Effects of diet on spontaneous locomotor activity and oxygen consumption in Adriatic sturgeon (Acipenser naccarii)

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Abstract

Adriatic sturgeon (Acipenser naccarii) were maintained on a commercial diet enriched either in long chain polyunsaturated fatty acids of the α₃ series (α₃ LPCUFA) or in saturated fatty acids (SFA). The effects of dietary fatty acid composition on spontaneous locomotor activity in normoxia and hypoxia (O₂ tension = 10.5 ± 0.8 kPa), and on oxygen consumption (MO₂) in normoxia, in hypoxia (O₂ tension = 6.6 ± 0.8 kPa) and during recovery were then investigated. The effects of adding supplementary vitamin E to the fat-enriched diets were also studied.

Dietary fatty acid composition had effects on spontaneous locomotor activity and MO₂ in normoxia. Activity levels were higher in all sturgeon fed extra dietary fats (without vitamin E), when compared with control animals, but fish fed α₃ LPCUFA had a significantly lower MO₂ than those fed SFA, with intermediate MO₂ in controls. In hypoxia, sturgeon α₃ LPCUFA did not alter activity or MO₂ whereas those fed SFA reduced both and control reduced MO₂. During recovery, both animals fed SFA and controls had a higher MO₂ than sturgeon fed α₃ LPCUFA. The data indicate that fish fed α₃ LPCUFA are more tolerant of hypoxia than controls or those fed SFA, as they did not reduce either activity or MO₂, and consumed less O₂ during recovery.

Vitamin E supplements modified the effects elicited by dietary fats. All sturgeon fed vitamin E had low activity levels in normoxia and hypoxia. Sturgeon fed vitamin E with α₃ LPCUFA had a higher MO₂ in normoxia than those fed α₃ LPCUFA alone; reduced MO₂ in hypoxia, and during recovery increased MO₂ to a rate higher than that of animals fed α₃ LPCUFA alone. In normoxia, sturgeon fed vitamin E with SFA had a similar MO₂ to those fed SFA alone but did not change MO₂ in hypoxia or during recovery. Thus, the effects of vitamin E were dependent on fat composition of the diet. Vitamin E with α₃ LPCUFA removed the beneficial effects on MO₂ and responses to hypoxia obtained with α₃ LPCUFA alone, but vitamin E with SFA allowed sturgeon to maintain aerobic metabolism in hypoxia, a more effective response than that observed in fish fed SFA alone.

Introduction

There is a great deal of evidence to suggest that, in mammals, increases in the amounts of long chain polyunsaturated fatty acids (LPCUFA) of the α₃ series and vitamin E (α-tocopherol) in the diet can inhibit various pathological processes, such as chronic degenerative diseases of the cardiovascular system.
system and issue damage following hypoxia or ischaemia (Dehmer et al. 1985; Leaf and Weber 1988; Budowski and Sklan 1989; Hornstra 1989; Diplock 1991; Packer 1991). These compounds also affect mammalian respiratory physiology, as an increase in dietary intake of o3 long chain polyunsaturated acids ameliorates the hypoxaemia associated with endotoxic shock in pigs (Murray et al. 1993) and vitamin E influences the susceptibility of pigs to stresses such as exercise and hyperthermia (Dubie et al. 1988).

Temperate freshwater fish is an interesting group of vertebrates because, unlike many terrestrial animals, they have tissues particularly rich in o3 LCPUEA and vitamin E, as a result of their dietary fatty acid composition and as an adaptation to life at relatively low ambient temperatures (Watanebe 1981; Henderson and Tocher 1987). They have an absolute requirement for these compounds in their diet and will otherwise exhibit specific pathologies due to deficiency (Cowey and Sargent 1979; Cowey 1986; Bell et al. 1986). Freshwater fish frequently suffer from hypoxia, as a result of the relatively low solubility of O2 in water (see Randall 1982, for a review) and it is interesting, therefore, to assess the influence of dietary o3 LCPUEA and vitamin E content on responses to hypoxia in these animals.

A previous study reported that when Adriatic sturgeon (Acipenser naccarii, Bonaparte) were placed in a recirculating system and allowed to create hypoxia by gradually consuming the available O2, animals that had received o3 LCPUEA and vitamin E supplements consumed the O2 less quickly than sturgeon fed a control diet, and maintained blood O2 content and pH unchanged at hypoxic water O2 tension that elicited significant hypoxaemia and acidosis in control animals (Randall et al. 1992). These authors also reported that the control sturgeon became very agitated as the water O2 tension (PwO2) fell whereas the animals that received the supplements did not, and suggested that these behavioural differences might have been important in eliciting the differences in O2 consumption and blood gas and pH homeostasis. It is not known whether the differences were a result of the effects of o3 LCPUEA or vitamin E alone, or of an interaction between the two compounds, or simply because the two groups received different amounts of fat in their diet.

A number of issues arising from the study by Randall et al. (1992) were investigated in the present study. These include: whether there are differences in spontaneous locomotor activity and behavioural responses to hypoxia in A. naccarii fed differing amounts of o3 LCPUEA and vitamin E; whether these dietary components affect oxygen consumption in hypoxia, when the sturgeon are exposed to a flow of hypoxic water at a constant PwO2, rather than progressively declining PwO2 in a recirculating system, and the relationship between behaviour and O2 uptake under these conditions. Further, an attempt was made to determine the individual and combined effects of o3 LCPUEA and vitamin E in eliciting any differences in response. To this latter end, 5 diets were formulated and spontaneous activity and O2 uptake measured in sturgeon fed these diets, in normoxia and hypoxia.

Materials and methods

Animals

Two hundred Adriatic sturgeon (Acipenser naccarii, Bonaparte) were maintained at the Experimental Thermal Aquaculture Plant, Le Caselle [via Argine del Battollino, 29010, Sarmato (PC), Italy] in 5 groups of 40 animals, in indoor 4 m2 fibreglass tanks (volume 2000 l) with a continuous supply of water at 23 ± 1°C and pH 7.9.

Administration of fatty acids and vitamin E

For a period of 6 months, the 5 separate groups of sturgeon received one of 5 diets, each with a different fatty acid and vitamin E content: the control diet (CD) was a commercial formulation (Anma Storioni Prima Fast, Agros, Bolzano, Italy), with the composition supplied by the manufacturers reported in Table 1. This feed contained nutritionally adequate levels of o3 LCPUEA and vitamin E in order to avoid any effects on physiology that might result from dietary deficiencies (Watanebe
Table 1. Composition of commercial feed used for control diet

| Protein(1) | 9.1 |
| Lipid(1) | 9.5 |
| Ash | 10.2 |
| Latter | 2.0 |
| Cellulose(1) | 0.8 |
| Vitamin E(1) | 42 |
| Vitamin C | 954 |
| Vitamin A | 6760 |
| Vitamin D | 400 |
| Vitamin B1 | 8.5 |
| Vitamin B2 | 9.3 |
| Vitamin K | 4.4 |
| Vitamin K3 | 3.0 |
| Vitamin K | 2.8 |
| Vitamin H | 0.04 |
| Para-aminohippuric acid(2) | 1408 |
| Choline(2) | 220 |
| Isoleucine | 46.4 |
| L-Cysteine | 36.8 |
| Isoleucine | 14.4 |
| D-cystine | 12.1 |
| Mannose(2) | 8.7 |
| Zinc | 6.4 |
| Iron | 6.2 |
| Copper | 1.4 |
| Sulfur(3) | 0.01 |

(1) g (w/w); (2) mg kg⁻¹; (3) μg kg⁻¹.

1981; Henderson and Tocher (1987). Diet 2 was enriched in fish oil (FOD), composed of 850 g of the commercial feed + 150 g of menhaden (Brevoortia tyrannus) oil, an oil particularly rich in ω3 LPCPUFA, to assess the effects of these compounds on responses to hypoxia. Diet 3 was enriched with coconut oil (CODO), composed of 850 g of the fish meal + 150 g of hydrogenated coconut (Cocos nucifera) oil, an oil composed of short-chain saturated fatty acids, compounds that do not have the biological activity of polyunsaturated fatty acids, to investigate any influence on responses to hypoxia of an increase in fats in general. Diet 4 was enriched in both fish oil and vitamin E (FODV), composed of 850 g of fish meal + 150 g of menhaden oil + 500 mg of vitamin E (α-tocopherol acetate), to assess the combined effects of ω3 LPCPUFA and vitamin E. Diet 5 was enriched in both coconut oil and vitamin E (CODO), composed of 150 g of fish meal + 150 g of hydrogenated coconut oil + 500 mg of vitamin E, to assess the effects of vitamin E in a diet enriched with SFA but not with ω3 LPCPUFA.

The oils were obtained from ICN Biomedicals and the α-tocopherol acetate from Hoffmann La Roche. All diets were mixed with 30% (w/w) water and 10% (w/w) of a commercial binder (Integra-tore R, A.C.E.F., Florenzoula d’Arda (PC), Italy) and prepared as moist pellets at La Cazeolla, divided into daily aliquots of 3% of the total weight of the dietary group, and stored at −20°C until used.

The fatty acid composition of the pellet diets is reported in Table 2.

### Table 2. Content of principle fatty acids (%) and vitamin E (μg kg⁻¹) in the 5 diets.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>CD</th>
<th>FOD</th>
<th>COD</th>
<th>FODV</th>
<th>COVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>3.9</td>
<td>7.1</td>
<td>16.0</td>
<td>7.1</td>
<td>16.0</td>
</tr>
<tr>
<td>16:0</td>
<td>22.7</td>
<td>23.3</td>
<td>24.3</td>
<td>23.3</td>
<td>24.3</td>
</tr>
<tr>
<td>18:0</td>
<td>6.2</td>
<td>4.8</td>
<td>6.7</td>
<td>4.8</td>
<td>6.7</td>
</tr>
<tr>
<td>18:2 n6</td>
<td>19.2</td>
<td>8.2</td>
<td>12.8</td>
<td>8.2</td>
<td>12.8</td>
</tr>
<tr>
<td>18:3 n3</td>
<td>2.8</td>
<td>1.9</td>
<td>1.7</td>
<td>1.9</td>
<td>1.7</td>
</tr>
<tr>
<td>20:3 n6</td>
<td>0.1</td>
<td>3.2</td>
<td>0.5</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>20:4 n6</td>
<td>1.0</td>
<td>1.2</td>
<td>1.1</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>22:5 n3</td>
<td>8.7</td>
<td>13.5</td>
<td>7.0</td>
<td>13.5</td>
<td>7.0</td>
</tr>
<tr>
<td>22:6 n3</td>
<td>10.1</td>
<td>9.4</td>
<td>6.8</td>
<td>9.8</td>
<td>6.8</td>
</tr>
<tr>
<td>SAT</td>
<td>33.8</td>
<td>33.4</td>
<td>47.2</td>
<td>33.4</td>
<td>47.2</td>
</tr>
<tr>
<td>MONO</td>
<td>21.6</td>
<td>24.2</td>
<td>20.6</td>
<td>24.2</td>
<td>20.6</td>
</tr>
<tr>
<td>POLY</td>
<td>45.6</td>
<td>42.3</td>
<td>32.2</td>
<td>42.3</td>
<td>32.2</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>42</td>
<td>42</td>
<td>42</td>
<td>42</td>
<td>42</td>
</tr>
</tbody>
</table>

CD, control diet; FOD, fish oil diet; COD, coconut oil diet; FODV, fish oil and vitamin E diet; COVD, coconut oil and vita-min E diet; SAT, total saturated fats; MONO, total monoun-saturated fatty acids; POLY, total polyunsaturated fats.

**Spontaneous locomotor activity.**

The apparatus used for measurement of spontaneous locomotor activity was identical to that described in Schurmann and Steffens (1994). Briefly, a sturgeon was placed in 20 cm of water in a 1 m² fiberglass tank. The water in the tank was recirculated through a biofilter to remove wastes and a gas-exchange column to control dissolved gas levels. The tank was in a controlled temperature chamber maintained at 23 ± 1°C. The floor of the tank was covered with a reflective material (LM Scotchlite 6060) and a CCD video...
camera and a source of infrared light were posi-
tioned above the tank. The camera was connected
to a video monitor in an adjacent room. The moni-
tor was connected, in turn, to a computer (Olivetti
M24) equipped with a video frame-grabber (Visio-
netics VFG-512 BC) that digitized single video
frames with a resolution of 256 × 256 pixels at a
speed up to real time (25 frames sec⁻¹). The materi-
al on the floor of the tank reflected a uniform back-
ground of light against which the fish could be dis-
inguished as a dark object. The software allowed
discrimination of 64 tones of grey and was thus able
to detect the fish. The position of the fish was deter-
mined by calculating the centre of gravity of the
space (calculated as pixels) occupied by the animal.
The x, y coordinates of the position were imported
into a data acquisition program (Labyte Note-
book).
Position data were logged every 1.3 sec, and
every 13 min, calculations of distance traveled,
mean swimming speed and maximum swimming
speed were made, and stored on disk for subsequent
analyses. At each 13 min interval, the data acqui-
sition program also calculated the frequency with
which the sturgeon swam at the following speeds: 0
to 5, 5 to 15, 15 to 25, 25 to 35, 35 to 45, 45 to
55 and >55 cm sec⁻¹. This then allowed calcula-
tion of the frequency distribution of swimming
speeds.
Animals were placed in the tank and allowed to
recover overnight, and then all activity measure-
ments were made between 09:30 and 17:00, to
minimize the influence of circadian rhythms. Prior
to and during measurements, the animals were not
disturbed by the experimenters, who remained in
an adjoining laboratory. Spontaneous activity was
measured for 6 sturgeon from each dietary group
for 3h of normoxia and for 3h of hypoxia at a
P₈₅₀, of 10.5 ± 0.2 kPa. The mean (± SD) weight
of the sturgeon in the 5 groups was CD, 758 ±
153 g; FOD, 795 ± 204 g; COD, 939 ± 229 g;
FOVD, 1088 ± 166 g; COVD, 957 ± 340 g. The
CD group had a significantly lower mean weight
than the FOVD group, but there were no other
differences. The mean (± SD) total bodylengths
of the sturgeon were CD, 54 ± 6 cm; FOD, 55 ± 5 cm;
COD, 56 ± 5 cm; FOVD, 62 ± 3 cm; COVD,
58 ± 4 cm. There were no significant differences in
length between the groups. Hypoxia was created with
100% N₂ and a gas-exchange column that was
positioned within the recirculating system. Water
P₈₅₀ was measured with a Yellow Springs O₂ elec-
trode (model 5311) and Yellow Springs Biological
Oxygen Monitor. Approximately 30 min were
required to reduce P₈₅₀ from air saturation to
10.5 kPa.
Oxygen uptake
For measurements of oxygen uptake (MO₂, mg
kg⁻¹ h⁻¹), sturgeon were placed in a plexiglass respirometer chamber (22 l) and allowed to recover
overnight. The respirometer chamber was im-
mersed in 25 cm of water in a 1 m³ tank. The water
in the large tank was recirculated through a biofilter,
to remove wastes, and a gas-exchange column,
to control dissolved gas levels. 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 (Eliehr 1048) that
continuously recyled water back into one of the
entry ports. The other entry port was connected to
another pump (Eliehr 1060) that flushed the cham-
ber with water drawn from the outer tank. The
flushing pump was controlled, via an AD/DA in-
terface (Data translation DT2801) to a computer
(Zenith 433D+ ×) containing the program Labyte
Notebook. The activity of the flushing pump was
controlled by Labyte Notebook such that it was
active for 4 min in every 10 min. When it was not
active, there was a decline in O₂ tension within the
chamber, due to O₂ uptake by the sturgeon. The
rate of O₂ decline was measured by a Radiometer
O₂ electrode (model E8047) that received continu-
ous water samples from the recirculating pump circuit.
This electrode was connected to a Radiometer
PHM 73 blood gas analyser which was in turn con-
nected, via the interface board, to the computer.
The weight of the fish and the volume of the recir-
culating chamber system were registered in the pro-
gram and used by Labyte Notebook to calculate
O₂ uptake by the fish, as mg kg⁻¹ h⁻¹. Thus, O₂
consumption by the fish was measured for 6 min in every 10 min, and then the flushing pump was acti-
vated in order to re-equilibrate PwO2 in the chamber with that in the large outer tank.

The PwO2 of the water in the outer tank was monitored by another Radiometer electrode (model E5047) attached to a Radiometer PHM 73 blood gas analyser and the computer, as described above. Water PwO2 in the tank could be made hypoxic by a solenoid valve that opened a flow of 100% N2 through the gas-exchange column. The electrode in the tank measured the PwO2, and the computer controlled the aperture of the solenoid, until the re-
quired hypoxic PwO2 was reached.

Oxygen consumption was measured for 7 sur-
geon from each dietary group (for a control period, for 3h of hypoxia (6.6 ± 0.2 kPa) and for 3h of recovery in normoxia. The mean weight of the sur-
geon from the 5 dietary groups was CD, 1406 ± 500 g; FOD, 1195 ± 297.3 g; COD, 1373 ± 199 g; FODV, 1242 ± 329 g; COVD, 1186 ± 294 g. There were no significant differences in weight between the groups. The system required approximately 40 min to reach a PwO2 of 6.6 kPa from saturation. The computer controlling the measurements of MwO2 was in an adjacent room, in order to min-
imize the disturbances to the fish. All measure-
ments were made between 09:00 and 18:00, to avoid any influence of circadian rhythms on MwO2.

Data analysis

Within each dietary group, the mean swimming speed was calculated for each 13 min measurement period during the 3h of normoxia, the 30 min of reducing PwO2, and during the 3h of hypoxia, to low description of the activity patterns during these periods. In order to describe the effects on spon-
taneous activity of dietary enrichment with fats, both menhaden and coconut oil, the mean swim-
ning speed of all the animals in the FOD and COD groups together was calculated, as described above. This procedure was repeated for the animals from groups FODV and COVD together, to describe the effects on spontaneous activity of the addition of vitamin E to a diet enriched in fats (both menhaden and coconut oil). All swimming speeds were calcu-
lated as (total body length sec−1) (body length sec−1); the exception of the frequency distribution of swimming speeds, which was in cm sec−1. In some groups, there was a noticeable increase in activity during the period when PwO2 was dropping and the water becoming hypoxic. The significance of these increases in activity was assessed for this peri-
od by analysis of variance (ANOVA) for repeated samples. Swimming speed at any given interval was compared between groups by one way ANOVA.

Within each dietary group, total distance swum, mean swimming speed and maximum swimming speed were calculated for the entire 3h of normoxia or 3h of hypoxia, as BL sec−1. These values were also calculated for groups FOD + COD and FOD + COVD. These variables in normoxia were com-
pared with those in hypoxia with a paired t-test. Variables were compared between groups by one way ANOVA.

As regards oxygen consumption, to describe the characteristics of MwO2 in each dietary group under the different experimental conditions, mean MwO2 was calculated at each time interval, beginning under control conditions, then during the 40 min of decreasing PwO2, during the 3h of hypoxia and the 3h of recovery. In some groups, there was a notice-
able increase in mean MwO2 during the period when PwO2 was dropping and the water becoming hy-
poxic. The significance of these increases in mean MwO2 was assessed for this period by analysis of variance (ANOVA) for repeated samples. Oxygen consumption at any given interval was compared between groups by one-way ANOVA. Mean MwO2 under control conditions, during hypoxia and dur-
ing recovery were compared within groups with ANOVA for repeated samples and between groups by one-way ANOVA. For the recovery period, differences amongst groups were also assessed by a non-parametric, Kruskal-Wallis ANOVA, as the 5 dietary groups exhibited heterogeneous variances. No attempt was made to calculate the effects on MwO2 of added fats in the diet (groups FOD + COD) or the effects of the addition of vitamin E to a diet enriched in fats (groups FODV + COVD).
because there were marked differences between the FOD and COD groups in $O_2$ uptake, and clearly no general effect of increased dietary fat content on this variable, unlike the case for spontaneous activity. In those cases where analysis of variance indicated a significant difference, Tukey’s post-hoc pairwise comparison of means was employed to determine the site of the differences; $p = 0.05$ was taken as the limit for statistical significance.
Results

Spontaneous locomotor activity

The sturgeon from all groups tested to swim constantly around the perimeter of the tank, with some periods of immobility and occasional bursts of high velocity movement. There were, however, differences in activity levels between the dietary groups. The mean swimming speed of the five groups during 3h of normoxia and 3h of hypoxia is shown in Figure 1. Activity was very variable in all the groups in normoxia, with the exception of the sturgeon fed SFA and vitamin E (COD group). The sturgeon fed extra SFA (COD group) were the most active, and they exhibited periods of activity where mean swimming speed was significantly higher than the control diet (CD) and FOVD groups at the analogous measurement interval. There were no other significant differences between groups, although the sturgeon fed eicosapentaenoic acid (EPA) a (FOD group) showed a relatively elevated mean swimming speed and the FOVD group a relatively low one (Fig. 1). When considered together, the sturgeon that received dietary fish supplements, i.e., those of the FOD and COD groups, had a significantly higher swimming speed, at certain measurement intervals, than the CD animals (Fig. 2). The sturgeon from the FOD and COD groups also had a significantly higher swimming speed than the sturgeon fed both fats and vitamin E, i.e., those from the FOVD and COVD groups (Fig. 2). Thus, the addition of vitamin E to the fat-enriched diets inhibited the effects on spontaneous activity of a diet enriched in fats alone.

During the 30 min period when the water was made hypoxic, i.e., when the sturgeon were exposed to a declining P<sub>0</sub><sub>2</sub>, the sturgeon of the CD and COVD groups showed a significant increase in mean swimming speed as compared with the speed measured immediately prior to the initiation of hypoxia (Fig. 1). The other dietary groups did not exhibit this behavioural response (Fig. 1). Once the P<sub>0</sub><sub>2</sub> reached 10.5 kPa, activity in the CD and COVD groups returned to levels similar to those in normoxia. In fact, the only dietary group that showed a significant change in mean swimming
Table 3. Total distance swum by sturgeon from the 5 dietary groups as a result of spontaneous activity in normoxia and hypoxia.

<table>
<thead>
<tr>
<th>Dietary Group</th>
<th>Normoxia</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>1700 ± 44*</td>
<td>1806 ± 509</td>
</tr>
<tr>
<td>FOD</td>
<td>2767.688</td>
<td>2966 ± 628</td>
</tr>
<tr>
<td>COD</td>
<td>3739 ± 12*</td>
<td>2342 ± 576**</td>
</tr>
<tr>
<td>FOVD</td>
<td>1786 ± 92*</td>
<td>1833 ± 582</td>
</tr>
<tr>
<td>COVD</td>
<td>1600 ± 217*</td>
<td>1853 ± 242*</td>
</tr>
<tr>
<td>FOD + COD</td>
<td>3233 ± 53K</td>
<td>2458 ± 602</td>
</tr>
<tr>
<td>FOVD + COVD</td>
<td>1697 ± 2.241*</td>
<td>1843 ± 397*</td>
</tr>
</tbody>
</table>

Mean ± SEM; Units: Body length; n = 6 in all cases, except FOD = COD and FOVD = COVD, where n = 12; CD, control diet; FOD, fish oil diet; COD, coconut oil diet; FOVD, fish oil and vitamin E diet; FOVD, coconut oil and vitamin E diet; COD, coconut oil and vitamin E diet; FOD + COD, animals that received supplementary fats; FOD + FOVD, animals that received both fats and vitamin E; * = significantly different from FOD; ** = significantly different from normoxic value.

Table 4. Swimming speed of sturgeon from the 5 dietary groups as a result of spontaneous activity in normoxia and hypoxia.

<table>
<thead>
<tr>
<th>Dietary Group</th>
<th>Normoxia</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>0.170 ± 0.04*</td>
<td>0.177 ± 0.05</td>
</tr>
<tr>
<td>FOD</td>
<td>0.207 ± 0.06</td>
<td>0.273 ± 0.08</td>
</tr>
<tr>
<td>COD</td>
<td>0.394 ± 0.09</td>
<td>0.254 ± 0.07**</td>
</tr>
<tr>
<td>FOVD</td>
<td>0.165 ± 0.05</td>
<td>0.166 ± 0.05</td>
</tr>
<tr>
<td>COVD</td>
<td>0.165 ± 0.02</td>
<td>0.161 ± 0.02</td>
</tr>
<tr>
<td>COD + COD</td>
<td>0.321 ± 0.06</td>
<td>0.259 ± 0.06</td>
</tr>
<tr>
<td>FOVD + COVD</td>
<td>0.165 ± 0.03*</td>
<td>0.355 ± 0.03</td>
</tr>
</tbody>
</table>

Mean ± SEM; Units: BL sec⁻¹; n = 6 in all cases, except FOD = COD and FOVD = COVD, where n = 12; CD, control diet; FOD, fish oil diet; COD, coconut oil diet; FOVD, fish oil and vitamin E diet; COD, coconut oil and vitamin E diet; FOD + COD, animals that received supplementary fats; FOD + FOVD, animals that received both fats and vitamin E; * = significantly different from FOD; ** = significantly different from normoxic value.

The speed between normoxia and hypoxia was the group fed saturated fats (COD group), which showed a significant reduction from the elevated swimming speed in normoxia during hypoxia (Fig. 1). In hypoxia, all of the dietary groups showed similar mean swimming speeds (Fig. 1).

Values of total distance swum and mean swimming speed, in normoxia and hypoxia, are shown for all dietary groups in Tables 3 and 4. These data confirm that the COD group were the most active in normoxia, although there were no significant differences between the groups when assessed by ANOVA (p = 0.058). On the other hand, the COD group exhibited a significantly higher total distance swum and mean swimming speed than the CD and COD groups when assessed by t-test. The sturgeon that received supplementary fats (FOD + COD) swam a significantly longer distance and had a significantly higher mean swimming speed than the CD group and the sturgeon that received both fats and vitamin E (groups FOD + FOVD), when assessed by ANOVA. In hypoxia, the COD group exhibited a significant reduction in total distance swum and mean swimming speed that was not observed in any other group.

The maximum swimming speed of the five dietary groups, in normoxia and hypoxia, is shown in Table 5. The five dietary groups did not show any differences in maximum swimming speed, indicating that the capacity for muscular work was probably similar between the groups. There were no effects of hypoxia on maximum swimming speed.

The frequency distribution of swimming speeds for all dietary groups in normoxia are shown in Figure 3. The distributions were unimodal in all groups, but the COD group had a distribution skewed towards higher velocities whereas the other groups all had distributions skewed towards the lower velocities. In hypoxia, all distributions remained unimodal (Fig. 3) and the COD group exhibited a distribution similar to all other groups.
Fig. 3. Frequency distribution of swimming speeds (cm sec⁻¹) in sturgeon fed 5 different diets, during 3h in normoxia (closed bars) and 3h hypoxia (hatched bars). CD, control diet; FOD, fish oil diet; COD, coconut oil diet; FOVD, fish oil and vitamin E diet; COVD, coconut oil and vitamin E diet.

**Oxygen uptake**

There were significant differences in oxygen uptake (M\(_{O_2}\)) between the dietary groups in normoxia. All groups had M\(_{O_2}\) values similar to those of the CD group (Table 6), but the sturgeon fed α3 LCPUFA (FOD group) had a relatively low M\(_{O_2}\), whereas that of the animals fed SFA (COD group) or α3
Fig. 4 Mean ± SEM O₂ consumption, in normoxia, during 3h hypoxia (6.4 kPa) and 3h recovery in normoxia, in mussels fed 5 different diets. Open symbols, M₀; solid line, Pw₀; CON, control, normoxic conditions; ΔP₀ = period of decline in water O₂ tension. CD, control diet; FO/D, fish oil diet; COD, coconut oil diet; FOVD, fish oil and vitamin E diet; COVD, coconut oil and vitamin E diet.

was true of the FOVD group. The data do, however, indicate that changes in spontaneous activity levels may influence O₂ uptake. Thus, the CD and COVD groups showed an immediate increase in activity and also M₀, during the period when Pw₀ was dropping at the beginning of hypoxia, indicating that the increased activity may have stimulated an increase in M₀. On the other hand, the FO/D and COD groups exhibited immediate increases in M₀ when Pw₀ started to decline at the
beginning of hypoxic exposure, but there was no parallel effect of declining \( P_{O_2} \) on spontaneous activity levels.

**Discussion**

The data indicate that dietary fatty acid (ω3 polyunsaturated vs saturated) and vitamin E composition can significantly influence spontaneous locomotor activity and oxygen consumption in sturgeon. The data confirm that these dietary constituents can have significant effects on the response to hypoxia in sturgeon. The interactions of fats and vitamin E in determining these variables are, however, rather complex. The data indicate that, while an increase in dietary fats creates a more active animal, the costs of such increases in activity are less if the diet is rich in LCPUFA ω3 rather than SFA. Furthermore, as the FOD group had a low \( M_{O_2} \) in normoxia, they were able to maintain uptake and spontaneous activity in hypoxia. On the other hand, at the levels of hypoxia employed in this study, the COD group was unable to maintain either \( M_{O_2} \) or activity at the elevated values measured in normoxia and also had a significantly higher \( O_2 \) consumption than the FOD fish during recovery. These results indicate that the FOD group was better able to tolerate hypoxia and are in general agreement with mammalian studies, indicating that increases in dietary ω3 LCPUFA can have beneficial effects on responses to such stresses as ischemia and hypoxia, when compared with diets rich in SFA (Leaf and Weber 1988; Honsdra 1989; Murray et al. 1993). Effects of dietary fat composition on \( O_2 \) uptake were not reported in those studies, so it is unclear whether there were differences in \( O_2 \) demand similar to those observed in this study. The reduction in activity in hypoxia observed in the animals fed SFA may be similar to that observed in the Crucian carp (Nilsen et al. 1993) and the Atlantic cod (Schramm and Steffensen 1994) where reductions in spontaneous activity in anoxia and hypoxia are considered to be an adaptation to reduce energy expenditure, an interpretation consistent with the fact that the COD group also reduced \( M_{O_2} \) in hypoxia whereas the FOD group, which had a low \( M_{O_2} \) in normoxia, was not obliged to change activity or uptake in hypoxia.

The data also indicate that there is a complex interaction between dietary fats and vitamin E in determining responses to hypoxia. Addition of vitamin E to a diet enriched in ω3 LCPUFA appeared to abolish the effects on spontaneous activity and \( O_2 \) uptake elicited by ω3 LCPUFA alone, and led to responses to hypoxia that were similar to those of animals fed the control diet or SFA. On the other hand, addition of vitamin E to a diet enriched in SFA allowed the animals to maintain \( M_{O_2} \) in hypoxia, a response unlike that of sturgeon fed SFA alone. Thus, vitamin E supplements may be considered to have a beneficial effect on responses to hypoxia only when animals are fed a diet rich in SFA, and these supplements actually had a pejorative effect on responses to hypoxia when administered to animals receiving a diet rich in ω3 LCPUFA. It is not clear whether the effects of vitamin E on spontaneous activity are of any functional significance.

In a previous study (Randall et al. 1992), sturgeon of the same species fed a diet enriched in fish oil and vitamin E (the equivalent of the FOD group in the present study) consumed significantly less \( O_2 \) during progressive hypoxia than animals fed the control diet (the equivalent of the CD group in the present study). The data presented herein indicate that the differences in \( M_{O_2} \), measured by Randall et al. (1992) were due to the combined presence of vitamin E and ω3 LCPUFA in the diet and were a result of differences between the two groups in the behavioural response to declining water \( O_2 \) tensions. Indeed, in that study, the control group was reported to be considerably more agitated than those receiving the dietary supplements, and in this study, the CD group showed a significant increase in activity and \( O_2 \) uptake during the period when \( P_{O_2} \) was declining, whereas the FOD group did not change either of these variables (unlike the group fed ω3 LCPUFA alone). In a closed system such as that used by Randahl et al. (1992) to create hypoxia, differences in agitation and \( M_{O_2} \) would exacerbate problems with homeostasis of blood oxygen content and pH. To this ex-
tent, a diet enriched in both vitamin E and ω3 LCPUFA may be considered beneficial. That is, under conditions where there is a finite supply of oxygen, if animals become agitated as O2 availability decreases, this could influence the rate at which the O2 is consumed and therefore be important in determining survival. It is interesting that the increase in activity and/or M03 observed in all groups (except the FOVD group) occurred only during the period when P02 was changing and did not continue once O2 tension had stabilized. It would be interesting to determine the basis for the lack of reactivity in the FOVD group.

A comparison of spontaneous activity levels with M03 in the various dietary groups is complicated by the fact that the two variables were measured under dissimilar conditions. Thus, while the CD and COVD groups showed an increase in both activity and M03 during the period when M03 was dropping at the beginning of hypoxia, indicating that increased activity may have stimulated the increase in M03, the FOD and COD groups also exhibited immediate increases in M03 during this period but there was no parallel effect of declining P02 on activity levels. It is not clear whether this is because the increases in M03 in those groups were not related to activity but to other aspects of metabolism, or whether it is because the surgeon in the FOD and COD groups became agitated when exposed to hypoxia in a confined space such as the respirometer used for O2 uptake measurements (thereby raising M03) but did not become equally agitated when exposed to hypoxia in a 1 m3 tank. Thus, confinement of the surgeon in respirometers for measurements of O2 uptake may have led to different behavioural responses to hypoxia to those observed in free-swimming animals in a 1 m3 tank. The increase in M03 was not a result of differences in the severity of the hypoxic stress employed for the two sets of measurements because the increase in M03 occurred almost immediately after P02 began to decline and not once it had gone below 10.5 KPa (the level of hypoxia utilized in the behavioural study). The increase in activity observed in the CD and COVD groups at the entrance into hypoxia probably reflected an attempt to escape to more favourable surroundings.

It is not, perhaps, surprising that diet should influence spontaneous activity and metabolic rate. Diet composition determines tissue composition and therefore also determines substrate availability for a wide range of metabolic processes. The precursor for the formation of ω3 LCPUFA is n-3 linolenic acid, an essential fatty acid that, along with linoleic acid (the precursor of LCPUFA of the ω6 series), cannot be synthesized de novo in animals but must be obtained in the diet (Ball et al. 1980; Henderson and Tocher 1987). Animals cannot synthesize vitamin E and it must also, therefore, be obtained via the diet (Cowey 1986). In A. naccarii, tissue fatty acid and vitamin E composition has been shown to reflect dietary levels of these nutrients (Randall et al. 1992; Agradi et al. 1993) and this is known to be true, at least as dietary fat is concerned, in another sturgeon species (Xu et al. 1993). If A. naccarii fed the diets used in the present study, liver and heart fatty acid composition reflected that of the diet (C. Galli and L. Bolis, unpublished observations).

Long chain polyunsaturated fatty acids of the ω3 and ω6 series are precursors for the formation of the eicosanoids, metabolites that have a profound influence on cell, tissue and organ function. Increases in dietary and tissue ω3 LCPUFA levels lead to differences in the amount and type of eicosanoids produced and this is considered to be one possible reason for the effects of these fatty acids on mammalian cardiac physiology (Lands 1991). Vitamin E is the most important liposoluble antioxidant in animals (see Packer 1991, for a review of the literature), and, as such, influences processes of LCPUFA oxidation, including the formation of eicosanoids (Meydani et al. 1991). The exact mechanisms by which ω3 LCPUFA and vitamin E exert their effects on biological systems are not yet fully elucidated, particularly as their insertion into cell membranes throughout the organism is known to influence metabolic activities in a number of different tissues and organs (Diplock 1992; Fitzgerald 1993). The mechanism behind the differences in whole-animal physiology reported in the present study is worthy of investigation. There is a great deal of literature on the effects of diet on behaviour (see Miller 1981, for a review), but the ef-
fects of dietary fatty acid composition on spontaneous activity and behaviour have not been studied. It is well known, however, that α3 LCP/FFA have an important role in the development of the brain in young mammals (Neuringer et al. 1988) and they also have an effect on cerebral microcirculation in rabbits (Ellis et al. 1992).

This study revealed that A. naccarii can reduce M30 in hypoxia, as originally reported for A. transmanitana by Burgren and Randall (1978) but then discounted by Nonnotte et al. (1993) in A. baeri and by Randall et al. (1992) in A. naccarii. The present data obtained during exposure to steady-state hypoxia indicate that in some cases there can be a reduction in M30. The degree of reduction in M30 in hypoxia is dependent on M03 in normoxia, to the extent that those animals with a high M03 in normoxia tended to reduce M30 in hypoxia. Given that M03 in normoxia and the response to hypoxia were dependent on dietary fatty acid content, it is clear that specific components of a diet can influence an animal’s response to environmental change.

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