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Oxygen consumption and ventilatory reflex responses are influenced by dietary lipids in sturgeon

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Abstract

The effects of one year's feeding with diets enriched either in highly unsaturated fatty acids of the ω3 series (ω3 HUFA) (fish oil-supplemented diet, FOD) or in saturated fatty acids (SFA) (coconut oil-supplemented diet, COD) on fatty acid composition of tissue lipids, on patterns of resting oxygen consumption and on responses to hypoxia and hypercapnia were investigated in the Adriatic sturgeon (*Acipenser naccarii*). FOD sturgeon had higher levels of ω3 HUFA in liver and muscle lipids than COD fish, which had higher levels of SFA. A frequency distribution of instantaneous oxygen uptake rates ($\dot{M}_{O_2}$), as measured every 10 min for 8 h, revealed a different pattern of resting oxygen consumption between the two groups. The FOD sturgeon consumed oxygen in a narrow range of low rates, with a lower mean $\dot{M}_{O_2}$ than COD sturgeon, which showed a wide range of more elevated rates. FOD sturgeon had a lower opercular pressure amplitude than COD fish in normoxia.

Exposure to three levels of hypoxia (P_{O_2}s = 10.8±0.2; 6.6±0.2 and 4.6±0.2 kPa) or mild hypercapnia (P_{CO_2} = 1.0±0.2 kPa) did not affect ventilation in FOD fish but elicited hyperventilation in COD animals. Mild hypoxia (P_{O_2} = 10.8±0.2 kPa) and hypercapnia caused less reduction in blood oxygen content in FOD as compared with COD sturgeon. The effects of adding vitamin E supplements to the diets was investigated; groups fed vitamin E supplements had elevated $\dot{M}_{O_2}$ and hyperventilated in hypoxia.

The data indicate that dietary fatty acid composition influences resting $\dot{M}_{O_2}$ in sturgeon and that this influences the regulation of ventilation and blood O_2 levels in hypoxia and hypercapnia. The low resting $\dot{M}_{O_2}$ of fish fed ω3 HUFA supplements (the FOD group) obviated the need for hyperventilation in hypoxia or hypercapnia, thereby making them less sensitive to these stresses than sturgeon fed SFA (COD group) or sturgeon fed either diet supplemented with vitamin E.

Introduction

Tissues of temperate water fish generally have an elevated content of highly unsaturated fatty acids of the ω3 series (ω3 HUFA), such as eicosapentaenoic acid (EPA; 20:5 ω3) and docosahexaenoic acid (DHA; 22:6 ω3), and of vitamin E (Watanabe 1981; Henderson and Tocher 1987). These lipids are known to affect mammalian heart function and improve resistance to cardiac and cerebral ischaemia (Hock *et al.* 1990; Downey 1990; Charnock *et al.* 1992; Ellis *et al.* 1992; Paulson *et al.* 1992). An important element of tissue ischaemia is the associated hypoxia. Freshwater fish may often experience periods of hypoxia, as a result of the low solubility of O_2 in water, and there is recent evidence to suggest that part of their resistance to this stress may be related to the elevated levels of ω3 HUFA and vitamin E in their tissues (Randall *et al.* 1992; McKenzie *et al.* 1995a; Agnisola *et al.* 1996).

In fish, the fatty acid and vitamin E composition of tissue lipids generally reflects dietary fatty acid and vitamin E composition (Henderson and

Tocher 1987), and thus diet can be used as a means of manipulating the fatty acid and vitamin E profile of tissue lipids. Research designed to enrich farmed fish in ω3 HUFA and vitamin E, as healthy food for human consumption, stimulated studies on the effects of these compounds on responses to hypoxia in the Adriatic sturgeon (*Acipenser naccarii*). An initial study compared responses to progressive hypoxia of sturgeon fed either a normal commercial diet or the same diet supplemented with ω3 HUFA and vitamin E, and found that animals fed the standard diet exhibited significant hypoxaemia and acidaemia at levels of aquatic hypoxia that had no effect on blood O_2 content and pH in animals fed the supplemented diet (Randall *et al.* 1992).

Subsequent studies in the same species (McKenzie *et al.* 1995a; Agnisola *et al.* 1996) have compared the cardio-respiratory physiology of sturgeon fed a diet enriched either with ω3 HUFA or with a similar quantity of saturated fats (SFA). McKenzie *et al.* (1995a) found that sturgeon fed ω3 HUFA supplements had lower oxygen consumption in normoxia and were able to maintain whole animal oxygen uptake and spontaneous locomotor activity unchanged when exposed to hypoxia, unlike sturgeon fed SFA which exhibited a hypoxic depression of both of these variables. Agnisola *et al.* (1996) found that hearts isolated from the same group of sturgeon fed the ω3 HUFA-enriched diet exhibited lower instrinsic *in vitro* heart rates and power output than hearts from animals fed SFA, but had a greater scope for cardiac work and, unlike hearts from animals fed SFA, were able to maintain *in vitro* cardiac performance unchanged when perfusate oxygen levels were reduced.

McKenzie *et al.* (1995a) found that if vitamin E supplements were added to the diets enriched in ω3 HUFA or SFA, the sturgeon exhibited elevated oxygen consumption rates and responded to hypoxia in a manner similar to those fed SFA. On the other hand, Agnisola *et al.* (1996) found that vitamin E had little influence on the effects of dietary fatty acid composition on *in vitro* cardiac performance.

The presence study is a continuation of these investigations into the effects on sturgeon cardio-respiratory physiology of diets enriched either in ω3 HUFA or in SFA. The effects of these dietary lipids on resting oxygen consumption were assessed more thoroughly in sturgeon from the same experimental populations as those studied by McKenzie *et al.* (1995a) and Agnisola *et al.* (1996), by describing the frequency distribution of instantaneous oxygen uptake rates when measured every 10 min over an 8 h period. Another series of experiments determined whether the differences in oxygen consumption elicited by the experimental diets (ω3 HUFA *vs.* SFA supplements) influenced ventilatory responses and the regulation of blood gas and acid-base-related variables following exposure to hypoxia or hypercapnia. The effect of adding vitamin E to the diets was also studied.

Materials and methods

Animals

Immature Adriatic sturgeon (*Acipenser naccarii*, Bonaparte) of approximately 3 months of age and a mean (±SEM) weight of 0.198 ± 0.015 kg were maintained at the Experimental Thermal Aquaculture Plant “La Casella” [Via Argine del Ballottino, 29010, Sarmato (PC), Italy] in 8 groups of 20 animals, in indoor $4m^2$ fibreglass tanks (volume 2000 l) with a continuous supply of biofiltered water at 23 ± 1°C and pH 7.9.

Administration of fatty acids and vitamin E

For a period of 12 months, the 8 separate groups of sturgeon received one of 4 diets, each with a different fatty acid and vitamin E content, such that each diet was fed to two replicate groups of 20 animals. The proximate and fatty acid composition of the diets was as reported previously (McKenzie *et al.* 1995a). The fish oil diet (FOD) was composed of 850 g of a commercial feed (Agros Storioni 1ª Fase, Agros, Bolzano, Italy) + 150 g of menhaden (*Brevoortia tyrannus*) oil and was particularly rich in ω3 HUFA such as 20:5 ω3, 22:5 ω3 and 22:6 ω3. The coconut oil diet (COD) was composed of 850 g of the feed + 150 g of hydrogenated coconut oil and was rich in SFA such as 14:0 and 18:0, and poorer in ω3 HUFA. Thus, as reported by McKenzie *et al.*

(1995a), these diets had the following ω3 HUFA contents: FOD, 23.8 mg 20:5 ω3; 3.5 mg 22:5 ω3, and 15.4 mg 22:6 ω3 per g wet weight of pelleted diet; COD, 6.1 mg 20:5 ω3; 0.8 mg 22:5 ω3; 5.8 mg 22:6 ω3 per g wet weight of pelletted diet. The third diet (designated FOVD) was the fish oil diet + 500 mg of vitamin E (α-tocopherol acetate). The fourth diet (designated COVD) was the coconut oil diet + 500 mg of vitamin E.

The oils were obtained from ICN Biomedicals and the α-tocopherol acetate from Hoffman La-Roche. All diets were mixed with 30% (w/w) water and 10% (w/w) of a commercial binder (Integratore R, A.C.E.F., Fiorenzuola d'Arda (PC), Italy) and prepared as moist pellets at "La Casella", divided into daily aliquots of 3% of the total weight of each replicate in the dietary groups, and stored at –20°C until use.

At the end of the 12 month period, the sturgeon had grown to a mean (±SEM) weight of 1.63 ± 0.02 kg, with no significant differences in weight between the 4 dietary groups. Experimental animals were then chosen from each of these dietary groups. The same dietary groups have provided experimental subjects for previous studies (McKenzie *et al.* 1995a; Agnisola *et al.* 1996).

Analysis of fatty acid composition of tissue lipids

The fatty acid composition of the tissue lipids was measured on samples of muscle and liver from sturgeon from each dietary group, after feeding for a period of 12 months. Samples were collected from freshly sacrificed sturgeon, frozen on dry ice and stored at –20°C until analysis. Tissues were homogenised and total lipids were extracted with 20 ml g^{-1} of chloroform/methanol 2:1 (v/v), according to Folch *et al.* 1957, with 5 mg l^{-1} butylated hydroxytoluene as an anti-oxidant, and extracted lipids dissolved in a known volume of chloroform/methanol (2:1 v/v). Acidic transmethylation (3N HCl in methanol) was used to prepare methylesters from 400 μg of the lipid extracts. These were then analysed using a Dani 8510 gas chromatograph with a programmable temperature vapouriser injector and a column (Supelcowax 30 m, 0.30 mm diameter, 0.27 mm film thickness) with temperature programming (150–220°C at 2.5°min^{-1} increments). Fatty acids were quantified by reference to an internal standard, and the percentage composition in fatty acids determined. The percentage composition as SFA, mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) was also calculated, as was the unsaturation index. This latter value is the sum of the products: percentage content of fatty acid x number of double bonds, as calculated for each fatty acid in the mixture and describes the average number of double bonds (× 100) of the fatty acid mixture. Finally the ratio of the total percentage of ω6 fatty acids to the total percentage of ω3 fatty acids in the mixture (the ω6/ω3 ratio) was also calculated.

Resting oxygen consumption

For measurements of oxygen uptake ($\dot{M}_{O_2}$), the same system as described by McKenzie *et al.* (1995a) was used. Sturgeon were placed in a Plexiglass respirometer chamber with a volume of 22 l and allowed to recover for at least 10 h (overnight). The respirometer chamber was immersed in 25 cm of water in a 1 m^2 tank. The water in the large tank was recircled through a gas-exchange column counter-current to a flow of compressed air to maintain normoxia and through a biofilter, to remove wastes. The temperature of the water was maintained at 23 ± 1°C.

The respirometer chamber containing the fish was fitted with two entry and two exit ports. One exit was connected to a pump (Eheim 1048) that continuously recycled water back into one of the entry ports. The other entry port was connected to another pump (Eheim 1060) that flushed the chamber with water drawn from the outer tank. The flushing pump was connected, *via* an AD/DA interface (Data translation DT2801) to a computer (Zenith 433D+) containing the program Labtech Notebook. The activity of the flushing pump was controlled by Labtech Notebook such that it was active for 4 min in every 10 min. When it was not active, there was a decline in O_2 tension with the chamber, due to O_2 uptake by the sturgeon. The rate of decline in O_2 in the system was measured by a Radiometer O_2 electrode (model E5047) that received continuous water samples from the recycling pump circuit. This electrode was connected to a Radiometer PHM 73 blood

gas analyser which was in turn connected, via the interface board, to the computer. The weight of the fish and the volume of the recirculating chamber system were registered in the program and used by Labtech Notebook to calculate instantaneous O_2 uptake by the fish, as mg kg^{-1} h^{-1}. Thus, $\dot{M}_{O_2}$ was measured for 6 min in every 10 min, and then the flushing pump was activated in order to re-equilibrate the water O_2 partial pressure (Pw_{O_2}) in the chamber with that in the large outer tank. Labtech Notebook also performed a least-squares linear regression analysis of the decline in oxygen tension in the chamber during recirculation, and measures of $\dot{M}_{O_2}$ were considered reliable only if the regression coefficient (r^2) was over 0.90.

Oxygen consumption was measured in 7 sturgeon from each dietary group for 8h, with all measurements made between 04:00 and 12:00, to avoid any influence of circadian rhythms on $\dot{M}_{O_2}$. The computer controlling the measurements of $\dot{M}_{O_2}$ was in an adjacent room, in order to minimize disturbances to the fish.

Surgical preparation

Sturgeon were anaesthetised in a 1:10,000 w/v buffered solution of tricaine methane sulphonate (MS 222) and then transferred to a surgical table and artificially ventilated with an MS 222 solution at 1:20,000 w/v. A cannula (PE 50 Intramedic) was implanted, *via* the roof of the mouth, into the dorsal aorta. An opercular cannula was fitted using heat-flared cannula tubing (PE 190 Intramedic) passed through a small hole in the operculum and secured with a cuff and sutures. Care was taken to ensure that the opercular cannula was placed in the same relative position on each fish.

Following surgery, fish were transferred to individual darkened PVC chambers (volume 40 l) and allowed to recover for a minimum of 24 h with a continuous flow of water aerated by passing it through a gas-exchange column countercurrent to a flow of compressed air (normoxic Pw_{O_2} = 19.2 ± 0.5 kPa). The dorsal aortic cannula was flushed twice daily with heparinised (50 IU l^{-1}) saline.

Hypoxia and hypercapnia exposure

Experiments were performed on between 6 and 9 animals from each dietary group. Following 48 h recovery, sturgeon were exposed to hypoxia and hypercapnia, presented randomly. Measurements were made of the effects of these respiratory stresses on ventilation, blood gases and pH, and on intracellular pH of red blood cells using blood samples withdrawn from the arterial catheter. Effects of hypoxia on haematocrit and plasma lactate were also measured.

The water-filled opercular cannula was attached to a differential pressure transducer (Validyne 45DF), which was connected to a chart recorder (Gould Windograf) for display and recording of ventilatory activity as gill ventilation rate (f_G, beats min^{-1}) and opercular pressure amplitude (P_{OP}). Opercular pressure amplitude was used as an index of ventilatory effort (Smatresk 1990).

Arterial blood oxygen partial pressure (Pa_{O_2}) was measured with a Radiometer oxygen electrode, thermostatted to the same water temperature as the fish and attached to a Radiometer PHM73 acid-base analyser. Arterial blood total oxygen content (Ca_{O_2}) was measured with the technique described by Tucker (1967) and an Instrumentation Laboratories oxygen electrode and IL 1302 blood-gas analyser thermostatted to 37°C. Arterial blood pH (pHa) was measured with a Radiometer capillary pH electrode thermostatted to the same temperature as the fish, attached to a Radiometer PHM73 acid-base analyser. Arterial blood total carbon dioxide content (Ca_{CO_2}) was measured with the technique described by Cameron (1970) and an Instrumentation Laboratories carbon dioxide electrode and IL 1302 blood-gas analyser thermostatted to 37°C. Arterial plasma carbon dioxide partial pressure (Pa_{CO_2}) was calculated with the Henderson-Hasselbalch equation using the measured values of Ca_{CO_2} and pHa and apparent pK values for trout plasma taken from Boutilier *et al.* (1984).

Portions of whole blood were immediately centrifuged upon sampling and plasma decanted, weighed and frozen in liquid nitrogen for subsequent analyses of plasma lactate levels. The red cell pellet was also weighed and immediately frozen for subsequent analysis of red cell intracellular pH. Haematocrit was calculated from the

weights of the liquid and cellular portions of the sample, using previously determined measurements of density of each fraction to calculate their relative volumes. Plasma lactate was measured with a Sigma assay (Lactate Method 735). Red cell intracellular pH (pHi) was measured using the freeze-thaw method and a Radiometer capillary pH electrode thermostatted to the temperature of the fish and attached to a Radiometer PHM73 acid-base analyser.

To assess responses to hypoxia, normoxic resting ventilatory variables were recorded and a 1 ml blood sample was collected for measurement of control normoxic Pa_{O_2}, Ca_{O_2}, pHa, Ca_{CO_2}, Pa_{CO_2}, haematocrit and plasma lactate (blood was replaced with an equal volume of saline). Sturgeon were then sequentially exposed to three levels of hypoxia, designated mild, moderate and deep, with Pw_{O_2}'s of 10.8 ± 0.3 kPa, 6.6 ± 0.2 kPa, and 4.6 ± 0.2 kPa, respectively. Hypoxia was created by passing water counter-current to a flow of 100% N_2 through a gas-exchange column. Exposure lasted for 30 min in mild and moderate hypoxia and 20 min in deep hypoxia. The chosen levels of mild and moderate hypoxia were those used by McKenzie *et al.* (1995a) to study the effects of the same diets on spontaneous locomotor activity and oxygen uptake, respectively. At the end of each exposure period, measurements of ventilatory variables were made and a blood sample was taken for measurement of the abovementioned blood and plasma variables. Sturgeon were allowed at least 3h recovery from hypoxia before being exposed to any further stress.

To assess responses to mild hypercapnia, control measurements of the same variables as described above (except haematocrit and plasma lactate concentration) were made under normocapnic, resting conditions and then the sturgeon were exposed for 30 min to a flow of water equilibrated, in the gas-exchange column as described above, with 1% CO_2 in air (aquatic carbon dioxide partial pressure (Pw_{CO_2}) = 1.0 ± 0.1 kPa). At the end of the exposure period, measurements were made of the appropriate variables. Animals were allowed at least 1.5h recovery from hypercapnia.

Data analysis

Tissue fatty acid contents were compared between groups, for any particular fatty acid, by one-way ANOVA. A frequency distribution of oxygen uptake rates was described by combining all measurements of instantaneous oxygen uptake of the sturgeon from a particular dietary group (approximately 6 × 8 × 7 = 338 observations, with some measurements discarded as unreliable) and calculating the percentage of the observations that fell within given intervals of oxygen uptake: 40–60 mg kg^{-1} h^{-1}; 60–80; 80–100, and so on at intervals of 20 until 400 mg kg^{-1} h^{-1}. The mean oxygen uptake over the 8th measurement period was also calculated for each group and compared by one-way ANOVA.

To quantify ventilation, rate was counted for 2 min within each sampling period, and P_{OP} averaged from 10 measurements of individual waveforms within that period. The effects of hypoxia on measured variables within each dietary group were analysed by one-way ANOVA for repeated samples. An ANOVA for repeated samples was also performed on all of the combined data from all of the groups, to describe the general effects of hypoxia on the sturgeon. The effects of hypercapnia on measured variables within each dietary group were analysed by paired t-test, and a paired t-test was performed on all of the combined data from all groups, to describe the general effects of hypercapnia on the sturgeon. Comparisons between groups at any measurement interval were made by one-way ANOVA, with Tukey post-hoc test to determine where any differences lay. In those cases that responses were analyzed as % change from the control value, data were arc-sine transformed before analysis by ANOVA or T-test (as appropriate). Significance was attributed at the 95% level of confidence (p 0.05).

Results

Fatty acid composition of tissue lipids

FOD sturgeon had significantly higher total lipid content in their liver than the COD and FOVD groups, but similar levels to the COVD (Table 1). Fatty acid composition of lipids in the liver was

Table 1. Total lipid content in the tissues of sturgeon from the 4 dietary groups

	FOD	COD	FOVD	COVD
Liver	327 ± 16	217 ± 37*	260 ± 92*	312 ± 61
Muscle	78 ± 10	40 ± 16	54 ± 10	33 ± 4*

Values = mean ± SEM; units = mg g^{-1}; n = 5 in all cases; * = significantly different from FOD group ($p < 0.05$); FOD, fish oil diet; COD, coconut oil diet; FOVD, fish oil and vitamin E diet; COVD, coconut oil and vitamin E diet.

Table 2. Fatty acid composition of lipids in the liver of sturgeon from the 4 dietary groups. Fatty acids found at levels lower than 0.5% of total fatty acids are not reported

	FOD	COD	FOVD	COVD
14:0	5.7 ± 0.2	16.1 ± 8.9	7.0 ± 1.3	15.6 ± 10.2
16:0	22.5 ± 1.4	24.6 ± 2.3	22.8 ± 1.4	24.6 ± 2.2
18:0	3.2 ± 0.2	6.8 ± 1.3	3.6 ± 0.3	5.6 ± 1.9
16:1	9.1 ± 0.6	4.5 ± 0.9	9.3 ± 1.4	5.8 ± 2.1
18:1	23.4 ± 2.6	36.6 ± 13.0	22.3 ± 1.3	36.0 ± 11.4
20:1	3.2 ± 0.8	1.7 ± 0.6	2.5 ± 0.5	1.6 ± 0.5
18:2 ω6	6.6 ± 0.7	4.7 ± 2.1	7.2 ± 0.4	4.9 ± 2.0
20:4 ω6	0.9 ± 0.01	0.4 ± 0.2	1.0 ± 0.1	0.4 ± 0.1
18:3 ω3	1.2 ± 0.1	0.4 ± 0.2	1.3 ± 0.1	0.4 ± 0.2
18:4 ω3	1.9 ± 0.2	0.4 ± 0.2	1.9 ± 0.3	0.4 ± 0.1
20:5 ω3	8.4 ± 0.7	0.9 ± 0.6	7.7 ± 1.1	1.0 ± 0.4
22:5 ω3	3.4 ± 0.5	0.3 ± 0.2	3.6 ± 0.8	0.4 ± 0.2
22:6 ω3	9.0 ± 1.1	2.0 ± 1.4	8.4 ± 1.6	2.2 ± 1.1
SFA	31.5 ± 1.2	47.0 ± 8.1	33.4 ± 2.7	45.9 ± 10.0
MUFA	35.9 ± 2.0	42.9 ± 13.1	34.3 ± 1.5	43.5 ± 13.0
PUFA	32.6 ± 2.9	10.1 ± 5.5	32.3 ± 3.7	10.6 ± 4.3
U.I.	181.5 ± 11.7	77.9 ± 10.0	175.7 ± 18.0	80.9 ± 11.4
ω6/ω3	0.35 ± 0.01	1.46 ± 0.25	0.40 ± 0.05	1.31 ± 0.22

Values = mean ± SD, n = 5; FOD, fish oil diet; COD, coconut oil diet; FOVD, fish oil and vitamin E diet; COVD, coconut oil and vitamin E diet; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U.I., unsaturation index; ω6/ω3, ratio of ω6 to ω3 fatty acids.

significantly affected by dietary fatty acid composition (Table 2), with FOD and FOVD sturgeon exhibiting significantly higher levels of ω3 HUFA, such as eicosapentaenoate (20:5 ω3), docosapentaenoate (22:5 ω3), and docosahexaenoate (22:6 ω3) than the COD and COVD animals and, conversely, the COD and COVD groups exhibited significantly elevated levels of SFA such as myristate (14:0) and stearate (18:0) (Table 1). As a result of these differences in ω3 HUFA and SFA levels, the FOD and FOVD groups had a significantly higher unsaturation index and lower ω6/ω3 ratio (Table 2).

Table 3. Fatty acid composition of lipids in the muscle of sturgeon from the 4 dietary groups. Fatty acids found at levels lower than 0.5% of total fatty acids are not reported

	FOD	COD	FOVD	COVD
14:0	6.4 ± 0.6	17.5 ± 2.1	7.6 ± 1.6	12.7 ± 2.6
16:0	21.7 ± 1.2	21.0 ± 1.0	22.7 ± 1.7	20.8 ± 0.6
18:0	2.1 ± 0.4	5.2 ± 1.1	2.6 ± 0.5	5.8 ± 0.9
22:0	1.8 ± 0.4	0.8 ± 0.1	1.3 ± 0.3	0.7 ± 0.1
16:1	9.7 ± 0.7	4.6 ± 0.9	9.5 ± 1.3	4.2 ± 0.7
18:1	19.2 ± 1.2	22.8 ± 3.2	18.7 ± 1.2	24.4 ± 2.7
20:1	3.6 ± 0.4	2.8 ± 0.2	3.0 ± 0.4	3.0 ± 0.7
18:2 ω6	6.1 ± 0.2	7.1 ± 0.5	6.1 ± 0.1	7.6 ± 0.6
20:4 ω6	1.1 ± 0.1	1.4 ± 0.7	1.3 ± 0.2	1.6 ± 0.6
18:3 ω3	1.2 ± 0.1	0.8 ± 0.1	1.2 ± 0.1	0.7 ± 0.1
18:4 ω3	2.0 ± 0.3	0.5 ± 0.1	1.8 ± 0.2	0.4 ± 0.1
20:5 ω3	11.0 ± 0.8	4.5 ± 1.3	10.1 ± 1.4	4.8 ± 1.5
22:5 ω3	2.3 ± 0.2	1.0 ± 0.2	2.3 ± 0.4	1.2 ± 0.2
22:6 ω3	10.1 ± 0.8	8.2 ± 1.9	10.1 ± 1.9	10.1 ± 1.9
SFA	32.1 ± 1.3	44.5 ± 2.0	34.3 ± 2.6	40.0 ± 2.8
MUFA	33.0 ± 1.3	30.7 ± 3.8	31.8 ± 1.7	32.0 ± 3.5
PUFA	34.9 ± 2.1	24.9 ± 4.5	33.9 ± 3.8	28.0 ± 4.1
U.I.	192.4 ± 9.3	136.4 ± 19.0	186.6 ± 19.2	153.7 ± 19.5
ω6/ω3	0.30 ± 0.01	0.64 ± 0.07	0.32 ± 0.04	0.59 ± 0.08

Values = mean ± SD, n = 5; FOD, fish oil diet; COD, coconut oil diet; FOVD, fish oil and vitamin E diet; COVD, coconut oil and vitamin E diet; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U.I., unsaturation index; ω6/ω3, ratio of ω6 to ω3 fatty acids.

The muscle of sturgeon had a significantly lower total lipid content that the liver (Table 1), the muscle of FOD sturgeon had the highest total lipid content and this was significantly higher than the lipid content in the muscle of the COVD group (Table 1). As observed in the liver, fatty acid composition of muscle lipids tended to reflect the fatty acid composition of the diet (Table 3), but the differences between groups were less pronounced than those measured in the liver. In particular, although FOD and FOVD groups still had significantly higher 20:5 ω3 and 22:5 ω3 levels, 22:6 ω3 levels were similar amongst all groups. As measured in the liver, the COD and COVD groups had significantly higher 14:0 and 18:0 levels. Muscle of the fish oil groups had a significantly higher unsaturation index and lower ω6/ω3 ratio (Table 3). The COD and COVD groups had significantly higher levels of 20:4 ω6 (arachidonate); 20:5 ω3 and 22:6 ω3 in their muscle lipids when compared with their liver lipids (Table 3 cf. Table 2).

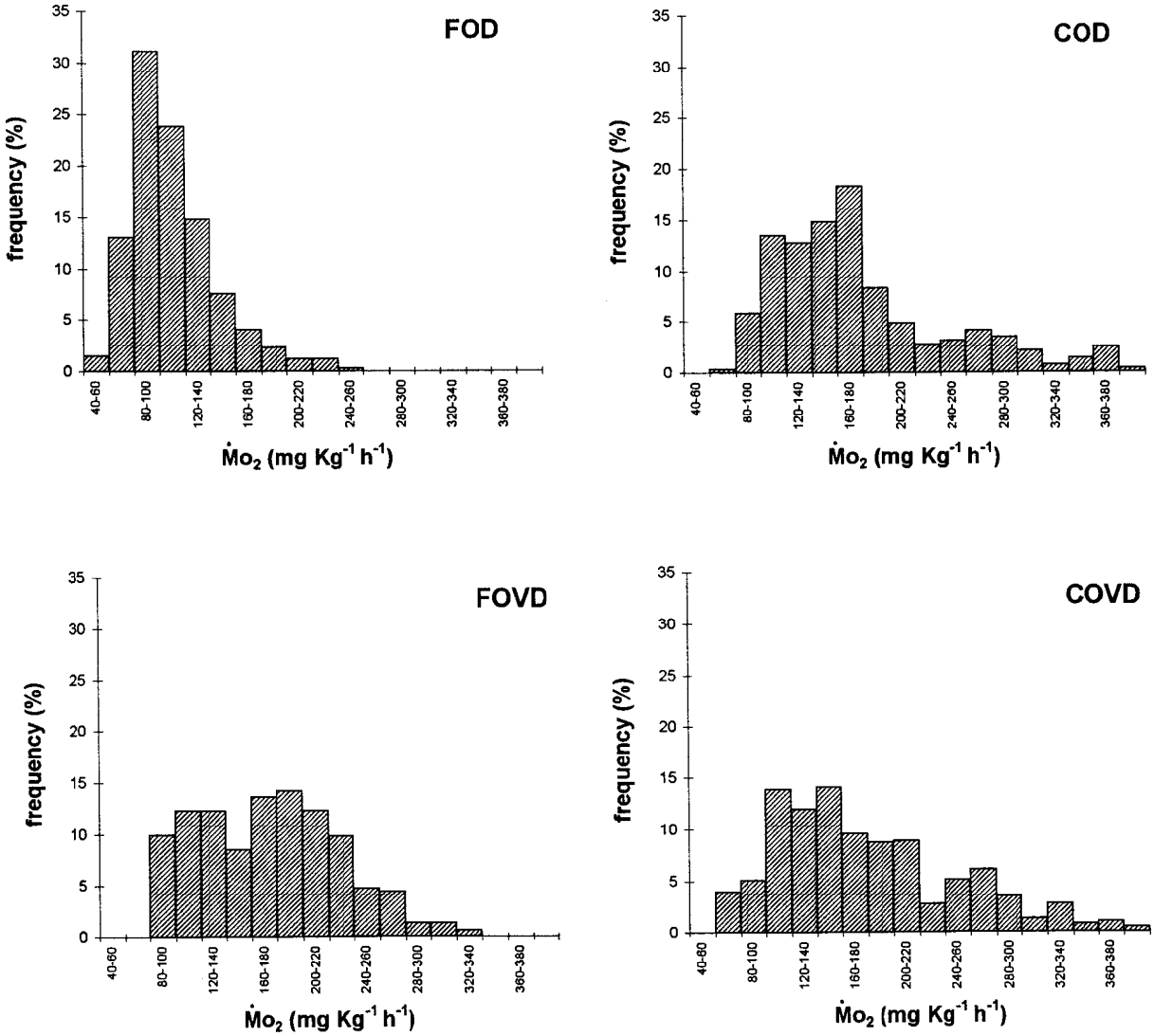

Fig. 1. Frequency distribution of rates of oxygen consumption as measured every 10 min for 8h in sturgeon fed four experimental diets. n = approx. 330 observations on 7 animals from each group. FOD, fish oil diet; COD, coconut oil diet; FOVD, fish oil plus vitamin E diet; COVD, coconut oil plus vitamin E diet; $\dot{M}_{O_2}$, oxygen consumption.

Resting oxygen consumption rates

Figure 1 shows the frequency distribution of instantaneous oxygen uptake rates as measured every 10 min over an 8h period in seven sturgeon from each of the four experimental groups. The FOD group exhibited a markedly different distribution from that of the other groups with a clearly unimodal distribution in a narrow range of low uptake rates, and no individuals in this group consumed at rates above 260 mg kg^{-1} h^{-1} (Fig. 1). The other groups showed a wide variation in oxygen uptake rates with more frequent consumption at high rates, up to 400 mg kg^{-1} h^{-1} (Fig. 1). As indicated by the differences in the distribution of oxygen uptake rates (Fig. 1), the FOD group had a significantly lower mean resting $\dot{M}_{O_2}$ than the COD and FOVD groups when considered over the 8 h measurement period (Table 4). Mean $\dot{M}_{O_2}$ in the COVD group was not significantly different from that of the FOD group due to the variability in uptake rates in the former group (Fig. 1, Table 4).

Table 4. Resting oxygen consumption rate and normoxic (Pw_{O_2} = 19.2 ± 0.7 kPa) ventilatory, blood-gas and acid-base-related variables in sturgeon from the 4 dietary groups

	FOD	COD	FOVD	COVD
$\dot{M}_{O_2}$ (mg $kg^{-1}h^{-1}$)	111 ± 9	181 ± 22*	186 ± 17*	175 ± 23
P_{OP} (kPa)	0.04 ± 0.001	0.09 ± 0.02*	0.05 ± 0.01	0.05 ± 0.01
f_G (beats min^{-1})	92 ± 4.5	97 ± 5.1	98 ± 5.0	91 ± 4.2
Pa_{O_2} (kPa)	11.5 ± 0.9	8.8 ± 0.9	8.0 ± 1.1	9.4 ± 0.4
Ca_{O_2} (vol %)	10.3 ± 0.8	11.1 ± 0.3	10.3 ± 0.9	11.5 ± 0.3
pHa	7.87 ± 0.03	7.89 ± 0.02	7.92 ± 0.02	7.90 ± 0.03
Ca_{CO_2} (mmol l^{-1})	8.79 ± 1.61	8.21 ± 0.83	7.94 ± 0.94	6.57 ± 0.89
Pa_{CO_2} (kPa)	0.35 ± 0.06	0.33 ± 0.05	0.27 ± 0.03	0.24 ± 0.03
Hcrit (%)	26.2 ± 3.0	21.2 ± 2.4	23.6 ± 2.2	21.7 ± 3.6
Lactate (mmol l^{-1})	0.98 ± 0.51	0.56 ± 0.11	0.42 ± 0.16	0.33 ± 0.11

Values = mean ± SEM; n = 7 for COVD; 9 for FOD and COD, and 11 for FOVD; * = significantly different from FOD group ($p < 0.05$); FOD, fish oil diet; COD, coconut oil diet; FOVD, fish oil and vitamin E diet; COVD, coconut oil and vitamin E diet; $\dot{M}P_{O_2}$, oxygen consumption rate; P_{OP}, opercular pressure amplitude; f_G, gill ventilation rate; Pa_{O_2}, arterial plasma oxygen partial pressure; Ca_{O_2}, arterial blood oxygen content; pHa, arterial blood pH; Ca_{CO_2}, arterial carbon dioxide content; Hcrit, haematocrit; Lactate, plasma lactate concentration.

Table 5. Effects of mild (Pw_{O_2} = 10.8 kPa), moderate (Pw_{O_2} = 6.6 kPa), and deep (Pw_{O_2} = 4.6 kPa) hypoxia on mean (± SEM) values of ventilatory, blood-gas and acid-base-related variables in the Adriatic sturgeon

		Hypoxia		
	Normoxia	Mild	Moderate	Deep
P_{OP} (kPa)	0.06 ± 0.008	0.07 ± 0.009	0.07 ± 0.008	0.09 ± 0.01
f_G (beats min^{-1})	95 ± 3.5	122 ± 3.1*	120 ± 2.2*	112 ± 2.6*
Pa_{O_2} (kPa)	9.59 ± 0.51	3.97 ± 0.24*	2.41 ± 0.10*	1.85 ± 0.08*
Ca_{O_2} (vol %)	10.1 ± 0.4	8.0 ± 0.4*	5.4 ± 0.4*	4.6 ± 0.3*
pHa	7.91 ± 0.01	7.95 ± 0.02	7.82 ± 0.02	7.71 ± 0.02*
RBC pHi	7.23 ± 0.02	7.20 ± 0.02	7.13 ± 0.03	6.91 ± 0.05*
Ca_{CO_2} (mmol l^{-1})	9.53 ± 0.87	7.70 ± 1.05	9.77 ± 0.80	4.73 ± 0.85*
Hcrit (%)	23.4 ± 1.3	23.9 ± 1.2	26.9 ± 1.5	26.6 ± 1.4*
Lactate (mmol l^{-1})	0.58 ± 0.13	1.04 ± 0.21	2.77 ± 0.29*	4.24 ± 0.31*

n = 19 for Ca_{CO_2}; 24 for pHi; 25 for P_{OP}, f_G, pHa, Pa_{O_2} and lactate; 26 for Ca_{O_2} and 27 for Hcrit; * = significantly different from normoxia ($p < 0.05$); P_{OP}, opercular pressure amplitude; f_G, gill ventilation rate; Pa_{O_2}, arterial plasma oxygen partial pressure; Ca_{O_2}, arterial blood oxygen content; pHa, arterial blood pH; RBC pHi, red blood cell intracellular pH; Ca_{CO_2}, arterial carbon dioxide content; Hcrit, haematocrit; Lactate, plasma lactate concentration.

Resting ventilatory blood gas and acid-base-related variables

There were intergroup differences in resting ventilatory variables, with the FOD group exhibiting a significantly lower P_{OP} than the COD group (Table 4). These differences were not observed in the two groups fed vitamin E supplements (FOVD and COVD), which both had similar, intermediate, levels of P_{OP} (Table 4). The differences in resting oxygen uptake were not reflected in differences in normoxic blood gas and acid-base-related variables or in plasma lactate concentration (Table 4).

Effects of hypoxia

Despite the marked difference in resting oxygen uptake between the FOD group and the other groups there were no significant differences amongst groups for any of the measured variables at any level of hypoxia. The data from all of the groups was, therefore, combined and Table 5 shows the general responses to hypoxia as described by the data from the groups considered together. Hypoxia elicited a significant increase in f_G and a significant reduction in Pa_{O_2} and Ca_{O_2} at all levels, and a reduction in pHa, Ca_{CO_2}, and RBC pHi in deep hypoxia. Plasma lactate was

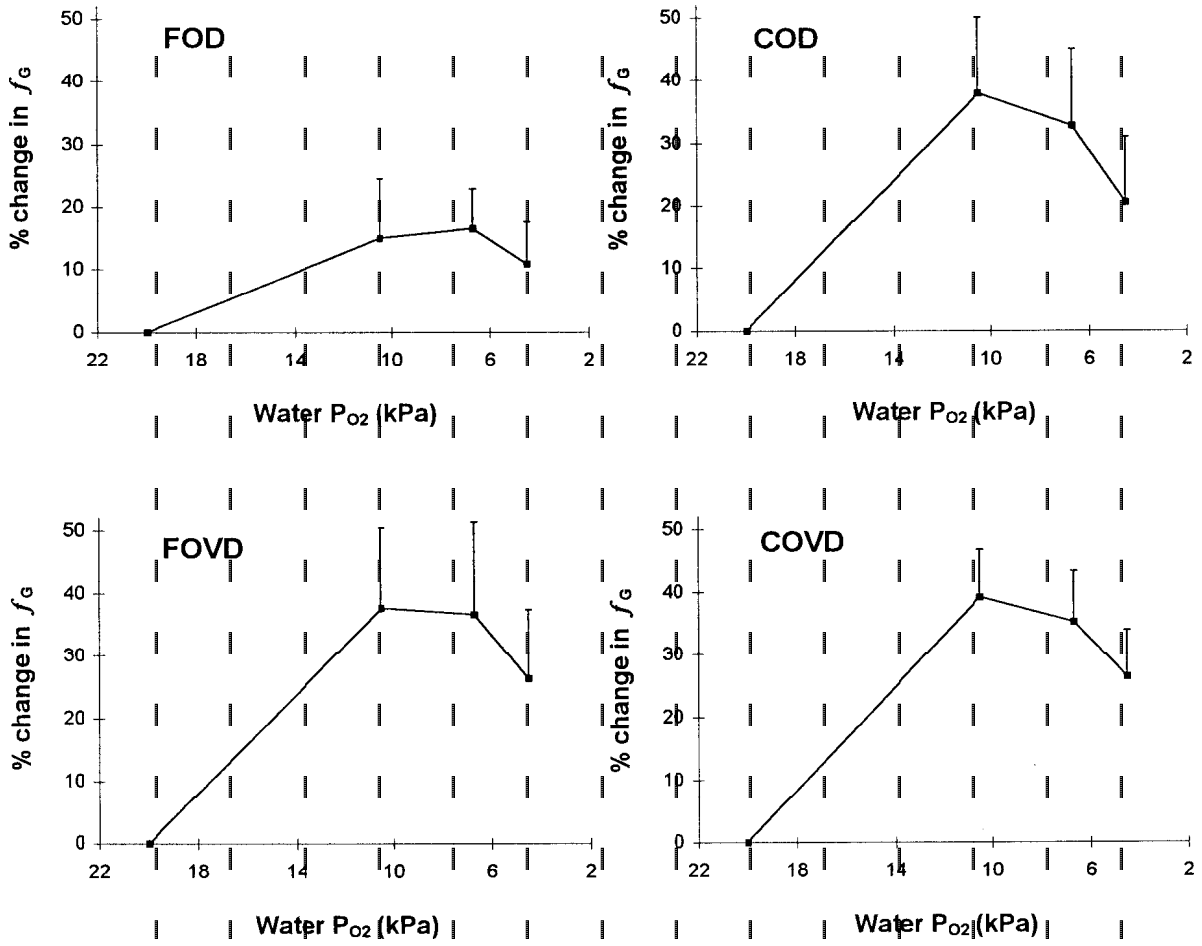

Fig. 2. Mean (± SEM) percent increase from normoxic (Pw_{O_2} = 19.2 kPa) value of gill ventilation frequency in mild (Pw_{O_2} = 10.8 kPa), moderate (Pw_{O_2} = 6.6 kPa) and deep (Pw_{O_2} = 4.6 kPa) hypoxia in sturgeon fed four experimental diets. n = 6 in all cases. FOD, fish oil diet; COD, coconut oil diet; FOVD, fish oil plus vitamin E diet; COVD, coconut oil plus vitamin E diet; f_G, gill ventilation frequency.

significantly elevated in both moderate and deep hypoxia and haematocrit in deep hypoxia (Table 5).

When considered individually, the dietary groups followed this general trend but there were some differences in response amongst the groups, and these will now be described in further detail. The FOD group did not hyperventilate significantly at any level of hypoxia whereas all other groups hyperventilated at all levels. Figure 2 illustrates the effects of hypoxia on f_G in the four individual dietary groups – the response by the FOD group was less marked than that of the other groups, and was not statistically significant. Figure 3 shows the effects of hypoxia on Ca_{O_2} in the dietary groups, and the net reduction in Ca_{O_2} at the three levels of hypoxia. Although mean Ca_{O_2} levels did not differ amongst the groups at any level of hypoxia, the FOD group showed a significantly smaller net reduction in Ca_{O_2} in mild hypoxia than that observed in the COD and COVD groups. The FOVD group showed a statistically similar net reduction in Ca_{O_2} to that seen in the COVD group but when animals fed ω3 HUFA (FOD and FOVD groups) were compared with all animals fed SFA (COD and COVD groups), the former showed a significantly smaller net reduction in Ca_{O_2} in mild hypoxia (–1.45 ± 0.32 *vs.* –3.23 ± 0.42 vol %, respectively, values are mean ± SEM, n = 12). Values were similar in moderate

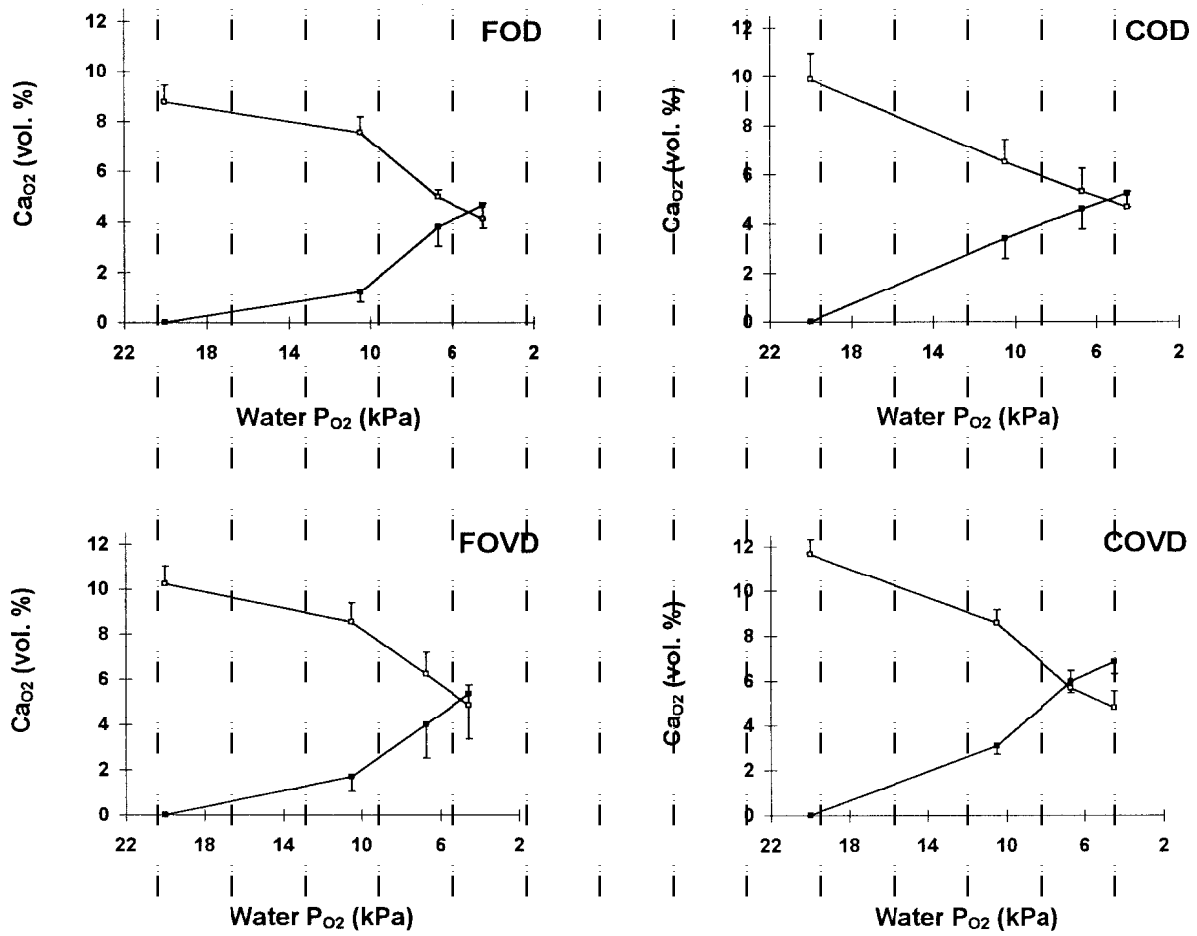

Fig. 3. Mean (± SEM) arterial blood oxygen content (open symbols) and net decline in arterial blood oxygen content (closed symbols) in mild (Pw_{O_2} = 10.8 kPa), moderate (Pw_{O_2} = 6.6 kPa) and deep (Pw_{O_2} = 4.6 kPa) hypoxia in sturgeon fed four experimental diets. n = 6 in all cases. FOD, fish oil diet; COD, coconut oil diet; FOVD, fish oil plus vitamin E diet; COVD, coconut oil plus vitamin E diet; Ca_{O_2}, arterial blood oxygen content.

and deep hypoxia. Although there were no significant differences in the increase in plasma lactate concentration in moderate and deep hypoxia between the FOD and COD groups, due to much individual variability, the FOD group consistently showed a smaller mean increase in lactate concentration for each hypoxic increment than the COD group (*e.g.*, 1.51 ± 0.54 *vs.* 2.37 ± 0.77 mM increase between normoxia and moderate hypoxia, respectively, n = 6 for FOD and 7 for COD).

Effects of hypercapnia

As for hypoxia, none of the four groups exhibited significant differences in any measured variable in hypercapnia, and Table 6 shows the general responses to hypercapnia as described by all the data from the groups considered together. Hypercapnia raised Ca_{CO_2}, Pa_{CO_2} and Pa_{O_2} significantly, and lowered pHa and RBC pHi. At 30 min exposure, CO_2 partial pressures in arterial blood and water had not yet equilibrated and Pa_{CO_2} was only approximately half of Pw_{CO_2} (Table 6). These changes were associated with an increase in P_{OP} but there were no changes in f_G (Table 6).

Table 6. Effects of mild hypercapnia (Pw_{CO_2} = 1 kPa) on mean (± SEM) ventilatory, blood gas and acid-base-related variables in the Adriatic sturgeon

	Normocapnia	Hypercapnia
P_{OP} (kPa)	0.059 ± 0.007	0.078 ± 0.010*
f_G (beats min^{-1})	95 ± 3.4	93 ± 3.5
Ca_{CO_2} (mmol l^{-1})	7.77 ± 0.57	10.62 ± 0.65*
pHa	7.90 ± 0.01	7.78 ± 0.01*
Pa_{CO_2} (kPa)	0.30 ± 0.02	0.56 ± 0.03*
RBC pHi	7.23 ± 0.03	7.11 ± 0.02*
Ca_{O_2} (vol %)	11.1 ± 0.4	10.7 ± 0.5
Pa_{O_2} (kPa)	9.45 ± 0.55	11.60 ± 0.37*

n = 26 in all cases; * = significantly different from normocapnia ($p < 0.05$); P_{OP}, opercular pressure amplitude; f_G, gill ventilation rate; Ca_{CO_2}, arterial carbon dioxide content; Pa_{CO_2}, arterial carbon dioxide partial pressure; pHa, arterial blood pH; RBC pHi, red blood cell intracellular pH; Ca_{O_2}, arterial blood oxygen content; Pa_{O_2}, arterial oxygen partial pressure.

In all individual groups, hypercapnia had the effects on plasma acid-base status described above, but in the COD group these were associated with a significant increase in P_{OP}, a response not observed in any other group (Fig. 4). The COD group also exhibited a significant reduction in Ca_{O_2} that was not observed in any other group, although mean Ca_{O_2} in hypercapnia was similar in all groups (Fig. 4). Once again, the FOD and FOVD groups showed a significantly smaller net reduction in Ca_{O_2} in hypercapnia as compared with the COD and COVD groups (+0.47 ± 0.72 *vs.* –1.42 ± 0.57 vol %, respectively, n = 12).

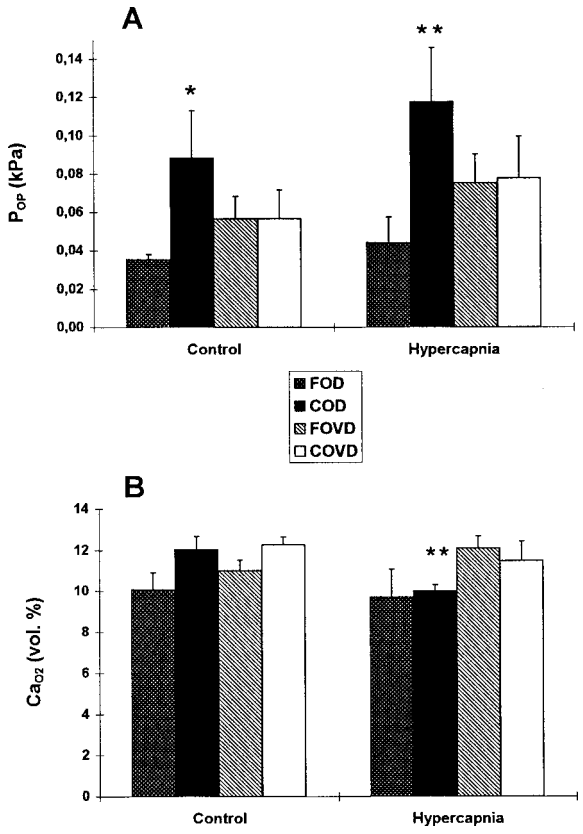

Fig. 4. Effects of mild hypercapnia (Pw_{O_2} = 1 kPa) on (A) mean (± SEM) opercular pressure amplitude and (B) mean (± SEM) arterial blood oxygen content in sturgeon fed four experimental diets. n = 6 for COD and COVD, 7 for FOD and 8 for FOVD. * = significantly higher than same value for FOD group; ** = significantly different from control value for same group; $p < 0.05$. FOD, fish oil diet; COD, coconut oil diet; FOVD, fish oil plus vitamin E diet; COVD, coconut oil plus vitamin E diet; P_{OP}, opercular pressure amplitude; Ca_{O_2}, arterial blood oxygen content.

Discussion

The results confirm that fatty acid composition of tissue lipids in the liver and muscle of the Adriatic sturgeon reflects dietary fatty acid composition (Randall *et al.* 1992; Agradi *et al.* 1993), and indicate that these differences in tissue fatty acid composition are associated with significant differences in patterns of whole-animal oxygen consumption under resting conditions and in reflex ventilatory responses to hypoxia and hypercapnia.

Fatty acid composition of lipids in the liver was more strongly influenced by diet than was muscle lipid composition. This reflects the fact that the liver is a lipid storage organ in fish, usually with a much higher total lipid and triacylglycerol content than the muscle, and is also the major site of fatty acid and lipid metabolism (Henderson and Tocher 1987). The fatty acid composition of the muscle lipids was somewhat different from that of the liver, particularly in the COD and COVD groups. The higher overall levels of HUFA such as 20:4 ω6; 20:5 ω3 and 22:6 ω3 probably reflect the fact that much of the muscle lipid is polar lipid with a characteristically high content of HUFA, whereas the liver contains larger quantities of triacyglycerols with a fatty acid composition reflecting dietary intake (Henderson and

Tocher 1987). An analysis of fatty acid composition of lipids in the hearts of sturgeon from the same dietary groups has previously been reported (Agnisola *et al.* 1996), demonstrating that similar differences in fatty acid composition are also observed in that organ. Administration of vitamin E to sturgeon at the doses used in this study (500 mg kg^{-1} dry weight of diet) leads to a significant accumulation in the lipids of the liver and muscle (Randall *et al.* 1992; Agradi *et al.* 1993).

The frequency distributions of oxygen rates over an 8 h period reported in this study demonstrate that dietary lipid composition has a profound effect on patterns of oxygen consumption in sturgeon, and confirms the previous report that sturgeon fed these diets exhibit differences in O_2 consumption in normoxia, with the FOD group consuming significantly less oxygen than the COD group (McKenzie *et al.* 1995a). There is much evidence to suggest that in fish ventilatory activity is geared primarily to meeting the animal's oxygen requirements (Randall 1982; Smatresk 1990), so the finding that the FOD group had a significantly lower resting normoxic P_{OP} than the COD group is consistent with their lower resting $\dot{M}_{O_2}$, as P_{OP} is an index of ventilatory effort (Smatresk 1990) and it would be expected that ventilatory effort be less in animals with a lower oxygen demand.

In bony fish, the matching of ventilatory activity to tissue oxygen requirements appears to be controlled with reference to oxygen-sensitive chemoreceptors, with a reduction in oxygen levels at the receptor site stimulating a reflex increase in ventilation (Smatresk 1990; Burleson *et al.* 1992). There is evidence for two populations of receptors; one receptor population is externally-oriented and sensitive to the P_{O_2} of the ventilatory water stream, while the other is internally-oriented and sensitive to blood oxygen content (Smatresk 1990; Burleson *et al.* 1992). A similar control system appears to be operant in sturgeon (McKenzie *et al.* 1995b). The hyperventilation by the COD group in hypoxia and hypercapnia presumably reflects greater stimulation of the internal oxygen receptors sensitive to Ca_{O_2}, as the external receptors sensitive to water oxygen levels should have been stimulated to an equal extent in both FOD and COD fish. Indeed, the small and non-significant increase in ventilation in the FOD group in hypoxia may reflect stimulation of the externally-oriented receptor group (McKenzie *et al.* 1995b). The existence of a significant hyperventilation in the COD group but not in the FOD group indicates that the greater net reductions in Ca_{O_2} in the former group in mild hypoxia and hypercapnia were of functional significance, despite the fact that the actual Ca_{O_2} levels in the blood were similar in the two groups under these two conditions (they started off higher in the COD group). That is, in the COD group the measured reduction in Ca_{O_2} elicited an increase in ventilatory drive, a reflex that was not stimulated in the FOD group.

Thus, the absence of a ventilatory response to reduced oxygen availability (hypoxia) or to impaired blood oxygen transport (hypercapnia) in the FOD sturgeon would appear to indicate that they were able to meet their low oxygen demands without the need to hyperventilate, whereas the higher oxygen demands of the COD group could not, presumably, be met without the need for adaptive hyperventilatory responses. The hyperventilation in the COD group could be taken to indicate that for any particular degree of hypox–aemia the COD fish experience greater problems with the maintenance of tissue oxygen delivery.

There is other evidence that the COD group experiences problems with the maintenance of oxygen delivery in hypoxia. McKenzie *et al.* (1995a) found that at the same degree of mild hypoxia as used in the present study (P_{O_2} = 10.8 kPa) sturgeon from the same COD group exhibited a significant reduction in spontaneous locomotor activity, an effect that was not observed in the FOD group. In fish, reductions in spontaneous activity in hypoxia are considered to be a response aimed at reducing energy expenditure (Nilsson *et al.* 1993; Schurmann and Steffensen 1994). Furthermore, McKenzie *et al.* (1995a) also found that at the same degree of moderate hypoxia as used in the present study (P_{O_2} = 6.6 kPa), sturgeon from the COD group of animals were unable to maintain O_2 uptake at normoxic levels whereas this degree of hypoxia had no effect on $\dot{M}_{O_2}$ in the FOD group.

The groups fed the experimental diets with vitamin E supplements (the FOVD and COVD groups) did not show the differences in resting $\dot{M}_{O_2}$ and reflex responses to hypoxia and hyper-

capnia observed in the groups fed the fat supplements alone (FOD and COD groups). The FOVD and COVD groups had elevated resting oxygen consumptions rates that were similar to those of the COD animals, and this appeared to make them more sensitive to hypoxia as both groups exhibited a significant hyperventilation. Thus, addition of vitamin E to the diets modified the effects elicited by the fatty acids (ω3 HUFA *vs*. SFA) when administered alone and caused the FOVD and COVD groups to behave in a similar manner. In this respect, addition of vitamin E to the fish oil diet was not advantageous as it led to an increased $\dot{M}_{O_2}$ and sensitivity to hypoxia, but addition of vitamin E to the coconut oil diet did appear to confer some advantages on the COVD versus the COD groups, as the former group did not hyperventilate or exhibit a significant hypoxaemia following exposure to mild hypercapnia. McKenzie *et al.* (1995a) reported that sturgeon fed fish oil and vitamin E supplements were unable to maintain oxygen uptake in hypoxia, unlike sturgeon fed fish oil supplements alone but, conversely, that sturgeon fed coconut oil plus vitamin E were able to maintain oxygen consumption in hypoxia, unlike those fed coconut oil supplements alone. Thus, there is a complex interaction between dietary fatty acid and vitamin E composition in influencing sturgeon respiratory physiology, and the effects of vitamin E appear to differ depending on the dietary fatty acid composition. Agnisola *et al.* (1996) found, on the other hand, that vitamin E supplements had little influence on the effects of dietary fatty acid composition on *in vitro* cardiac performance in sturgeon.

Randall *et al.* (1992) found that sturgeon fed ω3 HUFA and vitamin E supplements exhibited less severe reductions in Ca_{O_2} in progressive hypoxia than did control fish fed an unsupplemented standard diet, a result that is similar to the differences in Ca_{O_2} observed amongst the dietary groups in hypoxia and hypercapnia in this study. Data from the present study indicate that the lesser reductions in Ca_{O_2} in the animals fed ω3 HUFA versus those fed SFA were not simply because the FOD group had lower tissue oxygen requirements (lower $\dot{M}_{O_2}$) than the COD group, and therefore extracted less oxygen from the blood, because the FOVD group had a high $\dot{M}_{O_2}$ but nonetheless showed a smaller reduction in Ca_{O_2} than the COD animals. There were no differences in pHa, RBC pHi or haematocrit that might have influenced haemoglobin-oxygen affinity and blood-oxygen carrying capacity amongst the dietary groups. Dietary ω3 HUFA supplements ameliorate the hypoxaemia associated with endotoxic shock in mammals, but the mechanism behind this effect is unknown (Murray *et al.* 1993).

Randall *et al.* (1992) found that animals fed the ω3 HUFA and vitamin E supplements were less acidaemic in hypoxia than sturgeon fed an unsupplemented diet. The absence of any differences in pHa in hypoxia amongst the groups in the present study may reflect methodological differences between the two studies.

At present it is only possible to speculate about the basis for the differences in resting oxygen consumption between the FOD and COD groups. Studies in mammals comparing the effects on cardiac function of increased tissue ω3 HUFA *vs*. SFA content indicate that changes in the fatty acid composition of cardiac myocyte membrane phospholipids may lead to differences in the function of membrane-associated proteins such as adrenoceptors (Courtois *et al.* 1992), and also lead to differences in the relative production of the eicosanoids of the 2 and 3 series, local hormones derived, respectively, from the ω6 fatty acid arachidonate (20:4 ω6) and the ω3 fatty acid eicosapentaenoate (20:5 ω3) (Abeywardena *et al.* 1989). If the ω3 HUFA *vs*. SFA supplements have similar effects on these processes in fish tissues, then they might conceivably contribute to the differences in whole-animal metabolism and oxygen consumption reported here. Studies in elasmobranchs also indicate that there are differences in the way that HUFA *vs*. SFA are metabolised as energy substrates, with ß-oxidation of HUFA being initiated in peroxisomes but ß-oxidation of SFA occurring in mitochondria (Moyes *et al.* 1990). This also might conceivably contribute to differences in whole-animal metabolism and oxygen consumption. Clearly, this represents an interesting area for future research.

It is surprising that despite the significant differences in resting $\dot{M}_{O_2}$ elicited by the diets, there were few differences in the effects of hypoxia and hypercapnia on the diverse array of measured physiological and biochemical variables. Previous

studies demonstrated that dietary lipid composition had significant effects on such integrated measures of physiological status as the ability to maintain whole animal O_2 uptake and spontaneous locomotor activity in hypoxia (McKenzie *et al.* 1995a) and on *in vitro* cardiac performance in relation to oxygen supply (Agnisola *et al.* 1996). The absence of more pronounced differences in the variables measured in the present study may be because such individual physiological and biochemical variables are influenced (or possibly limited) by a complex array of nervous and hormonal control systems. For example, Agnisola *et al.* (1996) reported that despite the large differences in *in vitro* cardiac performance elicited by these diets in sturgeon, there was no evidence of consistent differences in *in vivo* cardiac activity at those same levels of aquatic hypoxia that McKenzie *et al.* (1995a) had found led to differences in the ability of the whole animal to maintain O_2 uptake or spontaneous locomotor activity. Nonetheless, the data reported here indicate that the low resting $\dot{M}_{O_2}$ of the FOD group makes them less sensitive to hypoxia than the animals with elevated $\dot{M}_{O_2}$ in the COD, FOVD and COVD groups, and add to the growing body of evidence indicating that fish physiology is influenced by dietary lipid composition, and that this affects the animal's responses to environmental change.

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