Journal of Experimental Biology
209, 3429-3439 (2006)
http://dx.doi.org/10.1242/jeb.02347

Associations between tissue fatty acid composition and physiological traits of performance and metabolism in the seabass (Dicentrarchus labrax)

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Abstract: Seabass were fed for 4 months with diets where the lipid was provided as either canola oil (CO), palm oil (PO) or fish oil (FO), to generate diversity in their tissue fatty acid (FA) composition and investigate how this influenced major traits of exercise performance, cardiac performance and respiratory metabolism. In particular, based upon previous observations, we investigated the hypothesis that enriching the fish tissues with oleic and linoleic acids (OA, 18:1n-9 and LA, 18:2n-6, respectively) from the CO and PO diets would improve maximum exercise and cardiac performance, and increase aerobic metabolic scope. This proved to be the case; exercise respirometry on bass fitted with cardiac flow probes revealed that those fed CO and PO diets had a significantly higher critical swimming speed (U_{crit}) than those fed the FO diet. The improved swimming performance in the CO and PO groups was accompanied by a higher maximum cardiac output ($\dot{Q}$) and net cardiac scope, and a higher active metabolic rate (AMR) and aerobic scope (AS) than in the FO group. Analysis of tissue FA composition revealed that the fish fed the CO and PO diets had accumulated significantly higher levels of OA and LA in their heart and muscle than the fish from the FO group, which had significantly higher levels of highly unsaturated FA of the n-3 series, such as EPA and DHA (20:5n-3 and 22:6n-3, respectively). Principal components analysis revealed significant positive associations between tissue OA and LA content and U_{crit}, maximum $\dot{Q}$, the increase in $\dot{Q}$ during exercise, AMR and aerobic scope. There was a negative association between these physiological traits and tissue content of EPA. Therefore, diet composition is an environmental factor that can generate significant phenotypic diversity in major physiological traits of performance and metabolism in the seabass, with increased intake of FAs such as OA and LA leading to improved cardiorespiratory performance.

Keywords: seabass, Dicentrarchus labrax, swimming, metabolism, cardiovascular performance, fatty acid, diet

Introduction

The study of exercise performance in fishes has a long history but, to date, most work has focused on investigating the mechanisms contributing to propulsion (e.g. Webb, 1993; 1998; Videler, 1993), the energetics of activity metabolism (e.g. Beamish, 1978; Jones and Randall, 1978; Webb, 1993), and the use of locomotor capacity as a gauge of fish stress level and/or water quality (e.g. Jain *et al.*, 1998; McKenzie *et al.*, 2003a). Logic would dictate that the ability to swim should factor into the Darwinian fitness of many fish species and it is, therefore, widely assumed that swimming performance will be critical to the success of individual fish. Intra-specific diversities in performance, and the potential sources of such diversity have, however, received little attention (reviewed in Kolok, 1999; Plaut, 2001; Nelson *et al.* 2002) despite the fact that this is the raw material upon which natural selection might act. Clearly, individual physiological diversity may have genetic or environmental origins but, at present, we have little understanding of the relative contributions from each of these sources.

One environmental factor that is emerging as a significant source of physiological diversity in fish is diet quality, and particularly the relative intake and subsequent tissue accumulation of certain fatty acids (FA; Tocher, 2003; McKenzie, 2005). Fishes accumulate FAs from their diet, storing them as neutral lipids (triacylglycerols) and inserting them into membranes as polar phosphoglycerides (Sargent *et al.* 1999; Tocher, 2003). McKenzie *et al.* (1998) found a direct positive relationship between the sustained aerobic exercise performance of Atlantic salmon (*Salmo salar*) and their muscle levels of oleic acid (OA; 18:1n-9) and linoleic acid (LA; 18:2n-6). On the other hand, highly unsaturated FA of the n-3 series (n-3HUFA), namely EPA (20:5n-3) and DHA (22:6n-3), have also been reported to have beneficial effects upon the exercise performance of Atlantic salmon (Wagner *et al.*, 2004). Indeed, the same n-3HUFA have a number of effects upon the cardiorespiratory

physiology of fish (McKenzie, 2001; 2005) that might impinge upon aerobic exercise performance. When compared with individuals fed a diet rich in saturated fatty acids (SFA, such as 14:0 and 16:0), whole-animal metabolic oxygen demand (standard and routine metabolic rates) was significantly lower in Adriatic sturgeon (*Acipenser naccarii*) and European eels (*Anguilla anguilla*) fed a diet rich in n-3 HUFA (McKenzie, 2001). These relative effects of SFA and n-3HUFA might influence aerobic swimming ability if changes in standard and routine metabolic rates influence metabolic scope for aerobic activities (Fry, 1971). Furthermore, *in vitro* experiments have demonstrated that hearts from the sturgeon fed the diet enriched in SFA were unable to maintain performance when oxygen supply was reduced, unlike hearts from sturgeon fed the n-3HUFA (Agnisola *et al.*, 1996). The myocardium is a predominantly aerobic muscle, and cardiac performance is believed to be one of the major factors underlying aerobic exercise capacity in active teleosts (Farrell, 1997; 2002; Claireaux *et al.*, 2005; Clark *et al.*, 2005). The mechanisms for these effects of tissue FA are not known, although they presumably derive from the manner in which different neutral triacylglycerols are used as oxidative fuels, and/or from changes in cell function and metabolism consequent to changes in the phosphoglyceride composition of membranes (McKenzie 2001; 2005).

The objective of the current study was, therefore, to investigate how the relative tissue levels of OA, LA, SFA and n-3HUFA influenced traits of exercise performance, respiratory metabolism and *in vivo* cardiac performance in the European seabass (*Dicentrarchus labrax*). Seabass are active predatory teleosts that feed primarily upon smaller fish and crustaceans, which they capture by pursuit (Pickett & Pawson 1994). Young stages are subject to predation by pelagic fishes and several species of bird (Pickett & Pawson, 1994). The species performs substantial migrations and is profoundly euryhaline (Chatelier *et al.*, 2005), colonising environments ranging from offshore oceanic waters to inshore, brackish and freshwaters in

estuaries and coastal lagoons (Pickett and Pawson 1994). Thus, exercise ability should be a correlate, and a predictor, of the fitness of an individual seabass in its natural environment. The availability of particular FAs (e.g. n-3HUFA and LA) can display large spatial and seasonal fluctuations between estuarine and coastal marine foodwebs (Galois *et al.*, 1996), so the different environments colonised by the seabass can be expected to provide different diets.

Three groups of fish were fed for 4 months with one of three diets in which lipids were provided as either canola oil (CO), palm oil (PO), or cod liver oil, a fish oil (FO), to supply different amounts of dietary OA, LA, SFA and n-3HUFA. Exercise respirometry and cardiac flow probes were then employed to measure a suite of physiological traits of metabolism and performance. The dietary oils provided a complex mixture of FA alongside specific ones of interest. Therefore, tissue levels of FA and the suite of traits were all measured upon the same fish, such that principal components analysis (PCA) could be performed to highlight associations between physiological traits and specific FA in the tissues. We investigated the hypothesis that fish with tissues rich in OA and LA (from CO and PO) would exhibit improved exercise performance by comparison with those with tissues rich in n-3HUFA (from FO). We also investigated the hypothesis that an improved exercise performance in seabass with tissues rich in OA and LA would be linked to greater aerobic metabolic scope and *in vivo* cardiac performance. Furthermore, we investigated the hypothesis that fish with tissues rich in SFA (from PO) would have a higher metabolic rate than the fish with tissues rich in n-3HUFA (from FO). Tissue polar and neutral lipid fractions were analysed separately, to provide some insight into whether any associations between physiological traits and tissue FA levels might derive from changes to membrane polar lipid composition, or from the quality of neutral lipids as oxidative fuels (McKenzie 2001; 2005).

Materials and methods

All experiments were performed at the CNRS/Ifremer Centre de Recherche sur les Écosystèmes Marins et Aquacoles (CREMA, l'Houmeau, France).

Experimental diets

The experimental diets were prepared at the Ifremer Centre de Nutrition des Poissons (Brest, France) as 4.5 mm pellets. The basal composition of these diets was identical. The addition of CO as dietary lipid provided a FA mixture dominated by OA, LA and α-linolenic acid (18:3n-3). Palm oil (PO) provided a dietary FA mixture dominated by OA and LA but also by palmitic acid (16:0), a SFA. The fish oil (FO) provided a mixture of many FA but in particular relatively high quantities of EPA and DHA, n-3HUFA which were only present at low levels in the two vegetable oils. The FA composition of the three diets is shown in Table 1.

Experimental animals and feeding

European seabass (*Dicentrarchus labrax*) with a mean (± SD) mass of 200 ± 10 g and length of 26.56 ± 0.28 cm were obtained from a commercial supplier on Ile de Ré (Charente Maritime, France). They were maintained at CREMA in 1 m^2 fibreglass tanks (water volume approximately 400 L) under a natural photoperiod. Tanks were provided with biofiltered seawater (SW) at a temperature of 20 °C and salinity of 30‰. A total of 72 fish were slightly anaesthetised, fitted with a subcutaneous passive integrated transponder (PIT) tag for individual identification, then allocated randomly to one of 6 experimental groups (2 replicates per diet, 12 individuals per replicate). The fish were then allowed month of acclimation to the prevailing holding conditions, during which they were fed a commercial diet (Bar D Perform Natura 4.5; Sica du Gouessant, Lamballe, France). They were then fasted for two weeks prior to the beginning of the feeding protocol, at which point they accepted the novel feeds eagerly. Fish were then fed by hand daily to satiation, with their designated

experimental diet. After 4 months the fish had approximately doubled in mass. Daily growth and condition factor were calculated as follows:

$$\text{Daily growth} = \frac{\frac{fW - iW}{iW} \times 100}{n},$$

$$\text{Condition factor} = \frac{fW}{L^3} \times 100$$

where *fW* is final weight in Kg, *iW* is initial weight in Kg, *n* is the number of feeding days and L is length in cm.

Surgical preparation

At this stage of the experiment, bass with a mean mass and forklength of 395 ± 10 g and 30.58 ± 0.28 cm, respectively, were anaesthetised with tricaine methane sulphonate (MS-222) at a concentration of 0.1g L^{-1}, and transferred to an operating table where their gills were irrigated with aerated water containing 0.05g L^{-1} MS-222. A 2S-type Transonic (EMKA, Paris, France) ultrasound flow probe (resolution 0.1 $ml.min^{-1}$; absolute accuracy ± 15%) was placed around the ventral aorta, as described by Axelsson *et al.* (2002). Following surgery, the animals were allowed 48 h recovery in opaque PVC chambers provided with a flow of water.

Exercise and cardiac performance

Swimming respirometry was performed with an automated Brett-type swim-tunnel respirometer designed to exercise fish in a non-turbulent water flow with a uniform velocity profile (McKenzie *et al.*, 2001). Fish were transferred individually to the respirometer and allowed to recover for at least 12 hours (overnight) at a current speed of 0.5 $bodylengths.s^{-1}$ ($BL.s^{-1}$). At this low current speed bass rested on the bottom and maintained position by gentle sculling of their pectoral fins and occasional tail flicks. The following day, fish were exposed to progressive increments in swimming speed at 1, 2, 3, 3.5 and 4 $BL.s^{-1}$, every 30 min, until fatigue. Fish were considered to be fatigued when they were unable to remove themselves from the posterior screen of the swimming chamber despite gentle encouragement

by sudden increases in current velocity. Measurements of O_2 uptake (M_{O2}, in mg kg^{-1} h^{-1}) were collected automatically at each swimming speed with the custom-designed data-acquisition system described in McKenzie *et al.* (2001) and custom-made software (G. Guillou, CREMA). Water O_2 saturation in the sealed respirometer was measured with an Orbisphere clarke-type polarographic oxygen electrode and associated meter (Orbisphere Laboratory, Geneva, Switzerland). The measurements of M_{O2} during exercise were used to derive the notional metabolic rate of the immobile fish (IMR), as described by McKenzie *et al.* (2003b). The maximum metabolic rate of activity (AMR) was identified during swimming (this occurred at speeds approaching U_{crit}) and used to calculate net aerobic scope relative to IMR (McKenzie *et al.*, 2003b). Critical swimming speed (U_{crit}) was calculated in $BL.s^{-1}$ as described by Brett (1964).

At each swimming speed, cardiac output (Q) was measured in $ml.min^{-1}.kg^{-1}$ with the signal from the flowprobe displayed on the Transonic amplifier and acquired by a PC with the custom-designed labview software described in Axelsson *et al.* (2002). The signal was used to calculate f_H in $beats.min^{-1}$ and, together with the data for Q, used to calculate V_{SH}, in $ml.beat^{-1}.kg^{-1}$. Cardiac scope during exercise was calculated as maximum Q minus "routine" Q. Maximum Q always occurred at swimming speeds approaching U_{crit}, routine Q was taken as the lowest Q measured when the fish was swimming very gently at a speed of 5 cm s^{-1}, prior to the exercise protocol. Routine f_H and V_{SH} were derived from the measures of routine Q. Increase in f_H and V_{SH} during exercise was calculated respectively as maximum f_H minus routine f_H and maximum V_{SH} minus routine V_{SH}.

After experiments, animals were rapidly removed from the respirometer and killed with a blow to the head. The ventricle and a piece of white muscle were taken and stored at -80°C until fatty acids analysis.

Tissues fatty acid analysis

Total lipid extraction and measurement:

Lipids were extracted from tissues following a procedure derived from that of Folch *et al.* (1957). A double step extraction was carried out on rehydrated samples by grinding them in chloroform: methanol mixtures (1:2 then 2:1, v/v) with an all-glass Potter homogeniser. For each sample, the two homogenates were filtered on GF/F pre-combusted filters and pooled in a conical glass centrifuge tube. Following the addition of a 1% NaCl solution, the crude extract separated into two phases. After decantation, the lower phase containing lipids was recovered by suction and stored in PTFE capped glass tubes at -20°C until analysed.

Total lipids were measured with a Chromarod SIII - Iatroscan TH-10 system (TLC-FID) connected to a Shimadzu CR3A integrator. For each sample, four successive volumes (1 µl) of a concentrated extract aliquot were spotted on a Chromarod with a 2 µl microsyringe. After drying in a dessicator, the rod was read directly by the Iatroscan without any development. A calibration curve based on a mixture of pure standards (Sigma) was used to calculate the total lipid concentration of the lipid extracts (Parrish & Ackman, 1985).

Separation of polar and neutral lipids

Total lipids were separated into polar and neutral fractions by HPLC (Kontron Analytical) coupled with an ELSD-500 detector (Alltech). The separation was carried out on a preparative 50 mm x 10 mm ID stainless steel column filled with an SPE Si-diol phase (IST). The polarity gradient was obtained with increasing proportions of methanol in chloroform. The column effluent was by-passed in such a way that 90% of the separated lipid fractions were recovered for FAME analyses, while the detector measured 10%.

FAME preparation, purification and measurement:

Fatty acid methyl esters (FAME) from the separated lipid fractions were obtained after a base-catalysed transesterification with sodium methylate (0.5 M, 1 h, 80°C; Christie, 1984).

FAME were purified by HPLC, using a preparative 100 mm x 10 mm ID stainless steel column filled with an SPE Si-NH_2 phase (IST). The polarity gradient was obtained by increasing the proportions of chloroform and methanol in heptane. As above, the column effluent was by-passed so that 90% of the purified FAME was recovered for gas chromatography, while the detector measured 10%.

Purified FAME were then analysed by gas chromatography (Packard 439) on a SGE BPX-70 highly polar capillary column (30 m x 0.25 mm ID x 0.25 μm film), with hydrogen as carrier gas at 1.1 $ml.min^{-1}$. The temperature gradient ran from 90 to 210°C at a rate of $1°C.min^{-1}$. Identification of FAME was completed by comparison with pure individual standards (Sigma), standard mixtures (Supelco) and hydrogenated samples.

Statistical analysis

Differences amongst the dietary groups for any given variable were assessed by one-way analysis of variance (ANOVA) with Bonferroni post-hoc tests to identify where significant differences lay. In those cases where the groups did not exhibit homogeneity of variance, a Kruskal-Wallis non-parametric ANOVA and Mann-Whitney post-hoc tests were used. Links between heart polar lipids and physiological traits were explored by Principal Component Analysis (PCA) followed by Pearson correlation tests, using StatBox6® software. Only FA that represented at least 1% of tissue FA in at least one lipid fraction (polar or neutral) were used for the PCA and Pearson correlation tests. A probability of less than 5% ($p<0.05$) was taken as the fiducial level for statistical significance.

Results

The FO group exhibited a significantly higher daily percentage increase in mass than the other two dietary groups, mean (± SEM) specific growth rate was 0.51 ± 0.10; 0.31 ± 0.03, and 0.36 ± 0.03 %.day^{-1} in the FO, CO and PO groups, respectively. The FO group also exhibited a significantly higher mean condition factor, being 1.46 ± 0.04; 1.36 ± 0.02, and 1.33 ± 0.03 in the FO, CO and PO groups, respectively.

Exercise performance, cardiac performance and respiratory metabolism

Seabass fed the CO and PO diets achieved a mean (± SEM) U_{crit} of 3.2 ± 0.04 and 3.24 ± 0.05 BL.s^{-1}, respectively, which were not significantly different (Fig. 1). In contrast, fish fed FO only achieved a U_{crit} of 2.93 ± 0.12 BL.s^{-1}, which was significantly lower than the other two groups (Fig. 1).

There was no significant difference in routine Q between diets (Fig 2), nor in routine V_{SH} and f_H (Table 2). There were, however, differences in the cardiac response to exercise. As shown in Fig 2, maximum Q was significantly higher in CO and PO fish than in those from the FO group. As a consequence, the increase in Q during exercise was also significantly higher for CO and PO fish (Fig 2). In all fishes, the increased Q during exercise was primarily a consequence of increased f_H, with a smaller contribution from increases in V_{SH} (Table 2), but there were no differences in the maxima for these latter two variables amongst the three diets (Table 2).

IMR was not significantly different between diets (Fig 3) but there were significant differences in respiratory metabolism during exercise. Exercise elicited an exponential increase in MO_2 in all fish (data not shown) until a plateau was reached just prior to fatigue (U_{crit}). As shown in Fig. 3, AMR was significantly higher in the CO and PO groups relative to the FO group. As a direct consequence, net aerobic scope was also significantly higher in the CO and PO groups, relative to the FO group (Fig 3).

Tissue FA composition

As anticipated, there was a clear influence of dietary FA supply upon the FA composition of neutral and polar lipids in the heart (Table 3) and muscle (Table 4). In polar lipids of the heart (Table 3), the PO fish had higher levels of stearic acid than found in the other two groups. Both the CO and PO fish had higher levels of OA and LA in their polar lipids than the FO fish did (Table 3). On the other hand, the FO fish had higher levels of n-3HUFA such as EPA and its elongation product 22:5n-3. DHA comprised about 28% of the heart polar lipids in all groups, with no differences between them. The groups did not differ in their overall levels of SFA, MUFA and PUFA but the FO group had a much higher ratio of n-3 to n-6 PUFA in its polar lipids by comparison with the other two groups. In the neutral lipids, stearic acid, OA, LA and n-3HUFA exhibited the same overall pattern of distribution as seen for the polar lipids (Table 3). However, the dominant FA in the neutral lipids of all groups was not DHA but, rather, OA. Furthermore, for their neutral lipids, the PO group had a higher total content of SFA than the other two groups, and it also had a higher total content of MUFA than the FO group (heart neutral MUFA levels varied greatly in the CO fishes, Table 3). Conversely, the PO group had a significantly lower level of total PUFA than the CO and FO groups. Finally, the FO group, as expected, had a much higher ratio of total n-3 to n-6 PUFA than in the other groups, although the differences were less pronounced than in the polar lipids (Table 3).

As can be seen in Table 4, skeletal white muscle also exhibited this general pattern of diversity in FA distribution between the three dietary groups. Indeed, the differences observed in the white muscle were, if anything, more pronounced than in the ventricular muscle (Table 3 cf Table 4). This demonstrates that the diets had generated systemic differences in tissue FA profile amongst the three groups.

Principal component analysis

The PCA revealed some clear associations between the measured physiological traits and levels of particular FA in the polar and neutral lipids of the heart (fig 4). The analysis also separated the groups along the primary axis, with the CO and PO fish localised close together and apart from the FO fish (fig 4). In particular, on axis 1, fishes from the CO and PO groups with elevated levels of OA, LA and total MUFA in their lipids had high Ucrit, maximum Q, scope to increase Q during exercise, AMR, and aerobic scope (fig 4). On the other hand, fishes from the FO group with high levels of n-3PUFA and a high ratio of n-3 to n-6 PUFA had low values for these traits of exercise and cardiac performance but high values for growth and condition factor (fig 4).

The axes 2 and 3 showed some further associations between tissue FA and physiological traits. These associations were not strictly linked to dietary group but were a function of the intrinsic individual diversity amongst all of the animals studied (fig 4). Axis 3 shows an effect of polar lipids, whereby fishes with high levels of stearic acid and total SFA tended to have high IMR, whereas IMR was low in fishes with high levels of DHA and total PUFA in their polar lipids (fig 4). Axis 2 shows an effect predominantly of neutral lipids, whereby fishes with high levels of stearic acid, OA, total SFA and total MUFA in neutral lipids tended to have low routine V_{SH}, whereas routine V_{SH} was high in fish with high levels of AA and DHA in neutral lipids, or of AA in polar lipids (fig 4).

These associations between FA and physiological traits were borne out by the Pearson correlations derived from the PCA. Table 5 shows the correlations for polar lipids. Specific growth rate showed a weak negative correlation with LA levels and a positive correlation with n-3 to n-6 PUFA ratio. Immobile metabolic rate showed a strong positive correlation with stearic acid and total SFA in polar lipids, and a negative correlation with DHA and total PUFA, this latter probably because total PUFA were dominated by DHA (Table 5). The performance traits (U_{crit}, maximum Q, scope to increase Q during exercise, AMR, and aerobic

scope) were all correlated positively with LA and OA in polar lipids, but negatively with EPA, 22:5n-3 and ratio of n-3 to n-6 PUFA (Table 5). Table 6 shows the correlations for neutral lipids. Once again, growth was correlated negatively with stearic acid and total SFA levels in neutral lipids, while both growth and condition factor were related positively with EPA levels. Routine V_{SH} was correlated positively with AA, DHA and total PUFA in neutral lipids (Table 6). Performance traits were related positively to LA levels in neutral lipids (but not neutral OA levels, which did not emerge from the PCA), and related negatively to EPA levels and ratio of n-3 to n-6 PUFA (Table 6).

Discussion

The results demonstrate that diet-related changes in the FA composition of the tissues can have significant effects upon major physiological traits of performance and metabolism in seabass. This overall result is consistent with previous studies showing an impact of tissue FA composition on the cardiorespiratory physiology of fish (Randall *et al.*, 1992; McKenzie *et al.*, 1995; 1997; 1998; 2000; Agnisola *et al.*, 1996; Wagner *et al.*, 2004). As expected, the best physiological performance was observed in the animals fed the CO and PO diets, rich in OA and LA. The PCA revealed a number of associations between the percentage content of specific FAs in the polar and/or neutral pools of ventricular muscle and particular traits of growth, metabolism and performance.

The significantly higher U_{crit} measured in the CO and PO groups, as compared to the FO group, was linked to the high levels of OA and LA in their tissues. These results are consistent with a previous study on Atlantic salmon (McKenzie *et al.*, 1998) which found that incremental substitutions of fish oil by canola oil in the dietary lipids led to incremental increases in swimming performance, with a direct positive relation between tissue OA and LA (derived from the dietary canola oil) and U_{crit}. The current data extend these observations to demonstrate that improved exercise performance was linked to improved maximum cardiac

performance, higher active metabolic rates and greater aerobic scope in fish with tissues rich in OA and LA.

It is possible that the greater aerobic scope and higher swimming performance of the CO and PO fish was a consequence of the improved cardiac performance. It has been argued that aerobic myocardial performance may be a primary factor limiting AMR and U_{crit} in active teleosts (Farrell, 2002; Claireaux *et al.*, 2005; Clark *et al.*, 2005). A recent study has demonstrated that intrinsic individual diversities in maximum Q are directly linked to parallel diversities in AMR, aerobic scope and U_{crit} in rainbow trout (Claireaux *et al.*, 2005). This linkage has yet to be demonstrated for seabass, but there is some evidence for a role of cardiac performance in defining aerobic scope and U_{crit}. In many active teleosts, including rainbow trout and seabass, both Q and M_{O2} plateau as fish approach U_{crit} in swim tests (Kiceniuk and Jones, 1977; Kolok and Farrell 1994; Thoraresen *et al.*, 1996; Gallaugher *et al.*, 2001; Chatelier *et al.*, 2005) and this has been taken as circumstantial evidence that it is limitations to cardiac work that are constraining aerobic scope (Farrell, 2002). In the seabass, the simultaneous plateau of both Q and M_{O2} during exercise corresponds with the initiation of an intermittent "burst and coast" swimming pattern (Chatelier *et al.*, 2005) that indicates recruitment of anaerobic white muscle (Day and Butler, 1996) and which precedes fatigue. Visual observation of the ventral aortic probe trace at this time of the experiment also revealed significant cardiac arrhythmias, further circumstantial evidence that impaired cardiac performance was linked to the onset of fatigue. This arrhythmia always occurred at higher swimming speeds in the CO and PO fish, when compared to the FO animals.

At present, it is only possible to speculate about the mechanism by which the tissue OA and LA might exert their effects upon cardiorespiratory performance. They may be related to the fact that aerobic metabolism, and aerobic work, is fuelled primarily by β-oxidation of neutral FA in fish (Hochachka and Somero, 1984; Richards *et al.*, 2002). There is

in-vitro evidence to suggest that OA and LA are preferred over other FA, especially HUFA, as substrates for β-oxidation (Sidell and Driedzic, 1985; Henderson and Sargent, 1985; Egginton, 1996). It is conceivable, therefore, that higher levels of these preferred substrates in the tissues might allow the animals to achieve higher rates of aerobic work (McKenzie, 2001). It was unexpected, therefore, that only levels of neutral LA in tissue lipids emerged as being related to high performance in the PCA, and that levels of neutral OA and total neutral MUFA showed no association. This might argue against such a "substrate" mechanism.

The linkage between levels of OA and LA in polar lipids and increased cardiac (and exercise) performance may also be a result of membrane processes. Studies on isolated mammalian cardiomyocytes revealed that extracellular application of EPA and other PUFA produced a prompt and reversible concentration-dependent inhibition of the L-type calcium current, thereby limiting calcium entry into the cells (Xiao *et al.*, 1997; Leaf *et al.*, 1999). It was proposed that such effects should protect against calcium overload and arrhythmia (Xiao *et al.*, 1997; Leaf *et al.*, 1999). Any such beneficial effect of EPA on cardiac arrhythmia was not revealed in our study. There is, however, preliminary evidence to indicate that OA has an inhibitory effect on L-type calcium channels and could, therefore, protect hearts against arrhythmia at high workloads (Chatelier, A., Imbert, N., Zambonino Infante, J.L., McKenzie, D.J. and Bois, P. manuscript submitted). Such an effect might have contributed to the improved cardiac performance in the CO and PO fish.

The relatively poorer exercise performance of the seabass with tissues rich in EPA and with a high n-3 to n-6 PUFA ratio is also consistent with previous results of McKenzie *et al.* (1998) on Atlantic salmon. These results are, however, in direct contrast to those reported by Wagner *et al.* (2004), who found improved exercise performance in salmon fed a diet rich in n-3HUFA. The explanation for these opposing results presumably lies in the enormous complexity of factors within such diet studies. In particular, the oils used in dietary studies all

provide a complex mixture of FA. Many of these FA have specific biological roles that interact with each other (Sargent *et al*., 1999), such that each study is effectively unique unless great care is taken to match the ingredients. There may also be minimum threshold of action for some FA. In the current study, the relatively poor cardiorespiratory performance of fish with tissues rich in EPA should not be taken as an indicator of overall "reduced fitness", since tissue levels of this FA were correlated positively with high fish growth rates. It is interesting that the PCA revealed that these effects were correlated with EPA (and its elongation product 22:5 n-3) rather than DHA, the other essential n-3HUFA provided in the FO diet.

The high IMR observed in fish with high tissue total SFA, revealed in the PCA and Pearson correlations, are consistent with previous studies (McKenzie *et al.*, 1997; 2000) where sturgeon and eels fed SFA had significantly higher rates of metabolism than those fed n-3HUFA. The results of the PCA indicate that this effect of SFA on IMR in seabass may have been a result of a membrane-related mechanism, as the associations were only observed for polar lipids. It is also interesting that it was high tissue levels of DHA in polar lipids that were correlated with low IMR in the PCA, with no other HUFA implicated.

Once again, it is only possible to speculate about the mechanism(s) for the effects on metabolic rate of SFA versus DHA (McKenzie, 2001). Hulbert and Else (1999) and Hulbert *et al.* (2005) suggest that effects of n-3HUFA versus SFA on metabolic rate are due to membrane-related processes, and particularly to differences in energy consumption by membrane-bound ATPases, consequent to changes in membrane FA composition and physico-chemical properties. These authors, however, report that n-3HUFA raise metabolic rate, while SFA lower it, in a number of terrestrial endotherms (birds and mammals) (Hulbert and Else, 1999; Hulbert *et al.*, 2005). This is in exact opposition to the effects on metabolic rate reported here and previously by McKenzie et al (1997; 2000). On the other hand, Pepe

and MacLennan (2002) reported that hearts isolated from rats fed with n-3HUFA (from fish oil) had significantly lower rates of myocardial oxygen consumption than hearts from rats fed SFA (from coconut oil). They attributed this to differences in mitochondrial membrane composition that influenced proton leak (Pepe and MacLennan, 2002). The basis for such contrasting results is not clear and, while there can be no doubt that dietary and tissue FA exert profound effects upon the metabolic and cardiorespiratory physiology of vertebrates, further work is needed to explore the mechanisms for these effects and thereby, hopefully, to explain contrasting results.

The positive correlation between routine V_{SH} and tissue total PUFA levels, and in particular HUFA such as AA and DHA, cannot be explained at present. Agnisola et al. (1996) found that, when working spontaneously *in vitro*, hearts isolated from sturgeon fed a diet rich in n-3HUFA had a greater routine V_{SH}, than hearts isolated from sturgeon fed a diet rich in SFA.

In the present study at 20°C, seabass exhibited a much greater AMR, aerobic scope, maximum Q and U_{crit} than the seabass at 15°C studied by Chatelier *et al.* (2005). This demonstrates that temperature exerts a profound effect upon the cardiorespiratory performance of seabass. In both this study and the study of Chatelier *et al.* (2005), the increase in Q during exercise was attributable to an increase in f_H rather than V_{SH}. Chatelier *et al.* (2005) found that rapid changes in water salinity had no effect on cardiac or swimming performance. This indicates that diet may be at least as important an environmental variable as salinity for defining the performance of seabass in their natural environment. Migrations of seabass may expose the animals to entirely different FA availabilities, particularly between marine foodwebs that are rich in n-3HUFA and estuarine foodwebs where FA such as LA and OA may be more common (Galois et al. 1996). Exploring the associations between tissue FA

composition and traits of growth, performance and respiratory metabolism in wild seabass is, therefore, an interesting avenue for future research.

Acknowledgements

AC was funded by a doctoral fellowship from IFREMER and the Conseil Régional Poitou-Charentes. DJM contributed to this study while employed upon the EU project Ethofish. The authors are grateful to G. Guillou, P. Pineau and M. Prineau for their assistance.

List of symbols and abbreviations

AMR: active metabolic rate

AS: aerobic scope

BL: body length

CO: canola oil

FA: fatty acids

f_H: heart rate

FO: Fish oil

IMR: metabolic rate of the immobile fish

LA: linoleic acid

MO_2: rate of oxygen uptake

n-3HUFA: highly unsaturated fatty acids of the n-3 series

OA: oleic acid

PCA: principal component analysis

PO: Palm oil

Q: cardiac output

SFA: saturated fatty acid

SGR specific growth rate

U_{crit}: critical swimming speed

V_{SH}: cardiac stroke volume

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Table 1. Fatty acid (FA) composition of lipids in the diets as a percentage of total fatty acids. FO, fish oil diet; CO, canola oil diet; PO, palm oil diet; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. For the individual FA, only those that occurred at more than 0.5% of lipids are reported, with the exception of 20:4n-6.

Fatty acid	CO	PO	FO
14:0	0.7	0.9	3.3
16:0	8.5	20.4	13.3
18:0	1.7	2.5	2.4
20:0	0.5	0.7	0.5
16:1n-7	0.9	0.8	4.1
18:1n-7	2.9	1.6	2.6
18:1n-9	43.9	43.5	27.3
20:1n-9	3.5	2.6	5.3
20:1n-11	0.1	0.2	0.5
22:1n-11	0.9	1.1	3.7
18:2n-6	19.2	14.9	10.2
20:4n-6	0.11	0.09	0.38
18:3n-3	8.5	4.0	4.5
18:4n-3	0.4	0.4	1.6
20:4n-3	0.1	0.1	0.6
20:5n-3	2.3	1.1	7.0
22:5n-3	0.2	0.1	1.2
22:6n-3	2.2	1.9	6.3
SFA	12.7	26.1	20.6

MUFA	53.8	51.0	45.5
PUFA	33.5	23.0	33.9
n3/n6	0.72	0.50	2.00

Table 2. Mean (± SEM) values for routine and maximum heart rate (f_H, beats min^{-1}) and ventricular stroke volume (V_{SH}, ml), and the resulting increase in each variable during exercise, in seabass fed one of three diets where lipids were provided as canola oil (CO), palm oil (PO) or fish oil (FO).

	CO	PO	FO
routine f_H	44.0 ± 7.0	45.2 ± 2.4	48.6 ± 5.2
max f_H	95.1 ± 3.8	96. 7 ± 1.3	90.1 ± 2.3
increase in f_H	51.1 ± 5.9	51.5 ± 3.4	41.6 ± 5.5
routine V_{SH}	0.96 ± 0.20	0.78 ± 0.07	0.73 ± 0.18
max V_{SH}	1.28 ± 0.20	1.06 ± 0.08	0.98 ± 0.18
increase in V_{SH}	0.32 ± 0.05	0.28 ± 0.04	0.25 ± 0.05

Values are means, n = 6 in all cases. There were no significant differences between the groups.

Table 3. Fatty acid (FA) composition of polar and neutral lipids in ventricular muscle of seabass fed one of three diets where lipids were provided as canola oil (CO), palm oil (PO) or fish oil (FO).

	Polar FA			Neutral FA		
	CO	PO	FO	CO	PO	FO
14:0	< 0.5	< 0.5	< 0.5	1.0 ± 0.2[a]	1.2 ± 0.2 [a]	1.6 ±0.4 [a]
16:0	16.5±1.1[a]	19.0±1.5[a]	17.5±0.3[a]	11.3 ± 0.6[a]	16.1 ± 0.5[b]	11.2 ± 0.6[a]
18:0	5.5±0.9[a]	5.1±1.3[a]	7.6±0.4[a]	3.8 ± 0.4[a]	3.4 ± 0.2[a]	3.8 ± 0.5[a]
16:1n-7	0.3 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	2.4 ± 0.4[a]	2.6 ± 0.3[a]	3.9 ± 0.4[b]
18:1n-7	2.7±0.1[a]	2.0±0.1[b]	2.8±0.1[a]	2.7 ± 0.5[a]	2.5 ± 0.1[a]	3.2 ± 0.1[a]
18:1n-9	17.2±0.4[a]	15.5±0.8[b]	13.2±0.3[c]	27.6 ± 1.4[a,b]	31.7 ± 1.1[a]	24.1 ± 0.9[b]
20:1n-9	1.6±0.1[a]	1.6±0.1[a]	1.9±0.1[b]	0.8 ± 0.6[a]	1.2 ± 0.6[a]	0.6 ± 0.0[a]
22:1n-11	< 0.5	< 0.5	< 0.5	1.0 ± 0.2[a]	0.8 ± 0.3[a]	1.5 ± 0.4[a]
18:2n-6	9.4±0.4[a]	9.1±0.4[a]	4.2±0.1[b]	13.9 ± 0.7[a]	12.7 ± 1.0[a]	9.1 ± 0.6[b]
20:2n-6	0.8 ± 0.1[a]	0.7 ± 0.0[a]	0.6 ± 0.0[a]	0.9 ± 0.2[a]	0.7 ± 0.1[a]	0.9 ± 0.2[a]
20:4n-6	3.5±0.1[a]	3.5±0.2[a]	3.9±0.2[a]	1.3 ± 0.2[a]	0.9 ± 0.1[a]	1.1 ± 0.2[a]
18:3n-3	1.9±0.1[a]	1.1±0.1[b]	1.0±0.0[b]	3.9 ± 0.2[a]	2.5 ± 0.1[b]	2.7 ± 0.1[b]
18:4n-3	< 0.5	< 0.5	< 0.5	0.7 ± 0.1[a]	0.5 ± 0.1[b]	1.0 ± 0.1[a]
20:4n-3	< 0.5	< 0.5	< 0.5	0.3 ± 0.1[a]	0.2 ± 0.1[a]	0.6 ± 0.0[b]
20:5n-3	6.7±0.3[a]	8.0±0.6[b]	9.8±0.3[c]	4.8 ± 0.4[a]	3.8 ± 0.3[b]	6.3 ± 0.2[c]
22:5n-3	1.6±0.1[a]	1.7±0.2[a]	2.2±0.1[b]	1.1 ± 0.0[a,b]	0.7 ± 0.1[a]	1.4 ± 0.3[b]
22:6n-3	28.7±1.9[a]	28.5±2.3[a]	28.3±1.1[a]	12.9 ± 1.6[a,b]	9.7 ± 0.4[a]	13.3 ± 1.0[b]
SFA	23.0±1.7[a]	25.1±2.6[a]	26.6±0.5[a]	16.3 ± 0.7[a]	20.9 ± 0.7[b]	17.0 ± 1.9[a,b]
MUFA	22.7±0.4[a]	20.6±1.0[a]	20.7±0.6[a]	34.8 ± 2.4[a,b]	39.0 ± 1.3[a]	33.8 ± 1.0[b]
PUFA	54.2±1.9[a]	54.3±3.4[a]	52.7±1.0[a]	40.1 ± 2.3a	31.9 ± 1.4[b]	37.0 ± 1.8[a,b]

n-3/n-6	3.0 ± 0.3^{a}	3.0 ± 0.3^{a}	4.8 ± 0.2^{b}	1.5 ± 0.1^{a}	1.3 ± 0.1^{a}	2.3 ± 0.1^{b}

Values = mean ± SEM, n = 7 in all cases. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids, n-3/n-6, ratio of total n-3 to total n-6 PUFA. Dissimilar letters indicate significant differences ($P<0.05$) between dietary treatments for that fatty acid fraction (polar or neutral).

Table 4. Fatty acid (FA) composition of polar and neutral lipids in white skeletal muscle of seabass fed one of three diets where lipids were provided as canola oil (CO), palm oil (PO) or fish oil (FO).

	Polar FA			**Neutral FA**		
	CO	PO	FO	CO	PO	FO
14:0	< 0.5	< 0.5	< 0.5	2.0 ± 0.1[a]	1.2 ± 0.5[a]	3.3 ± 0.3[b]
16:0	16.7 ± 0.5[a]	24.3 ± 1.2[b]	19.0 ± 1.2[a]	14.2 ± 1.4[a]	19.1 ± 2.5[b]	16.3 ± 0.8[a]
18:0	6.1 ± 0.6[a]	5.4 ± 0.9[a]	6.3 ± 0.6[a]	2.7 ± 0.5[a]	3.2 ± 0.6[b]	3.1 ± 0.1[b]
16:1n-7	0.4 ± 0.1[a]	0.5 ± 0.1[a]	0.7 ± 0.1[a]	3.3 ± 0.2[a]	3.0 ± 0.6[a]	5.4 ± 0.3[b]
18:1n-7	1.5 ± 0.1[a]	1.1 ± 0.1[b]	1.6 ± 0.1[a]	3.0 ± 0.1[a]	2.3 ± 0.4[b]	3.2 ± 0.1[a]
18:1n-9	20.3 ± 0.4[a]	16.9 ± 1.8[a]	14.0 ± 0.4[b]	29.3 ± 3.6[a]	27.1 ± 5.3[a]	19.9 ± 4.0[b]
20:1n-9	1.0 ± 0.0[a,b]	0.9 ± 0.1[b]	1.1 ± 0.1[a]	0.9 ± 0.5[a]	1.1 ± 0.7[a]	0.3 ± 0.1[a]
20:1n-11	< 0.5	< 0.5	< 0.5	0.9 ± 0.4[a]	0.7 ± 0.4[a]	1.1 ± 0.7[a]
18:2n-6	10.9 ± 0.3[a]	11.5 ± 0.5[a]	4.1 ± 0.1[b]	12.9 ± 0.6[a]	12.0 ± 0.6[a]	8.4 ± 0.4[b]
20:2n-6	< 0.5	< 0.5	< 0.5	0.6 ± 0.0[a]	0.5 ± 0.1[a]	0.6 ± 0.0[a]
20:4n-6	1.7 ± 0.0[a]	1.6 ± 0.1[a]	2.2 ± 0.0[b]	0.3 ± 0.0[a]	0.3 ± 0.0[a]	0.4 ± 0.0[a]
22:4n-6	0.5 ± 0.0[a]	0.5 ± 0.0[a]	0.6 ± 0.0[a]	< 0.5	< 0.5	< 0.5
18:3n-3	2.6 ± 0.1[a]	1.6 ± 0.2[b]	1.0 ± 0.0[c]	4.7 ± 0.1[a]	3.0 ± 0.2[b]	3.2 ± 0.2[b]
18:4n-3	< 0.5	< 0.5	< 0.5	0.7 ± 0.1[a]	0.8 ± 0.1[a]	1.4 ± 0.1[b]
20:4n-3	< 0.5	< 0.5	< 0.5	0.3 ± 0.0[a]	0.3 ± 0.1[a]	0.7 ± 0.0[b]
20:5n-3	6.4 ± 0.2[a]	6.3 ± 0.5[a]	10.4 ± 0.2[b]	3.8 ± 0.3[a]	3.8 ± 0.4[a]	6.5 ± 0.5[b]
22:5n-3	1.1 ± 0.0[a]	1.0 ± 0.1[a]	1.9 ± 0.2[b]	0.9 ± 0.1[a]	0.9 ± 0.0[a]	1.4 ± 0.3[a]
22:6n-3	26.7 ± 1.3[a]	23.3 ± 2.6[a]	32.5 ± 1.2[b]	7.3 ± 0.6[a]	8.0 ± 0.4[a]	9.8 ± 0.9[a]
SFA	23.8 ± 0.8[a]	30.7 ± 1.6[b]	26.5 ± 1.3[a]	19.9 ± 1.8[a]	24.2 ± 3.5[a,b]	23.6 ± 1.1[b]
MUFA	24.4 ± 0.5[a]	21.9 ± 1.6[a]	18.9 ± 0.4[b]	37.3 ± 3.8[a]	33.7 ± 4.4[a]	30.8 ± 3.3[b]

PUFA	51.8 ± 1.1[a,b]	47.4 ± 2.8[b]	54.6 ± 1.6[a]	32.3 ± 1.5[a]	31.6 ± 1.9[a]	33.7 ± 1.9[a]
n-3/n-6	2.8 ± 0.1[a]	2.4 ± 0.3[a]	6.4 ± 0.2[b]	1.3 ± 0.1[a]	1.4 ± 0.1[a]	2.4± 0.1[b]

Values = mean ± SEM, n = 7 in all cases. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids, n-3/n-6, ratio of total n-3 to total n-6 PUFA. Dissimilar letters indicate significant differences ($P<0.05$) between dietary treatments for that fatty acid fraction (polar or neutral).

Table 5. Significant Pearson correlations (i.e. coefficient greater than 0.5 derived from the principal components analysis) between traits of growth, performance or metabolism and the percentage content of polar fatty acids in ventricular muscle.

	SGR	CF	U_{crit}	max-Q	inc-Q	IMR	AMR	AS
16:0	-	-	-	-	-	0.73	-	-
18:1n-9	-	-	0.71	0.53	0.61	-	0.67	0.62
18:2n-6	-0.56	-	0.66	0.60	0.67	-	0.67	0.70
20:5n-3	-	0.51	-0.69	-0.52	-	-	-0.70	-0.64
22:5n-3	-	-	-0.59	-	-0.55	-	-0.59	-0.52
22:6n-3	-	-	-	-	-	-0.57	-	-
SFA	-	-	-	-	-	0.78	-	-
MUFA	-	-	0.60	-	-	-	0.55	-
PUFA	-	-	-	-	-	-0.73	-	-
n-3/n-6	0.63	-	-0.69	-0.59	-0.66	-	-0.68	-0.66

SGR, specific growth rate; CF, condition factor; U_{crit}, critical swimming speed; max-Q, maximum cardiac output; inc-Q, increase in Q during exercise; IMR, immobile metabolic rate; AMR, active metabolic rate; AS, aerobic scope; SFA, saturated FA; MUFA, monounsaturated FA; PUFA, polyunsaturated FA; n-3/n-6, ratio of total n-3 to n-6 PUFA.

Table 6. Significant Pearson correlations (i.e. coefficient greater than 0.5 derived from the principal components analysis) between traits of growth, performance or metabolism and the percentage content of neutral fatty acids in ventricular muscle.

	SGR	CF	U_{crit}	r-V_{SH}	max-Q	inc-Q	AMR	AS
16:0	-0.66	-	-	-	-	-	-	-
18:2n-6	-	-	0.52	-	-	0.58	0.71	0.76
20:4n-6	-	-	-	0.55	-	-	-	-
20:4n-3	0.53	0.52	-0.61	-	-0.58	-0.60	-0.62	-0.63
22:6n-3	-	-	-	0.58	-	-	-	-
SFA	-0.56	-	-	-	-	-	-	-
PUFA	-	-	-	0.54	-	-	-	-
n-3/n-6	-	-	-0.62	-		-0.61	-0.66	-0.70

SGR, specific growth rate; CF, condition factor; U_{crit}, critical swimming speed; r-V_{SH}, routine ventricular stroke volume; max-Q, maximum cardiac output; inc-Q, increase in Q during exercise; AMR, active metabolic rate; AS, aerobic scope; SFA, saturated FA; PUFA, polyunsaturated FA; n-3/n-6, ratio of total n-3 to n-6 PUFA.

Figure legends

Figure 1. . Effects of diet on mean (± SEM) critical swimming speed *(U_{crit})* in the three groups of seabass fed diets where lipids were provided as either canola oil (CO, green column), palm oil (PO, red column) or fish oil (FO, blue column). N = 6 in all cases, a dissimilar letter indicates a significant difference (P<0.05) between the dietary treatments.

Figure 2. Effects of diet on mean (± SEM) routine cardiac output, maximum cardiac output, and the resultant increase in cardiac output during exercise, in the three groups of seabass fed diets where lipids were provided as either canola oil (CO, green column), palm oil (PO, red column) or fish oil (FO, blue column). N = 6 in all cases, a dissimilar letter indicates a significant difference (P<0.05) between the dietary treatments. *Q*, cardiac output.

Figure 3. Effects of diet on mean (± SEM) immobile metabolic rate, active metabolic rate, and aerobic scope, in the three groups of seabass fed diets where lipids were provided as either canola oil (CO, green column), palm oil (PO, red column) or fish oil (FO, blue column). N = 6 in all cases, a dissimilar letter indicates a significant difference (P<0.05) between the dietary treatments. MR, metabolic rate.

Figure 4. Projections of individuals onto the plane of three main functions of a principal components analysis of individual data for ventricular muscle neutral fatty acids (FA), polar FA, and the measured traits of growth, metabolism and performance, in the three groups of seabass fed diets where lipids were provided as either canola oil (green symbols, N = 5), palm oil (red symbols, N = 5) or fish oil (blue symbols, N = 6). The upper panel shows projections for the first and second axes, which describe 36% and 20% of the variation, respectively. The lower panel shows the same first axis plotted against the third, which describes 15% of the variation.

Figure 1.

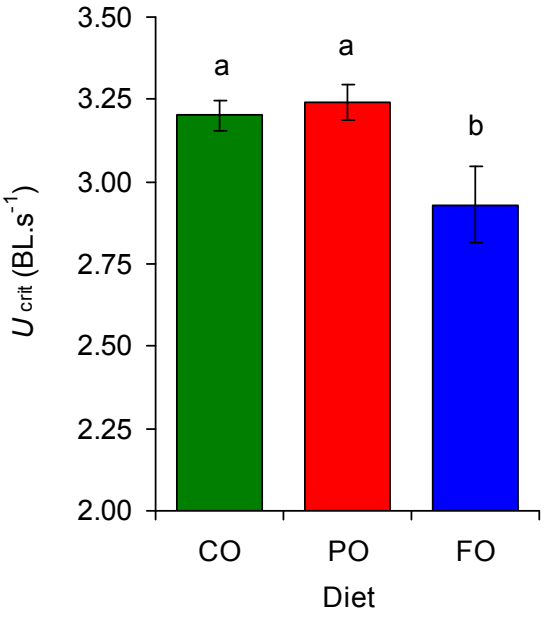


Figure 2.

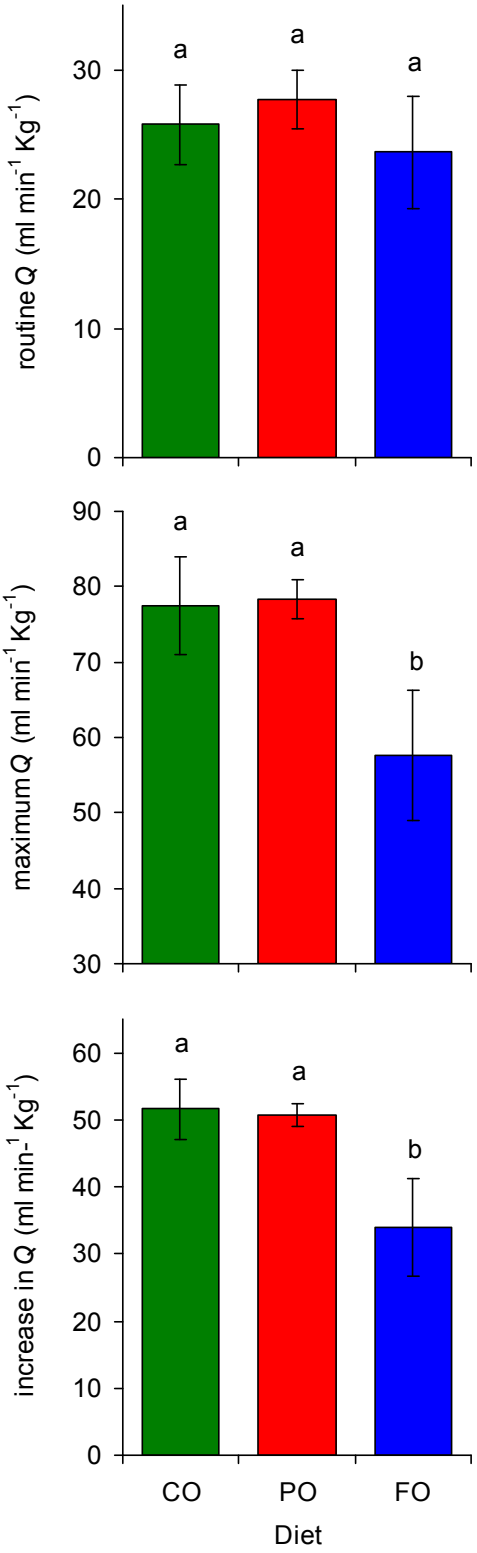

a
a
a
30
20
10
0
routine Q (ml min-1 Kg-1)
a
a
b
90
80
70
60
50
40
30
maximum Q (ml min-1 Kg-1)
a
a
b
60
50
40
30
20
10
0
increase in Q (ml min-1 Kg-1)
CO
PO
FO
Diet

Figure 3

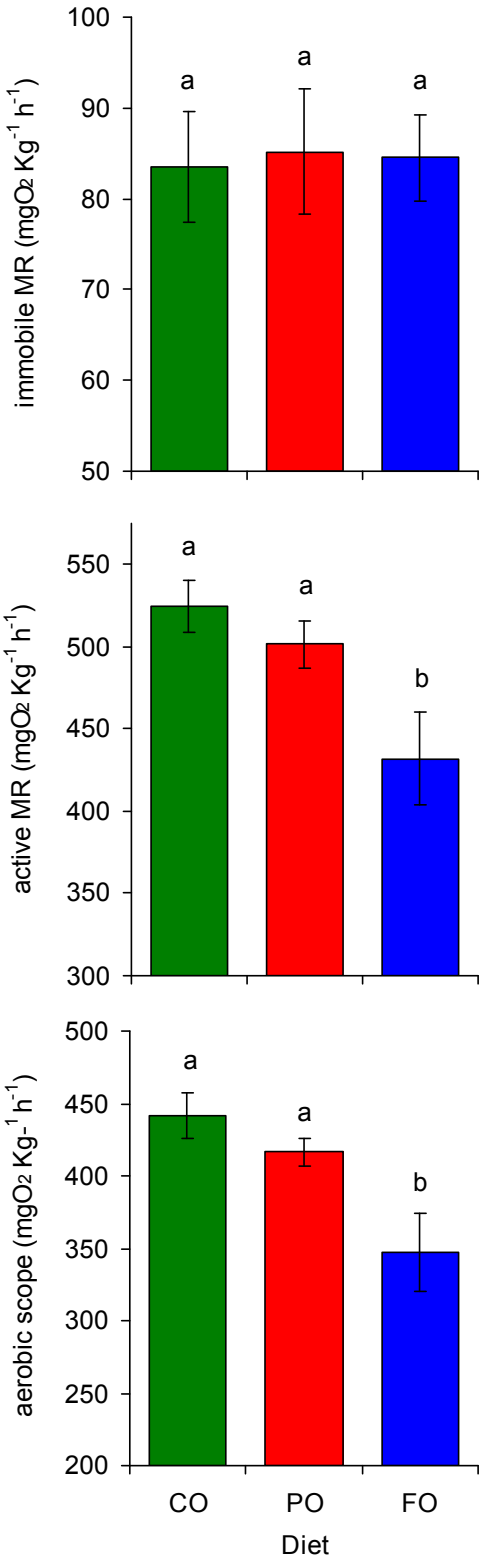
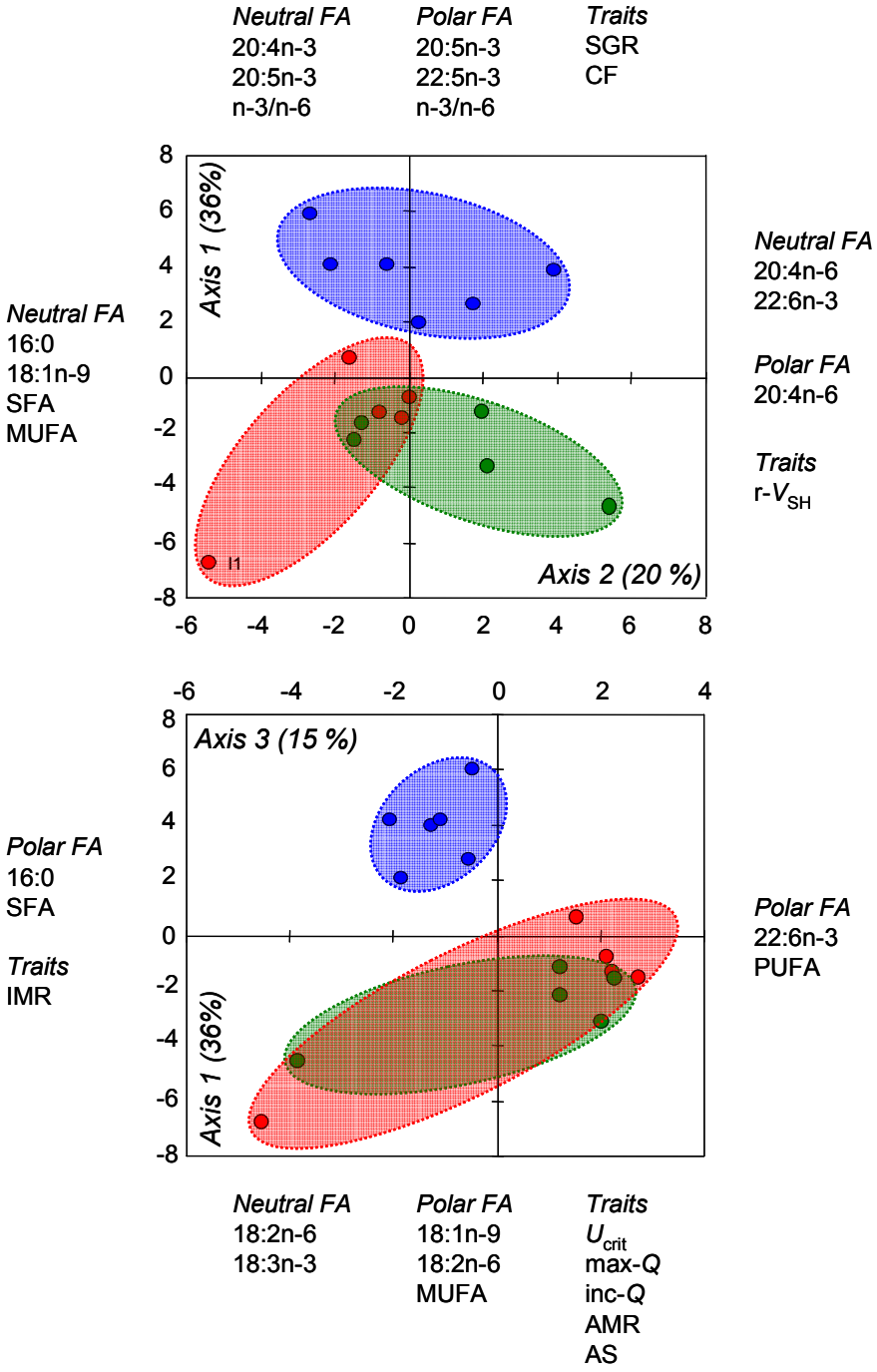

immobile MR (mgO2 Kg-1 h-1)
100
90
80
70
60
50
a
a
a
active MR (mgO2 Kg-1 h-1)
550
500
450
400
350
300
a
a
b
aerobic scope (mgO2 Kg-1 h-1)
500
450
400
350
300
250
200
a
a
b
CO
PO
FO
Diet

Figure 4.


Neutral FA
20:4n-3
20:5n-3
n-3/n-6
Polar FA
20:5n-3
22:5n-3
n-3/n-6
Traits
SGR
CF
Axis 1 (36%)
Axis 2 (20 %)
Neutral FA
16:0
18:1n-9
SFA
MUFA
Neutral FA
20:4n-6
22:6n-3
Polar FA
20:4n-6
Traits
r-V_{SH}
Axis 3 (15 %)
Axis 1 (36%)
Polar FA
16:0
SFA
Traits
IMR
Polar FA
22:6n-3
PUFA
Neutral FA
18:2n-6
18:3n-3
Polar FA
18:1n-9
18:2n-6
MUFA
Traits
U_{crit}
max-Q
inc-Q
AMR
AS