chamber and connected to a Loligo data acquisition system (Loligo Systems APS). Fish were left in the darkened chamber overnight prior to the commencement of measurements at 08:00 to 09:00 h the following day. The chambers were provided with a constant rate of flow-through water that was sufficient to ensure O_2 supply and nitrogenous waste removal, but were not high enough to induce swimming during this overnight holding period. At the start of the measurements, water flow through the chamber was stopped and the water within the chamber was continually mixed with a magnetic stir bar. Fish were monitored throughout these measurements for activity, and results from active fish were discarded. Oxygen content of the water was allowed to drop continuously from initial air saturated values (155 mm Hg) to a minimum of 20 mm Hg or until the PO_2 had remained constant at low levels for over 5 min. Resting metabolic rate was determined by fitting a linear regression to the water O_2 data as it dropped from saturation values to approximately 80 mm Hg, and the metabolic response to hypoxia was quantified as the critical oxygen tension (P_{crit}; defined as the oxygen tension at which an animal converts from regulating internal oxygen levels to conforming to the environmental oxygen tension; Pörtner and Grieshaber, 1993). The program Regress v0.99 (Jeffrey Muday, Wake Forest University, 1998), which uses an algorithm adapted from Yeager and Ultsch (1989), was used in data analysis to determine P_{crit}.

2.5. Analysis of whole body polar lipid fatty acid profiles

Polar lipid FA profiles were analyzed from 6 fish sampled immediately following U_{crit} #2 from each experimental diet group following 30 weeks of feeding. Total lipid, extracted from a sub sample of the whole body homogenates, was separated into neutral and polar lipid fractions *via* silica gel cartridges (Sep-Pack, Waters Co., Millford, U.S.A.), as described by Juaneda and Rocquelin (1985). The fatty acid composition of total polar lipids was determined from FA methyl esters (FAMEs) using gas chromatography as described by Huang et al. (2008). Individual FAME concentrations were calculated as a percentage area of the total of the identifiable FAs (>98%). FAME data for diet and fish whole body total lipids were obtained from Huang et al. (2008). Unsaturated FA index was calculated by dividing total indentified unsaturated FAs by the total indentified saturated FAs.

2.6. Statistics

The software SigmaStat v 5.0 and SigmaPlot v 8.0 were used to analyze data. Comparisons were made across diet groups and salinity using two-way ANOVA, or two-way ANOVA on ranks for data that were not normally distributed. The effect of diet on the recovery ratio, resting metabolic rate, and P_{crit} was tested using one way ANOVA. Post-hoc assessment of significant differences among means was made using Tukey's test. Comparison of plasma ions between weeks was done on data pooled across diets using a t-test. Significance was assumed at $P = 0.05$. Fatty acid percentage data and relationships between the selected FA contents of dietary lipids or total body polar lipids and U_{crit} #1 were analyzed using least squares linear regression and the t-statistic was used to determine whether the slope of the regression was significantly different from zero.

3. Results

3.1. Fatty acid composition of whole body total and polar lipids

The survival of fish reared on the experimental diets was ~97% in all groups, and major growth performance parameters such as specific growth rate, feed intake, and feed utilization were not influenced by dietary treatment. The foregoing findings have been reported by Huang et al. (2008) and are representative of the fish used in the present study.

Increases in dietary CO level led to increases in LNA, LA, and the FA unsaturation index in both dietary and whole body lipids. The opposite trend was shown among EPA and DHA percentages, as well as n−3:n−6 FA ratios (Huang et al., 2008). Regressions of the specific preceding FA levels in the whole body lipids or selected ratios of FAs against their respective percentages or ratios in the dietary lipids revealed statistically significant relationships in all cases (Figs. 1 and 2), with the exception of AA. In the latter, the slope was not statistically different from 0, indicating that whole body total lipid AA levels were quite well protected despite large changes in dietary levels. Percentages of FAs noted in the whole body polar lipids were not influenced by their respective dietary concentrations to nearly the same extent as was seen in the whole body lipids (Figs. 1 and 2). For instance, the slopes found for the relationships between the whole body polar lipid levels of LA, LNA, AA, DHA and EPA and their respective percentages in the dietary lipids were lower than those observed when each of these FAs in the whole body lipids was regressed against their respective concentrations in the dietary lipids. This finding suggests that alterations of dietary lipid composition had less of an effect on the concentrations of FAs in the polar lipids than in the whole body lipids. In the case of percentages for EPA and the FA unsaturation index, the slopes resulting from the

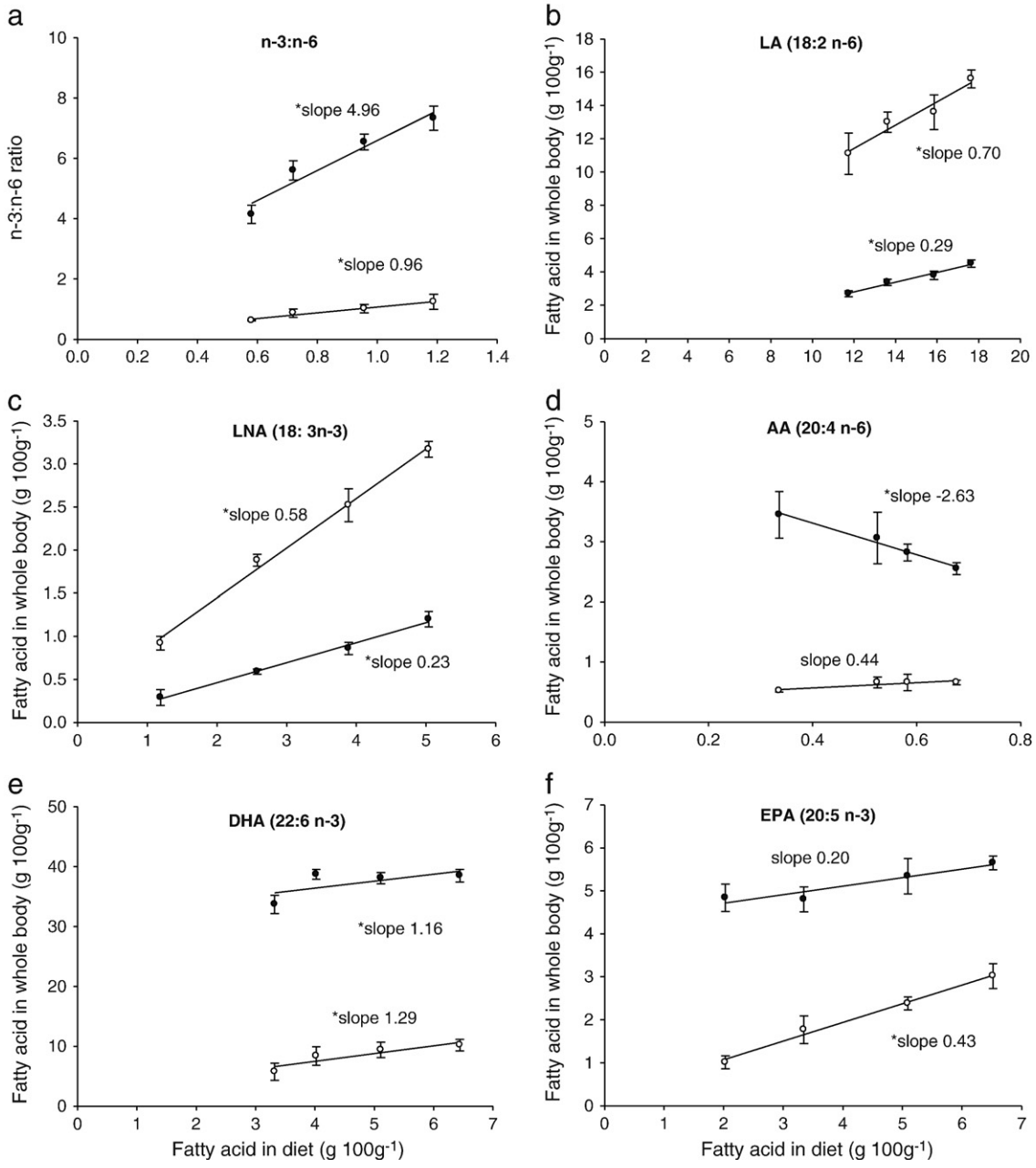

Fig. 1. The effect of dietary fish oil and poultry fat replacement with canola oil (as % CO of total lipid) in spring Chinook salmon fed diets for 30 weeks, on fatty acid (FA) composition of body and polar lipids. Specific fatty acids (g respective fatty acid/100 g total fatty acids) extracted from whole body total lipids (total lipids; open symbol; n = 3) and whole body polar lipids (polar lipids; closed symbol; n = 6) have been regressed against their respective dietary fatty acid levels. a) n−3:n−6 = omega-3:omega-6 ratio; b) LA = Linoleic acid (18:2n−6); c) LNA = Linolenic acid (18:3n−3); d) AA = arachidonic acid (20:4n−6); e) DHA = docosahexaenoic acid (22:6n−3); f) EPA = eicosapentaenoic acid (20:5 n−3);. *denotes a slope that is significantly different from zero. Specific FAs for whole body total lipids and diets are from Huang et al. (2008).

regression of their whole body polar lipid levels relative to their dietary levels were not significantly different from 0, and in the case of AA, the slope was negative. Once again, despite large changes in their dietary levels, these findings suggest that the Chinook salmon parr in this study had a remarkable ability to maintain these fatty acid levels and the polar lipid FA unsaturation index.

3.2. Swim performance and 24 h salinity exposure

For the most part, dietary treatment had no significant effect on U_{crit} #1, U_{crit} #2, or the recovery ratio (U_{crit} #2/ U_{crit} #1) in either FW or 75% SW (following 24 h acclimation period). The exception to this occurred during week 30 in 75% SW, where CO25 showed a significant reduction in Ucrit #1 and dAPF showed a significant increase in Ucrit #2, relative to fish consuming the other diets (Table 2). Twenty-four hour exposure to 75% SW significantly reduced U_{crit} #1 and U_{crit} #2 at 25 weeks in all dietary groups relative to FW values, while no significant differences were observed following 30 weeks of feeding. No significant differences in recovery ratio were observed between 75% SW values and FW values (Table 2).

3.3. Plasma and tissue analyses

Dietary treatment had no consistent or significant effects on values for plasma ion levels ($[Na^+]$ and $[Cl^-]$) or muscle water content following exercise within each salinity treatment or age group tested

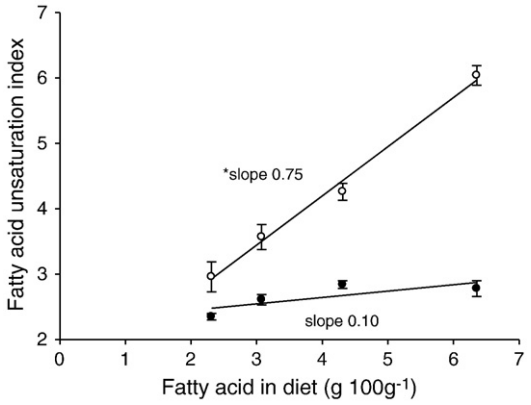

Fig. 2. The effect of dietary fish oil and poultry fat replacement with canola oil (as % CO of total lipid) in spring Chinook salmon fed diets for 30 weeks, on the fatty acid unsaturation index of whole body lipids (open symbols) and whole body polar lipids (closed symbols). FA unsaturation index was calculated by dividing total indentified unsaturated FAs by the total indentified saturated FAs.

(Table 3). There was, however, a significant increase in both plasma $[Na^+]$ and $[Cl^-]$, and reduction in muscle water content, after exercise in 75% SW relative to FW for all dietary groups combined at weeks 25 and 30. The magnitudes of these were significantly less at week 30 than at week 25 (Table 4), indicating improved hypoosmoregulatory ability over that five week period in Chinook salmon parr. These results corresponded with the measures of swimming performance, where a smaller decline in U_{crit} was recorded between FW and 75% SW in fish swum in week 30 versus those swum in week 25 (Table 2).

3.4. Metabolic rate and metabolic response to hypoxia

The resting metabolic rates and the metabolic responses of the fish to hypoxia as estimated by P_{crit} were not statistically different between dietary treatments (Table 5).

4. Discussion

Replacement of up to 72% of the total dietary lipid with refined CO in a feeding trial lasting for up to 40 weeks in stream-type spring Chinook salmon parr did not significantly affect repeated, sustained swimming performance (U_{crit}), ionoregulatory ability during exercise, resting metabolic rate, or metabolic response to hypoxia (P_{crit}). Although differences in dietary CO intake were reflected in the FA composition of whole body total lipids of these fish, there was comparatively little effect on the FA composition of whole body polar lipids (with the curious exception of AA, for reasons worthy of future investigation), which has also been observed in red sea bream (Huang et al., 2007). Since cell membranes are believed to play a role in setting the pace of basal metabolism and in ionoregulation, the minimal changes to the polar lipid composition may largely explain the lack of effects on physiological performance reported in this study. The data further support the conclusions of Huang et al. (2008), namely, that all of the dietary treatments met the EFA requirements of Chinook salmon parr. Thus, the rate and extent of bioconversion of LNA to EPA and DHA (and possibly of LA) to AA appears to have been adequate in fish fed the CO49 and CO72 diets. Other explanations of the relative lack of dietary influence on the polar lipids in the Chinook salmon may include, 1), an adequate bioconversion of LA and LNA, obtained mainly from dietary CO, 2), the other dietary lipid sources of n−3 HUFAs, and 3), the inclusion of some preformed EPA and DHA in the non-CO lipid sources present in each of the diets (refer to Huang et al., 2008 for detailed information on the ingredient compositions of all diets). The small changes in FA compositions of the polar lipids relative to the great differences noted in the FA compositions of the dietary and whole body lipids may have also been a consequence of the need for the fish to maintain optimal types and concentrations of eicosanoid compounds originating from non-esterified membrane EPA and AA for development of proper ionoregulatory function. It has been shown that wild Chinook salmon parr in freshwater encounter changes in the FA compositions of their dietary lipids similar to that fed to the salmon in the present study (Higgs et al., 1995). Thus, Chinook salmon parr (and perhaps other juvenile *Oncorhynchus* species) appear to have evolved adequate physiological mechanisms to minimize changes in polar lipid composition in the face of widespread alterations in the fatty acid compositions of their diet. The present findings support this hypothesis, but additional work is required on Chinook salmon at different life history stages to confirm this possibility.

In the present study, swimming performance in both freshwater and following 24 h exposure to 75% SW salinity was, for the most part, unaffected by the changes in dietary FA composition in Chinook salmon parr after 25 or 30 weeks of feeding the experimental diets (fish at 30 weeks in SW excepted). U_{crit} values determined for the fish in 75% SW were generally lower than U_{crit} values observed for the fish in FW, and were associated with a greater increase in plasma $[Na^+]$ and $[Cl^-]$ and reduction in muscle water content at fatigue. However, by week 30, these differences were greatly reduced. This result reflects that the fish used at week 25 were physiologically pre-smolt, and by week 30, had greatly improved their hypoosomoregulatory abilities. Similar effects have been observed in coho salmon parr, where U_{crit} was found to be negatively correlated with plasma $[Na^+]$ prior to, and following, a 24 h SW challenge (Brauner et al., 1992). The reduced aerobic swimming performance and ionoregulation in response to combined exercise and salinity exposure is not surprising given that fish gills are the primary site for both oxygen extraction and ionoregulation. In a physiologically-challenged fish, one or both of these functions may be compromised in order to maintain the other (Randall et al., 1972; Nilsson, 1986; Sardella and Brauner, 2007).

Our findings differ from those reported by McKenzie et al. (1998) in their study on post-smolt Atlantic salmon in seawater, where they observed higher U_{crit} values for fish as replacement of menhaden oil with CO increased. Also, they found a negative correlation between U_{crit} values and dietary n−3 HUFA and AA levels, but no relationship between U_{crit} values and concentrations of n−3 HUFA or AA in skeletal muscle. Their results indicate that the observed differences in swimming performance of the Atlantic salmon fed the different diets

Table 2
The effect of dietary canola oil level on mean U_{crit} #1 (Bl/s), mean U_{crit}#2, and recovery ratio (RR) of spring chinook salmon held in freshwater (FW) or 75% seawater (SW) after they had been fed their prescribed diets for either 25 or 30 weeks.

		Experimental diet			
		dAPF	CO25	CO49	CO72
25 weeks					
FW	U_{crit} #1	7.0 ± 0.4	6.8 ± 0.1	7.0 ± 0.5	6.7 ± 0.2
	U_{crit} #2	7.3 ± 0.4	6.8 ± 0.5	7.3 ± 0.3	7.2 ± 0.5
	RR	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
75% SW	U_{crit} #1	4.8 ± 0.2*	5.2 ± 0.2*	5.0 ± 0.3*	4.8 ± 0.3*
	U_{crit} #2	4.8 ± 0.4*	5.0 ± 0.4*	5.1 ± 0.4*	5.3 ± 0.4*
	RR	1.0 ± 0.0	0.9 ± 0.1	1.0 ± 0.0	1.1 ± 0.1
30 weeks					
FW	U_{crit} #1	5.3 ± 0.1	5.6 ± 0.2	5.2 ± 0.2	5.3 ± 0.3
	U_{crit} #2	5.1 ± 0.2	5.8 ± 0.2	5.4 ± 0.3	5.5 ± 0.3
	RR	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.1	1.0 ± 0.1
75% SW	U_{crit} #1	5.3 ± 0.3[a]	4.7 ± 0.2[b]	4.8 ± 0.3[a]	4.8 ± 0.2[a]
	U_{crit} #2	4.9 ± 0.1[a]	4.8 ± 0.2[b]	4.7 ± 0.3[b]	4.7 ± 0.3[b]
	RR	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1

Numerical values after CO refer to the percentage of total dietary lipid comprised of canola oil (CO). dAPF (control diet) was supplemented with only a 1:1 blend of anchovy oil and poultry fat. Letters that differ indicate statistically significant effects of diet on each U_{crit} measure. *Indicates a statistically significant effect of 75% SW relative to FW within a dietary group. (n = 7 (FW); n = 5–7 (SW)).

Table 3
The effect of dietary canola oil level on % muscle water, plasma [Na^+] and plasma [Cl^-] (mM) following determination of Ucrit 2 in spring chinook salmon that had been held in either freshwater (FW) or 75% seawater (75% SW) and fed their prescribed experimental diets for either 25 or 30 weeks.

Parameter	Experimental diet				
	Salinity	dAPF	CO25	CO49	CO72
Week 25					
% Muscle water	FW	78.3 ± 1.3 (7)	80.0 ± 1.2 (7)	76.2 ± 4.0 (7)	81.3 ± 2.7 (7)
	75% SW	74.1 ± 1.5 (7)[a]	73.2 ± 2.6 (7)[a]	71.9 ± 2.5 (7)[b]	68.5 ± 2.0 (7)[a]
Plasma [Na^+]	FW	134.3 ± 4.5 (4)	137.0 ± 4.4 (4)	149.3 ± 6.4 (4)	140.5 ± 8.8 (3)
	75% SW	220.8 ± 11.5 (3)	237.9 ± 9.2 (4)	219.3 ± 2.3 (2)	230.5 ± 4.5 (4)
Plasma [Cl^-]	FW	84.1 ± 4.5 (3)[a]	86.5 ± 5.9 (3)[a,b]	104.2 ± 4.9 (4)[b]	96.2 ± 2.0 (3)[a,b]
	75% SW	164.1 ± 5.6 (3)	175.3 ± 7.6 (4)	151.2 ± 10.6 (1)	166.8 ± 2.6 (4)
Week 30					
% Muscle water	FW	76.9 ± 1.3 (7)	75.4 ± 3.0 (7)	76.9 ± 1.3 (7)	76.5 ± 1.8 (7)
	75% SW	72.4 ± 3.4 (6)[a]	76.5 ± 1.7 (6)[b]	75.5 ± 1.5 (6)[b]	74.3 ± 1.4 (6)[a,b]
Plasma [Na^+]	FW	144.3 ± 4.6 (6)	151.1 ± 4.9 (5)	142.4 ± 6.3 (7)	145.1 ± 4.1 (7)
	75% SW	199.4 ± 5.0 (5)	202.0 ± 3.9 (6)	212.1 ± 1.8 (6)	208.0 ± 5.2 (6)
Plasma [Cl^-]	FW	115.5 ± 5.9 (5)	99.4 ± 7.6 (3)	95.5 ± 11.0 (4)	103.9 ± 6.4 (4)
	75% SW	145.0 (1)	144.8 ± 1.9 (5)	147.3 ± 1.1 (4)	155.3 ± 3.6 (3)

Numerical values after CO refer to the percentage of total dietary lipid comprised of canola oil (CO). dAPF (control diet) was supplemented with only a 1:1 blend of anchovy oil and poultry fat. Values without a letter in common are significantly different with respect to the effect of diet on that particular parameter. Numbers in parentheses indicate n.

Table 4
Plasma [Na^+] and [Cl^-] (mM) following Ucrit 2 of spring chinook (all diet groups combined) that were held in either freshwater (FW) or 75% seawater (75% SW) and fed their experimental diets for either 25 or 30 weeks.

	Salinity	Week 25	Week 30
Plasma [Na^+]	FW	143.7 ± 1.9	145.8 ± 2.5
	75% SW	228.8 ± 4.2[a*]	205.6 ± 2.2[b*]
Plasma [Cl^-]	FW	93.6 ± 3.4	104.5 ± 4.1
	75% SW	173.4 ± 6.4[a*]	148.0 ± 1.6[b*]
% Muscle water	FW	78.9 ± 1.1	76.9 ± 0.9
	75% SW	71.6 ± 0.8 [a*]	74.5 ± 1.1[b]

Variable letters indicate statistically significant differences between the duration over which the fish were fed their respective diets. *Indicates a statistically significant effect of 75% SW relative to FW within a duration group.

were not due to differences in n−3 HUFA metabolism, but possibly reflected increased metabolism of C-18 unsaturated FAs furnished by CO, such as oleic acid and LA, as an energy source. The dissimilar results of our study and those of McKenzie and colleagues may well be due to differences in species and/or experimental methods (e.g., life history stage of fish, water salinity, temperature). But they may also in part reside in the more adept ability of pre-smolt salmon to tolerate and/or bioconvert n−3 C18 PUFA (despite low dietary n−3 HUFA content) than post-smolt salmon. However, this idea is debatable. Several other studies have also found no effect on the swimming performance of salmon in response to changes in the FA composition of their diets. For example, Wilson et al. (2007) showed no effect of diet on the aerobic swimming performance of post-smolt Atlantic salmon fed diets where up to 60% of the total dietary lipid came from poultry fat, crude super de-gummed canola oil, and cold pressed flaxseed oil. Also, Chatelier et al. (2006) found no effect on the sustained swimming performance of seabass fed diets containing CO- or palm oil-derived EFAs compared to those fed diets containing MFO-derived EFAs. Despite similar results to our study, it is difficult to draw generalizations on the effects of alteration of dietary n−3:n−6 and n−3 HUFA concentrations on the swimming performance of the fish, as these studies differed with respect to the fish species and life history stages, the types and proportions of the supplemental dietary lipids, and the experimental duration.

Table 5
The effect of dietary canola oil (CO) level (expressed as % of total dietary lipid) on resting metabolic rate (MO_2; $\mu molO_2/h/g$) and P_{crit} (mmHg) in spring chinook salmon fed for 40 weeks.

%CO	Mean MO_2 ($\mu molO_2/h/g$)	Mean P_{crit} (mmHg)
0	4.32 ± 0.75	37.54 ± 2.83
25	4.37 ± 0.64	37.61 ± 3.34
49	3.79 ± 0.31	35.70 ± 1.91
72	3.63 ± 0.20	33.67 ± 2.55

No significant differences were observed among treatments. (n = 10).

In conclusion, the findings of this study demonstrate that incorporating CO by up to 72% of total dietary lipid had little to no effect on the sub-lethal physiological performance of Chinook salmon parr fed these diets for up to 40 weeks, even when the fish were subjected to arduous environmental challenges such as sustained exercise and hypoxic exposure. Indeed, we did not observe any adverse effects of dietary treatment on the aerobic swimming performance, resting metabolic rate, metabolic response to hypoxia, or ionoregulatory performance of the fish. Although the dietary FA compositions had a large effect on whole body FA composition, there were few effects on the FA compositions of polar lipids. The FA compositions of the latter may have been protected through bioconversion of LA and LNA to their more unsaturated derivatives and/or by selective incorporation and retention of pre-formed n−3 HUFAs into the polar lipids. Because the polar lipids are largely found in membranes, this may indicate that membrane lipid composition was relatively constant across dietary groups, and this, in turn, may partially explain the lack of physiological effects of the different diets observed in this study. Taken together with a companion study on this same group of fish (Huang et al., 2008), as well as another on juvenile fall Chinook salmon (Grant et al., 2008) where high dietary CO content was also found to not adversely affect the feed intake, growth, or ionoregulatory development of the fish, there appears to be great potential for inclusion of CO in the diets of juvenile Chinook salmon.

Acknowledgements

Funding for this project was provided by the AquaNet–Network Centres of Excellence in Aquaculture. We gratefully acknowledge Spius Creek Hatchery for donating the fish used in this study. Experiments conducted in this study were in compliance with the standards of and approved by the Animal Ethics Committee of the University of British Columbia, Canada.

References

Barlow, S., 2000. Fish meal and fish oil: sustainable feed ingredients for aquafeeds. Glob. Aquacult. Advocate 4, 85–88.
Beamish, F.W.H., 1978. Swimming capacity. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology, Vol. VII. Academic Press, Locomotion, New York, pp. 101–187.

Bell, M.V., Henderson, R.J., Pirie, B.J.S., Sargent, J.R., 1985. Effects of dietary polyunsaturated fatty acid deficiencies on mortality, growth and gill structure in the turbot, *Scophthalmus maximus*. J. Fish Biol. 26, 181–191.

Bell, J.G., Tocher, D.R., Farndale, B.M., Cox, D.I., McKinney, R.W., Sargent, J.R., 1997. The effect of dietary lipid on polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation. Lipids 32, 515–525.

Bendiksen, E.A., Jobling, M., 2003. Effects of temperature and feed composition on essential fatty acid (n−3 and n−6) retention in Atlantic salmon (*Salmo salar* L.) parr. Fish Physiol. Biochem. 29, 133–140.

Brauner, C.J., Shrimpton, J.M., Randall, D.J., 1992. Effect of short-duration seawater exposure on plasma ion concentrations and swimming performance in coho salmon (*Oncorhynchus kisutch*) parr. Can. J. Fish. Aquat. Sci. 49, 2399–2405.

Brauner, C.J., Iwama, G.K., Randall, D.J., 1994. The effect of short-duration seawater exposure on the swimming performance of wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kitsutch*) during smoltification. Can. J. Fish. Aquat. Sci. 51 (10), 2188–2194.

Brett, J.R., 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. 21, 1183–1226.

Chatelier, A., McKenzie, D.J., Prinet, A., Galois, R., Robin, J., Zambonino, J., Claireaux, G., 2006. Associations between tissue fatty acid composition and physiological traits of performance and metabolism in the seabass (*Dicentrarchus labrax*). J. Exp. Biol. 209, 3429–3439.

Food and Agriculture Organization – Fisheries Department (FAO-FD), 2006. State of World Fisheries and Aquaculture. http://www.fao.org/docrep/009/A0699e/A0699e00.htm 2006.

Grant, A.A.M., Baker, D., Higgs, D.A., Brauner, C.J., Richards, J.G., Balfry, S.K., Schulte, P.M., 2008. Long-term effects of dietary canola oil level on growth, fatty acid composition and osmoregulatory ability of juvenile fall Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 277, 303–312.

Higgs, D.A., Macdonald, J.S., Levings, C.D., Dosanjh, B.S., 1995. Nutrition and feeding habits in relation to life history stage. In: Groot, C., Margolis, L., Clarke, W.C. (Eds.), Physiological Ecology of Pacific Salmon. UBC Press, Vancouver, pp. 159–315.

Huang, S.S.Y., Oo, A.N., Higgs, D.A., Brauner, C.J., Satoh, S., 2007. Effect of dietary canola oil level on the growth performance and fatty acid composition of juvenile red sea bream, *Pagrus major*. Aquaculture 271, 420–431.

Huang, S.S.Y., Fu, C.H.L., Higgs, D.A., Balfry, S.K., Schulte, P.M., Brauner, C.J., 2008. Effects of dietary canola oil level on growth performance, fatty acid composition and ionoregulatory development of spring Chinook salmon parr, *Oncorhynchus tshawytscha*. Aquaculture 274, 109–117.

Hulbert, A.J., Else, P.L., 1999. Membranes as possible pacemakers of metabolism. J. Theor. Biol. 199, 257–274.

Hulbert, A.J., Else, P.L., 2005. Membranes and the setting of energy demand. J Exp. Biol. 208, 1593–1599.

Jain, K.E., Hamilton, J.C., Farrell, A.P., 1997. Use of a ramp velocity test to measure critical swimming speed in rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. 117A, 441–444.

Jain, K.E., Birtwell, I.K., Farrell, A.P., 1998. Repeat swimming performance of mature sockeye salmon following a brief recovery period: a proposed measure of fish health and water quality. Can. J. Zool. 76, 1488–1496.

Juaneda, P., Rocquelin, G., 1985. Rapid and convenient separation of phospholipids and nonphosphorus lipids from rat heart using silica cartridges. Lipids 20, 40–41.

McKenzie, D.J., 2001. Effects of dietary fatty acids on the resipiratory and cardiovascular physiology of fish. Comp. Biochem. Physiol. 128A, 607–621.

McKenzie, D.J., Higgs, D.A., Dosanjh, B.S., Deacon, G., Randall, D.J., 1998. Dietary fatty acid composition influences swimming performance in Atlantic salmon (*Salmo salar*) in seawater. Fish Physiol. Biochem. 19, 111–122.

Nendick, L., Grant, A., Gardner, M., Sackville, M., Brauner, C.J., Farrell, A.P., 2009. Swimming performance and associated ionic disturbance of juvenile pink salmon (*Oncorhyncus gorbuscha*) determined using different acceleration profiles. J. Fish Biol. 75 (7), 1626–1638.

Nilsson, S., 1986. Control of gill blood flow. In: Nilsson, S., Holmgren, S. (Eds.), Fish Physiology: Recent Advances. Croom Helm, London, pp. 87–101.

Pörtner, H., Grieshaber, M.K., 1993. Critical PO_2(s) in oxyconforming and oxyregulating animals: gas exchange, metabolic rate and the mode of energy production. CRC Press, Boca Raton.

Randall, D.J., Brauner, C.J., 1991. Effect of environmental factors on exercise in fish. J. Exp. Biol. 160, 113–126.

Randall, D.J., Baumgarten, M., Malyusz, D., 1972. The relationship between gas and ion transfer across the gills of fishes. Comp. Biochem. Physiol. 41A, 629–637.

Sardella, B.A., Brauner, C.J., 2007. The osmo-respiratory compromise in fish: the effects of physiological state and the environment. In: Fernandes, M.N., Rantin, F.T., Glass, M.L., Kapoor, B.G. (Eds.), Fish Respiration and Environment. Science Publishers, U.S.A.

Tacon, A.G.J., 2004. Use of fish meal and fish oil in aquaculture: a global perspective. Aquat. Resour. Cult. Dev. 1, 3–14.

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11, 107–184.

Tocher, D.R., Bell, J.G., Dick, R.J., Henderson, R.J., McGhee, F., Michell, D., Morris, P.C., 2000. Poly unsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation and the effects of dietary linseed and rapeseed oils. Fish Physiol. Biochem. 23, 59–73.

Wagner, G.N., Balfry, S.K., Higgs, D.A., Lall, S.P., Farrell, A.P., 2004. Dietary fatty acid composition affects the repeat swimming performance of Atlantic salmon in seawater. Comp. Biochem. Physiol. 137A, 567–576.

Welker, T.L., Congleton, J.L., 2003. Relationship between dietary lipid source, oxidative stress, and the physiological response to stress in sub-yearling Chinook salmon (*Oncorhynchus tshawytscha*). Fish Physiol. Biochem. 29, 225–235.

Wilson, C.M., Friesen, E.N., Higgs, D.A., Farrell, A.P., 2007. The effect of dietary lipid and protein source on the swimming performance, recovery ability and oxygen consumption of Atlantic salmon (*Salmo salar*). Aquaculture 273, 687–699.

Yeager, D.P., Ultsch, G., 1989. Physiological regulation and conformation—a basic program for the determination of critical points. Physiol. Zool. 62, 888–907.

Zall, D., Fisher, D., Garner, M., 1956. Photometric determination of chlorides in water. Anal. Chem. 28, 1665–1678.