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

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## Summary

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 $Q$, the increase in 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.

Key words: 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; Webb, 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 (FAs) (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. (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; McKenzie, 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; Farrell, 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; McKenzie, 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 and Pawson, 1994). Young stages are subject to predation by pelagic fishes and several species of bird (Pickett and 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; McKenzie, 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 that 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* L.) with a mean mass (±s.d.) 200±10 g and length 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 m³ fibreglass tanks (water volume approximately 400 liters) 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 1 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 2

Table 1. *Fatty acid composition of lipids in the diets as a percentage of total fatty acids*

| 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 |

FA, 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.

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:

$$\text{Daily growth} = \{[(M_f - M_i)/M_i] \times 100\} / n \,,$$

$$\text{Condition factor} = (M_f/BL^3) \times 100 \,,$$

where $M_f$ is final mass in kg, $M_i$ is initial mass in kg, $n$ is the number of feeding days and $BL$ is body length in cm.

### Surgical preparation

At this stage of the experiment, bass with a mean mass and fork length $FL$ of 395±10 g and 30.58±0.28 cm, respectively, were anaesthetised with tricaine methane sulphonate (MS-222) at a concentration of 0.1 g l$^{-1}$, and transferred to an operating table where their gills were irrigated with aerated water containing 0.05 g 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 (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 using 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 h (overnight) at a current speed of 0.5 $BL$ 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 ($\dot{M}_{O_2}$, in mg kg$^{-1}$ h$^{-1}$) were collected automatically at each swimming speed with the custom-designed data-acquisition system described elsewhere (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 $\dot{M}_{O_2}$ during exercise were used to derive the notional metabolic rate of the immobile fish (IMR) (McKenzie et al., 2003b). The maximum metabolic rate of activity (AMR) was identified during swimming (this occurred at speeds approaching critical swimming speed $U_{crit}$) and used to calculate net aerobic scope (AS) relative to IMR (McKenzie et al., 2003b). $U_{crit}$ was calculated in $BL$ s$^{-1}$ (Brett, 1964).

At each swimming speed, cardiac output ($\dot{Q}$) 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 elsewhere (Axelsson et al., 2002). The signal was used to calculate heart rate $f_H$ in beats min$^{-1}$ and, together with the data for $\dot{Q}$, used to calculate cardiac stroke volume $V_{SH}$, in ml beat$^{-1}$ kg$^{-1}$. Cardiac scope during exercise was calculated as $\dot{Q}_{max} - \dot{Q}_{routine}$. $\dot{Q}_{max}$ always occurred at swimming speeds approaching $U_{crit}$, $\dot{Q}_{routine}$ was taken as the lowest $\dot{Q}$ measured when the fish was swimming very gently at a speed of 5 cm s$^{-1}$, prior to the exercise protocol. $f_{H,routine}$ and $V_{SH,routine}$ were derived from the measures of $\dot{Q}_{routine}$. Increase in $f_H$ and $V_{SH}$ during exercise was calculated, respectively, as $f_{H,max} - f_{H,routine}$ and $V_{SH,max} - V_{SH,routine}$.

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 acid analysis.

### Tissue fatty acid analysis
#### Total lipid extraction and measurement

Lipids were extracted from tissues following a procedure derived from that of Folch et al. (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 (Whatman, Brentford, UK) 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 Teflon-capped glass tubes at –20°C until analysed.

Total lipids were measured with a Chromarod SIII–Iatroscan TH-10 system (TLC-FID, Iatron Laboratories, Tokyo, Japan) connected to a CR3A integrator (Shimadzu, Kyoto, Japan). 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-Aldrich, St Louis, MO, USA) was used to calculate the total lipid concentration of the lipid extracts (Parrish and Ackman, 1985).

### Separation of polar and neutral lipids

Total lipids were separated into polar and neutral fractions by high performance liquid chromatography (HPLC) (Kontron Analytical, Zürich, Switzerland) coupled with an ELSD-500 detector (Alltech, Deerfield, IL, USA). The separation was carried out on a preparative 50 mm ×10 mm i.d. stainless steel column filled with an SPE Si-diol phase (IST, Mid-Glamorgan, UK). 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 fatty acid methyl ester (FAME) analyses, while the detector measured 10%.

### FAME preparation, purification and measurement

FAME from the separated lipid fractions were obtained after a base-catalysed transesterification with sodium methylate (0.5 mol l$^{-1}$, 1 h, 80°C) (Christie, 1984).

FAME were purified by HPLC, using a preparative 100 mm×10 mm i.d. 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 BPX-70 highly polar capillary column 30 m×0.25 mm i.d.×0.25 μm film (SGE, Courtaboeuf, France), 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-Aldrich), standard mixtures (Supelco, Bellemonte, PA, USA) 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 specific growth rates (± s.e.m.) were 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, namely 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 (± s.e.m.) $U_{crit}$ 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 $\dot{Q}_{routine}$ 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, $\dot{Q}_{max}$ was significantly higher in CO and PO fish than in those from the FO group. As a consequence, the increase in $\dot{Q}$ during exercise was also significantly higher for CO and PO fish (Fig. 2). In all fishes, the increased $\dot{Q}$ during
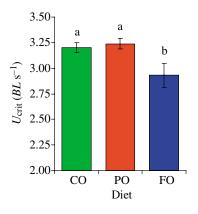


Fig. 1. Effects of diet on critical swimming speed ($U_{crit}$) in the three groups of seabass fed diets where lipids were provided as either canola oil (CO, green column), palm oil (PO, red column) or fish oil (FO, blue column). Values are means ± s.e.m.; $N$=6 in all cases. A dissimilar letter indicates a significant difference ($P<0.05$) between dietary treatments.
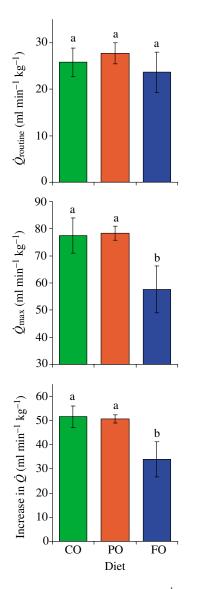
exercise was primarily a consequence of increased $f_H$, with a smaller contribution from increases in $V_{SH}$ (Table 2), but there were no differences in the maxima for these latter two variables amongst the three diets (Table 2).

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 $\dot{M}_{O_2}$ 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
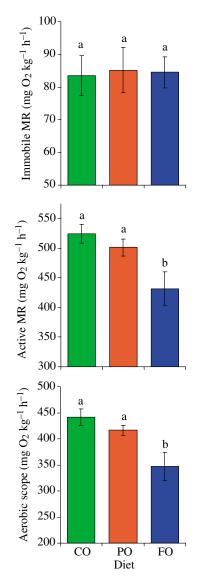


Fig. 2. Effects of diet on routine cardiac output $\dot{Q}$, maximum cardiac output $\dot{Q}_{max}$, and the resultant increase in cardiac output during exercise $\dot{Q}_{routine}$, in the three groups of seabass fed diets where lipids were provided as either canola oil (CO, green column), palm oil (PO, red column) or fish oil (FO, blue column). Values are means ± s.e.m.; $N$=6 in all cases. A dissimilar letter indicates a significant difference ($P<0.05$) between the dietary treatments.

Fig. 3. Effects of diet on 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). Values are means ± s.e.m.; $N$=6 in all cases. A dissimilar letter indicates a significant difference ($P<0.05$) between the dietary treatments. MR, metabolic rate.

Table 2. *Routine and maximum heart rate and ventricular stroke volume, and the resulting increase in each variable during exercise, in seabass fed one of three diets where lipids were provided as canola oil, palm oil or fish oil*

| Variable | CO | PO | FO |
|---|---|---|---|
| $f_{H,routine,}$ | 44.0±7.0 | 45.2±2.4 | 48.6±5.2 |
| $f_{H,max}$ | 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 |
| $V_{SH,routine}$ | 0.96±0.20 | 0.78±0.07 | 0.73±0.18 |
| $V_{SH,max}$ | 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 |

$f_H$, heart rate (beats min$^{-1}$); $V_{SH}$, ventricular stroke volume (ml); CO, canola oil; PO, palm oil; FO, fish oil.
Values are means ± s.e.m. ($N$=6 in all cases). There were no significant differences between the groups.

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

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

| | 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±2.3[a] | 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 are means ± s.e.m., $N$=7 in all cases.
CO, canola oil; PO, palm oil; FO, fish oil; FA, fatty acids; 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 composition of polar and neutral lipids in white skeletal muscle of seabass fed one of three diets where lipids were provided as canola oil, palm oil or fish oil*

| | 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 are means ± s.e.m., *N*=7 in all cases.

CO, canola oil; PO, palm oil; FO, fish oil; FA, fatty acids; 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).
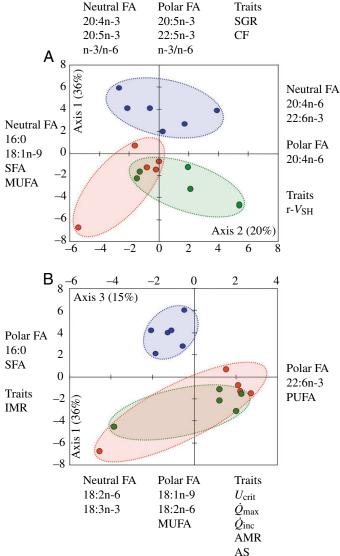
PO fish localised close together and apart from the FO fish (Fig. 4). In particular, on axis 1, fishes from the CO and PO groups with elevated levels of OA, LA and total MUFA in their lipids had high $U_{crit}$, $\dot{Q}_{max}$, scope to increase $\dot{Q}$ during exercise, AMR, and AS (Fig. 4). On the 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 arachidonic acid (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 ($U_{crit}$, $\dot{Q}_{max}$, scope to increase $\dot{Q}$ during exercise, AMR and AS) 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

Fig. 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, $N=6$). (A) Projections for the first and second axes, which describe 36% and 20% of the variation, respectively. (B) The same first axis plotted against the third, which describes 15% of the variation.

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; McKenzie et al., 1997; McKenzie et al., 1998; McKenzie et al., 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 $\dot{Q}_{max}$ are directly linked to parallel diversities in AMR, AS 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 $\dot{Q}$ and $\dot{M}_{O_2}$ 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 $\dot{Q}$ and $\dot{M}_{O_2}$ 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

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 | $U_{crit}$ | $\dot{Q}_{max}$ | $\dot{Q}_{inc}$ | 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; $\dot{Q}_{max}$, maximum cardiac output; $\dot{Q}_{inc}$, increase in $\dot{Q}$ 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.

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 (A. Chatelier, N. Imbert, J. L. Zambonino Infante, D. J. McKenzie and P. Bois, manuscript submitted for publication). 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 (McKenzie et al., 1998) on Atlantic salmon. These results are, however, in direct contrast to those reported by Wagner et al., who found improved exercise performance in salmon fed a diet rich in n-3HUFA (Wagner et al., 2004). 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 a 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.

Table 6. *Significant Pearson correlations between traits of growth, performance or metabolism and the percentage content of neutral fatty acids in ventricular muscle*

| | SGR | CF | $U_{crit}$ | $V_{SH,routine}$ | $\dot{Q}_{max}$ | $\dot{Q}_{inc}$ | 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 |

Significant Pearson correlations are coefficients greater than 0.5 derived from the principal components analysis.

SGR, specific growth rate; CF, condition factor; $U_{crit}$, critical swimming speed; r-$V_{SH,routine}$, routine ventricular stroke volume; $\dot{Q}_{max}$, maximum cardiac output; $\dot{Q}_{inc}$, increase in $\dot{Q}$ 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.

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; McKenzie et al., 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). It has been suggested 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 (Hulbert and Else, 1999; Hulbert et al., 2005). 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 exactly opposite to the effects on metabolic rate reported here and previously (McKenzie et al., 1997; McKenzie et al., 2000). On the other hand, 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) (Pepe and MacLennan, 2002), which was attributed 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. found, when working spontaneously *in vitro*, that 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 (Agnisola et al., 1996).

In the present study at 20°C, seabass exhibited a much greater AMR, aerobic scope, $\dot{Q}_{max}$ and $U_{crit}$ than the seabass at 15°C studied by Chatelier et al. (Chatelier et al., 2005). This demonstrates that temperature exerts a profound effect upon the cardiorespiratory performance of seabass. In both the present study and that of Chatelier et al. (Chatelier et al., 2005), the increase in $\dot{Q}$ during exercise was attributable to an increase in $f_H$ rather than $V_{SH}$. Rapid changes in water salinity had no effect on cardiac or swimming performance (Chatelier et al., 2005). 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.

### List of symbols and abbreviations

| | |
|---|---|
| AMR | active metabolic rate |
| AS | aerobic scope |
| *BL* | body length |
| CF | condition factor |
| CO | canola oil |
| DHA | docosahexaenoic acid |
| EPA | eicosapentaenoic acid |
| FA | fatty acid |
| FAME | fatty acid methyl ester |
| $f_H$ | heart rate |
| *FL* | fork length |
| FO | fish oil |
| HPLC | high performance liquid chromatography |
| HUFA | highly unsaturated fatty acids |
| IMR | metabolic rate of the immobile fish |
| LA | linoleic acid |
| *M* | mass |
| $\dot{M}_{O_2}$ | rate of oxygen uptake |
| MUFA | monounsaturated fatty acids |
| n-3HUFA | highly unsaturated fatty acids of the n-3 series |
| OA | oleic acid |
| PCA | principal component analysis |
| PIT | passive integrated transponder |
| PO | palm oil |
| PUFA | polyunsaturated fatty acids |
| $\dot{Q}$ | cardiac output |
| SFA | saturated fatty acids |
| SGR | specific growth rate |
| SW | seawater |
| $U_{crit}$ | critical swimming speed |
| $V_{SH}$ | cardiac stroke volume |

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