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Effects of diet on fatty acid composition of body zones in larvae of the sea bass *Dicentrarchus labrax*: a chemometric study

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Abstract Larvae of the sea bass *Dicentrachus labrax* were fed four *Artemia* sp. diets for 28 d. Three were nauplii enriched with emulsions of polyunsaturated fatty acids, and the fourth nauplii enriched with baker's yeast. At the end of the experimental period, the fatty acids of the bodies, heads and eyes of the larvae were analysed. A multivariate statistical method (discriminant analysis, DA) applied to the data revealed anatomical as well as dietary fatty acid pattern-discrimination. We propose here the use of discriminant analysis as a pattern-recognition method that will help to integrate the fatty acid information obtained in nutritional studies.

Introduction

Brine shrimp, *Artemia* sp. nauplii are the most-used larval food in marine aquaculture (Sorgeloos and Léger 1992). Despite their widespread use, *Artemia* sp. nauplii lack long-chain polyunsaturated fatty acids (PUFA), especially docosahexaenoic acid (22:6*n*-3) essential for marine organisms (Kanazawa et al. 1979; Léger et al. 1981; Bell et al. 1985; Watanabe 1988). This creates the necessity of enriching the nauplii, i.e. incubating them for 12 to 24 h in media containing PUFA in the form of microparticles or, more often, emulsions. The nauplii are passive filter-feeders that store the oil droplets from the emulsion or the microparticles in their digestive tract. This has the advantage of overcoming most of the problems caused by feeding non-enriched nauplii (sometimes complete mortality of the larval stock) to fish larvae, and offers the posibility of testing the effects of different levels of essential fatty acids on the body composition of the larvae. Several workers have already dealt with these aspects (Izquierdo et al. 1989; Koven 1991; Navarro et al. 1993). These studies have often resulted in long tables with more than 25 individual fatty acids and several fatty acid classes being reported for the dietary groups. The situation is even more complex when different factors such as the fatty acid composition of body zones (Navarro et al. 1993; Delgado et al. 1994; Webster et al. 1994), the fatty acid composition of the different lipid classes (Navarro et al. 1993), the age or origin of the fish (Hornung et al. 1994; Villarreal et al. 1994), etc., are considered. In addition, many other kinds of nutritional experiments have similarly complex designs (see for example Albentosa et al. 1994). To evaluate the results of such experiments, several Student's *t*-tests and/or unifactorial or multifactorial analyses of variance are performed. This is clearly a univariate – and sometimes wrongly applied – approach to a multivariate problem.

Multivariate analysis of fatty acid profiles has been used to compare fish eggs of different age and species (Vogt et al. 1986; Ulvund and Grahl-Nielsen 1988), different stocks of fish (Grahl-Nielsen and Mjaavatten 1992), populations of seals (Grahl-Nielsen et al. 1993), and even to perform chemotaxonomic studies on parasites (Berland et al. 1990) and bacterial communities (Haack et al. 1994). A study of dietary influence on the fatty acid composition of seals has been addressed by Grahl-Nielsen and Mjaavatten (1991), who concluded that in the blubber the dietary effect was insignificant compared with metabolic and other effects. Discriminant analysis (DA) is a pattern-recognition method (Wold et al. 1984) that helps to separate two or more groups from data provided for several variables (Manly 1994). Linear combinations of the independent (sometimes called predictor) variables are formed, and

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these serve for the classification of the cases into one of the various groups. The present paper integrates, in such a multifactorial approach, fatty acid data obtained from a nutritional study on sea bass larvae fed *Artemia* sp. nauplii enriched (Léger et al. 1986) with different oils. Fatty acids from the bodies, heads and eyes of the larvae have been analyzed, since neural (heads) and visual (eyes) lipids consisting mainly of long-chain *n*-PUFAs are selectively retained by vertebrates in general (Crawford et al. 1976; Neuringer et al. 1988; Bell and Tocher 1989; Bourre et al. 1989; Scott and Bazan 1989; Anderson et al. 1990; Bazan and Scott 1990; Lin et al. 1990). However, a dietary influence on the neural and visual lipids has been documented (Sastry 1985), especially during development (Martin et al. 1994; Bell et al. 1995), and thus they may be good indicators of *n*-3 PUFA nutritional deficiencies. In an earlier study, Navarro et al. (1993) reported the effects of dietary lipids on the lipid composition of neural and visual lipids of herring, *Clupea harengus*, larvae. Pagliarani et al. (1986) studied the effect of diets containing different fatty acids on the brain composition of adult sea bass and found that, in general, the fatty acids in the brains reflected the dietary history of the fish. These authors concluded that the brain of adult sea bass is capable of incorporating dietary fatty acids.

Our study had two objectives: (a) to determine the effect of experimental diets with different PUFA contents on various body regions of the larvae of the sea bass *Dicentrarchus labrax* as an example of a temperate fish-species, and (b) to integrate all the information in a pattern-recognition model capable of separating the groups and establishing a reference framework for further studies.

Materials and methods

Sea bass (*Dicentrarchus labrax*) larvae, 27 d old, were kept at 18 °C in sixteen 2-litre containers (Navarro 1990) at a density of 50 larvae per container. Using four replicate containers per treatment, the fish were fed ad libitum on enrichment-grade *Artemia* sp. nauplii (*Artemia* Systems, N.V., Ghent, Belgium, Lot: 0811) enriched in seawater (300 000 nauplii l^{-1}) for 24 h at 28 °C with one of the following enrichment diets (0.3 g diet l^{-1}, added every 12 h): (a) Diet T85: tuna orbital oil (ICI Bioproducts and Fine chemicals, Billingham, UK) containing 3.33% (wt:wt) Tween 85 (Sigma Chemical Co., Missouri, USA) and 1% (wt:wt) ethoxyquin (Sigma); (b) Diet T80: tuna orbital oil containing 3.33% (wt:wt) Tween 80 (Sigma) and 1% (wt:wt) ethoxyquin; (c) Diet SS: Super Selco (*Artemia* Systems, N.V., Ghent, Belgium); (d) Diet Y: yeast.

Every morning, the faeces accumulated at the bottom of the experimental containers were siphoned off, the number of survivors was counted, and the uneaten nauplii were removed and replaced with freshly enriched specimens. The naupliar concentration was again checked every afternoon. The experiment was continued for 28 d. At the end of the experimental period the fish were anaesthetized, counted, measured and weighed. Four replicates of five fish per treatment were used for dry weight determinations after drying in an oven for 48 h at 100 °C. Samples of enriched *Artemia* sp., plus the bodies, heads and eyes of the remaining sea bass were sampled and stored under nitrogen in chloroform:methanol (2:1, vol:vol) with 0.01% (wt:vol) butylated hydroxytoluene (BHT, Sigma) at − 20 °C. Lipids were extracted using the method of Folch et al. (1957), and transmethylation of fatty acids was carried out using Christie's (1982) method. Fatty acid methyl esters (FAME) were extracted with hexane:diethyl ether (1:1, vol:vol) and purified on thin-layer chromatography plates before gas-liquid chromatography analysis, as detailed by Navarro et al. (1992b).

Univariate statistical comparisons of the means were carried out using one-way analysis of variance (ANOVA) and subsequent Tukey tests (Zar 1984) after arcsine-transformation of the data (Zar 1984).

The amounts of linoleic acid (18:2*n*-6), linolenic acid (18:3*n*-3), arachidonic acid (20:4*n*-3), eicosapentaenoic acid (20:5*n*-3), docosapentaenoic acid (22:5*n*-3), docosahexaenoic acid (22:6*n*-3), total saturates (SATS), total monounsaturates (MONOS), as well as the 22:6*n*-3:20:5*n*-3 ratio of the bodies, heads and eyes of the fish were introduced into discriminant analysis (DA) models (Klecka 1980). Discrimination was based on the diets (T85, T80, SS, Y) and body zones: bodies, eyes, and heads. Further discrimination was also based on the diets within the different body zones. The pooled within-groups correlations between discriminating variables and the canonical discriminant functions (structure coefficients) were used to examine the loading of the functions, i.e. to analyse the contribution of a given variable in the discriminant function (Klecka 1980).

The calculations were performed with the statistical packages Stagraphics 6.0 (Statistical Graphics Corporation, Rockville, Maryland, USA) and the SPSS package (Nie et al. 1970).

Results

One diet was poor in *n*-3 long-chain PUFA (Diet Y) (4.9 mg g^{-1} dry wt), whereas the other three were rich in these fatty acids (Diet T85 = 32.9 mg g^{-1} dry wt, T80 = 31.1 mg g^{-1} dry wt and SS = 42.9 mg g^{-1} dry wt). The levels of 22:6*n*-3 were very similar in Diets T85, T80 and SS (17, 15.6 and 14.2 mg g^{-1} dry wt, respectively), but the ratio 22:6*n*-3:20:5*n*-3 in the first two treatments (ratio of 1.3 and 1.2, respectively) was twice that in the latter treatment (0.6) due to the higher level of 20:5*n*-3 in Diet SS (23.8 mg g^{-1} dry wt) than in the other two diets (T85 = 12.8 mg g^{-1} dry wt, T80 = 12.6 mg g^{-1} dry wt).

The weight, size and survival results (Table 1) varied little between the different dietary groups of *Dicentrarchus labrax*. Only the larvae fed Diet Y were significantly smaller than the other groups.

The results of the fatty acid analyses of total lipid from the bodies, heads and eyes of larvae fed different diets are shown in Tables 2, 3 and 4. The bodies of the fish fed nauplii supplemented with tuna oil (T85 and T80) had a higher level of 22:6*n*-3 than the bodies of those fed the SS diet. The ratio 22:6*n*-3:20:5*n*-3 in the bodies of the T85- and T80-fed fish was similar to that of their food. Likewise, the ratio 22:6*n*-3:20:5*n*-3 in the bodies of SS-supplemented larvae reflected the ratio in the nauplii diet. The heads of the larvae (Table 3) contained substantially more 22:6*n*-3 than the bodies, and in all cases the 22:6*n*-3:20:5*n*-3 ratio corresponded between larval bodies and nauplii diet. This resulted in substantially higher levels of 22:6*n*-3 in larval heads with tuna orbital oil supplementation in their

Table 1 *Dicentrarchus labrax*. Mean weight (mg), length (mm) and survival (%) of larvae fed four *Artemia* sp. diets. Standard deviations ($n = 4$) in parentheses. Means with different letter indicate significant differences ($P < 0.05$) (*T85*, *T80* tuna orbital oil; *SS* Super Selco; *Y* yeast; further details in "Materials and methods"

	Diets			
	T85	TT80	SS	Y
Wet weight	25.1 (5.6)	23.3 (3.6	23.1 (7.4)	19.8 (4.8)
Dry weight	5.1 (0.8)	4.8 (0.7)	4.9 (1.1)	4.2 (0.8)
Length	16.3 (1.6) a	16.2 (1.7) a	16.5 (1.6) a	15.5 (2.0) b
Survival	88 (4)	92 (3)	90 (8)	84 (9)

Table 2 *Dicentrarchus labrax*. Mean fatty acid composition (mg FA g^{-1} dry wt) of total lipid from bodies of larvae fed four types of enriched *Artemia* sp. nauplii. Standard deviations have been omitted for clarity. Within each row, means with different letter indicate significant differences ($P < 0.05$) (*PuFA* long-chain polyunsaturated fatty acids; *nd* not detected; *tr* trace)

Fatty acid	T85	T80	SS	Y
14:0	0.3	0.3	0.3	0.1
15:0	tr	tr	0.1	0.1
16:0	9.6 a	9.7 a	8.8 ab	6.7 b
16:1*n*-7	2.3 a	3.4 a	2.7 b	1.9 b
16:2	0.8	0.8	0.2	tr
17:0	0.7	0.7	0.6	0.5
16:3	0.9	1.1	0.8	0.9
18:0	5.3	5.2	5.4	4.7
18:1*n*-9	14.1 a	14.4 a	12.3 ab	9.8 b
18:1*n*-7	8.1 a	8.4 ab	7.0 ab	6.0 b
18:2*n*-6	3.1	3.2	3.0	2.7
18:3*n*-6	0.2	0.3	0.3	0.3
18:3*n*-3	9.8	9.9	9.3	8.5
18:4*n*-3	1.1 ab	1.2 a	1.1 ab	0.9 b
20:0	tr	tr	0.2	0.2
20:1*n*-9	1.7 ab	1.6 b	2.2 a	1.1 c
20:2*n*-6	0.3	0.9	0.8	0.5
20:3*n*-6	0.2	tr	tr	0.1
20:4*n*-6	4.4 ab	4.9 a	2.9 c	3.5 bc
20:3*n*-3	0.7	0.8	0.5	0.6
20:4*n*-3	0.5	0.4	0.5	0.4
20:5*n*-3	8.8 b	8.6 b	13.5 a	5.7 c
22:0	0.5	0.4	0.4	0.5
22:1*n*-11	0.2	tr	0.3	0.1
22:5*n*-6	1.0 a	1.1 a	0.4 b	0.2 b
22:5*n*-3	0.8 b	0.8 b	1.8 a	tr
22:6*n*-3	11.1 a	12.4 a	10.1 a	0.5 c
24:1	nd	nd	nd	0.1
Total saturates	16.5	16.2	15.7	12.7
Total monoenes	27.1 a	27.9 a	24.5 ab	19.0 b
Total PUFA	43.7 a	46.4 a	45.4 a	24.8 b
Total *n*-3	32.8 a	34.2 a	37.8 a	17.5 b
Total *n*-6	9.2 ab	10.5 a	7.6 bc	7.3 c
22:6*n*-3/20:5*n*-3	1.3 a	1.4 a	0.8 b	0.1 c

Table 3 *Dicentrarchus labrax*. Mean fatty acid composition (mg FA g^{-1} dry wt) of total lipid from heads of sea larvae fed four types of enriched *Artemia* sp. nauplii. Standard deviations omitted for clarity. Within each row, means with different letter indicate significant differences ($P < 0.05$)

Fatty acid	T85	T80	SS	Y
14:0	0.7 ab	0.8 a	0.5 ab	0.5 b
15:0	tr	0.4 a	0.2 b	0.1 b
16:0	18.1 a	18.0 a	16.2 ab	13.9 b
16:1*n*-7	7.4 ab	7.6 a	5.4 bc	4.7 c
16:2	1.4	1.5	nd	nd
17:0	1.3	1.4	1.1	1.1
16:3	2.2 a	2.1 a	1.7 b	1.8 ab
18:0	9.4	9.6	9.9	9.9
18:1*n*-9	27.7 a	25.8 ab	21.9 bc	19.5 c
18:1*n*-7	12.2	14.8	14.1	13.9
18:2*n*-6	5.6 ab	5.9 a	5.7 a	4.5 b
18:3*n*-6	0.5	0.5	0.5	0.4
18:3*n*-3	19.3	17.7	17.3	15.1
18:4*n*-3	2.2	2.3	2.1	1.9
20:0	tr	0.3	0.2	0.2
20:1*n*-9	3.2 a	3.0 a	3.8 a	2.2 c
20:2*n*-6	1.2	1.2	1.2	1.0
20:3*n*-6	0.2	tr	tr	tr
20:4*n*-6	7.2 a	7.1 a	5.2 a	5.6 a
20:3*n*-3	1.2	1.1	1.0	1.0
20:4*n*-3	0.8 ab	0.7 ab	0.9 a	0.6 b
20:5*n*-3	14.9 b	13.2 bc	22.4 a	9.6 c
22:0	0.9	0.4	0.6	0.5
22:1*n*-11	0.3	0.2	0.3	tr
21:5	nd	tr	0.2	tr
22:4*n*-6	0.2	tr	tr	nd
22:5*n*-6	1.3 a	1.5 a	0.5 b	0.3 b
22:5*n*-3	1.5 b	1.4 b	3.0 a	0.3 c
22:6*n*-3	20.0 a	19.0 a	16.8 a	1.3 b
24:1	nd	nd	nd	0.2
Total saturates	30.4	30.8	28.6	26.2
Total monoenes	50.6	51.4	45.5	40.4
Total PUFA	79.9 a	74.7 a	78.6 a	43.2 b
Total *n*-3	63.5 a	58.3 a	65.1 a	31.1 b
Total *n*-6	16.4 a	16.4 a	13.2 ab	11.8 b
22:6*n*-3/20:5*n*-3	1.3 a	1.4 a	0.8 b	0.1 c

diet than in those with SS supplementation (Table 3), whereas the latter treatment increased the content of 20:5*n*-3 in the heads. The eyes of the larvae had the highest 22:6*n*-3:20:5*n*-3 ratio (Table 4) for T85-, T80- and SS-fed fish. Here the 22:6*n*-3 level after tuna orbital oil supplementation was significantly higher than after SS supplementation (Table 4). As expected, the Y diet yielded larvae with the lowest levels of 22:6*n*-3 and the lowest 22:6*n*-3:20:5*n*-3 ratio. Interesting is the higher content of 18:3*n*-3 in the bodies and heads and the higher level of 22:5*n*-3 in the bodies and heads of SS-fed fish.

The DA showed that in all cases the first discriminant function accounted for ~80% of the variability, with the second function contributing almost all of the remainder. The DA model clearly separated fish fed the different diets, even when body zones were pooled (Fig. 1). The structure coefficients showed that 22:6*n*-3 and the 22:6*n*-3:20:5*n*-3 ratios contributed most to Function 1, with 22:5*n*-3, 20:5*n*-3 and 20:4*n*-6 producing the highest loadings on Function 2. The fish fed the low PUFA diet (yeast) were the most widely separated group, whereas those fed the two tuna oil

Table 4 *Dicentrarchus labrax.* Mean fatty acid composition (mg FA g^{-1} dry wt) of total lipid from eyes of larvae fed four types of enriched *Artemia* sp. nauplii. Standard deviations have been omitted for clarity. Within each row, means with different letter indicate significant differences ($P < 0.05$)

Fatty acid	T85	T80	SS	Y
14:0	0.2	0.2	0.2	0.2
15:0	tr	tr	tr	0.1
16:0	6.5	6.3	5.7	6.6
16:1*n*-7	1.2	2.0	1.5	2.0
16:2	0.3	0.3	tr	nd
17:0	0.5	0.5	0.4	0.5
16:3	0.5	0.5	0.4	0.6
18:0	4.6	4.4	4.1	5.6
18:1*n*-9	6.1	6.2	5.0	6.9
18:1*n*-7	4.5	4.3	3.8	5.5
18:2*n*-6	1.3	1.4	1.2	1.8
18:3*n*-6	tr	0.1	tr	0.2
18:3*n*-3	3.4	3.4	2.9	4.8
18:4*n*-3	0.4	0.4	0.3	0.5
20:0	tr	tr	tr	0.1
20:1*n*-9	0.7	0.7	0.8	0.6
20:2*n*-6	0.5	0.4	0.4	0.5
20:3*n*-6	tr	0.1	tr	0.1
20:4*n*-6	2.2	2.3	1.7	3.2
20:3*n*-3	0.4 ab	0.5 ab	0.2 a	0.8 b
20:4*n*-3	0.4 ab	0.3 ab	0.3 a	0.5 b
20:5*n*-3	3.8 a	3.6 a	4.6 ab	7.3 b
22:0	tr	tr	tr	0.2
22:5*n*-6	0.7	0.6	tr	tr
22:5*n*-3	1.2	1.2	1.7	1.8
22:6*n*-3	13.3 a	13.6 a	9.3 b	1.7 c
Total saturates	11.8	11.5	10.4	13.2
Total monoenes	13.4	13.2	11.1	15.0
Total PUFA	28.6	28.6	23.2	23.7
Total *n*-3	23.7	23.8	19.6	18.0
Total *n*-6	4.9	4.8	3.5	5.7
22:6*n*-3/20:5*n*-3	3.5 a	3.7 a	2.0 b	0.2

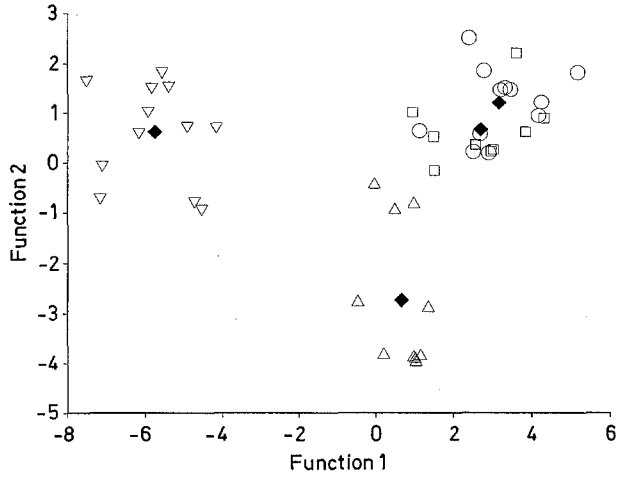

Fig. 1 *Dicentrarchus labrax.* Scores for first two discriminant functions of fatty acid data of larvae fed four *Artemia* sp. diets. Discrimination based on dietary groups (□ T85; ○ T80; △ SS; ▽ Y; ◆ group centroids, where Diets T85, T80 = tuna orbital oil; SS = Super Selco; and Y = yeast; further details in "Materials and methods")

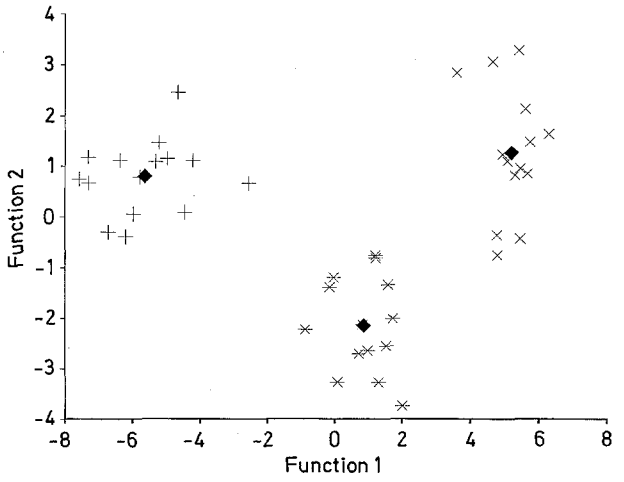

Fig. 2 *Dicentrarchus labrax.* Scores for first two discriminant functions of fatty acid data for zones of larvae fed different *Artemia* sp. diets. Discrimination based on anatomical zones (⁎ bodies; + heads; × eyes; ◆ group centroids)

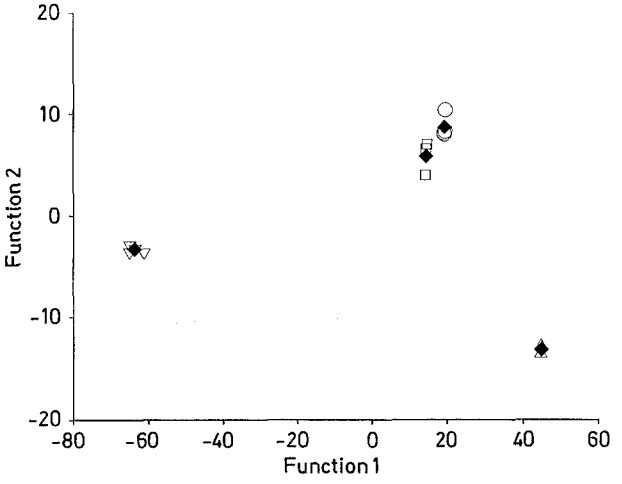

Fig. 3 *Dicentrarchus labrax.* Scores for first two discriminant functions of fatty acid data of bodies of larvae fed four *Artemia* sp. diets. Discrimination based on dietary groups. Symbols as in Fig. 1

diets (which varied only in their emulsifying agent) clearly overlapped.

Fig. 2 illustrates the separation of body-zone fatty acid patterns, irrespective of diet. Here, the highest loadings were produced by 18:3*n*-3, 18:2*n*-6, MONOS and SATS on Function 1, and by the 22:6*n*-3:20:5*n*-3 ratio and 22:6*n*-3 on Function 2.

Dietary patterns were particularly well discriminated within each body zone. In bodies (Fig. 3), the 22:6*n*-3:20:5*n*-3 ratio, 22:6*n*-3 and 20:5*n*-3 contributed most to Function 1, with the same three variables loading heavily on Function 2. In heads (Fig. 4), the most important variables in the first and second functions were the 22:6*n*-3:20:5*n*-3 ratio and 22:5*n*-3. Finally, the 22:6*n*-3:20:5*n*-3 ratio and 22:6*n*-3 contributed

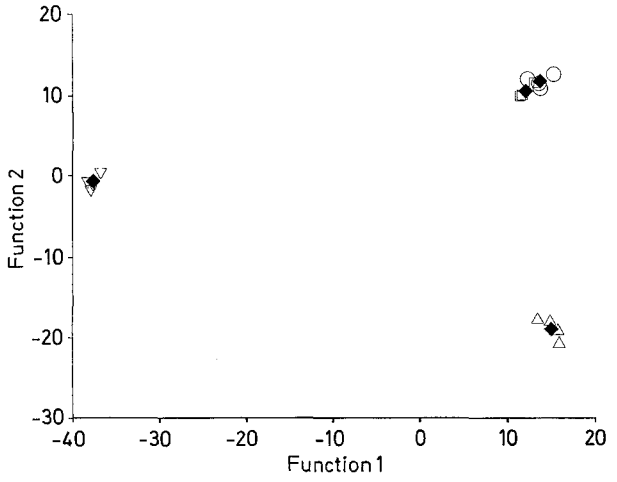

Fig. 4 *Dicentrarchus labrax*. Scores for first two discriminant functions of fatty acid data of heads of larvae fed four *Artemia* sp. diets. Discrimination based on dietary groups. Symbols as in Fig. 1

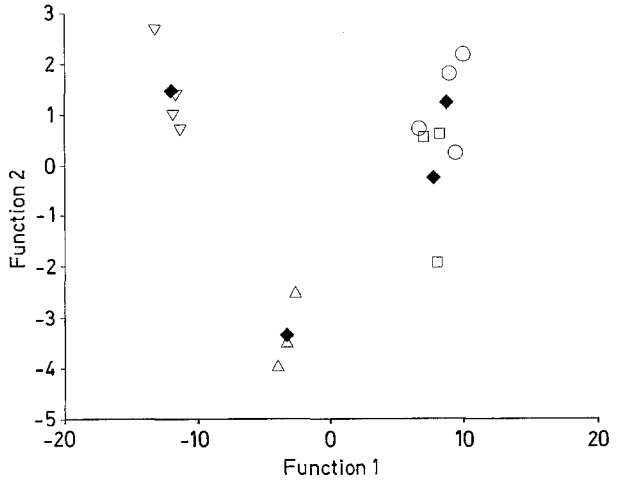

Fig. 5 *Dicentrarchus labrax*. Scores for first two discriminant functions of fatty acid data of eyes of larvae fed four *Artemia* sp. diets. Discrimination based on dietary groups. Symbols as in Fig. 1

most to Function 1 in the bass eyes, with 22:6*n*-3, 18:3*n*-3, 20:4*n*-6 and 18:2*n*-6 producing the highest loadings on Function 2 (Fig. 5).

Discussion and conclusions

The results obtained in the present study on *Dicentrarchus labrax* are in agreement with those of Navarro et al. (1993) for the Atlantic herring *Clupea harengus*. Navarro et al. showed that two *Artemia* sp. diets with different fatty acid content fed to the larvae for 30 d had a drastic effect on the fatty acid composition of the lipid classes of the bodies, heads and eyes of the fish. Although in the present work only the fatty acids from the total lipids were analysed, there is enough evidence to show that the neural and visual lipids of the larvae of a more temperate species such as the sea bass are also drastically affected by dietary lipids. Furthermore, and in accordance with the results of the present study, Navarro et al. reported that the biometrical differences between the two groups of herring were but slight.

The study of Navarro et al. (1993), among others (see for example Albentosa et al. 1994), provided a good example of the difficulties involved in interpreting results obtained in this sort of nutritional experiment. The multivariate approach employed in the present study clearly revealed different fatty acid patterns, both anatomical and dietary, and that the dietary influence was consistent in the different body zones of the larvae. Together with other multivariate methods (Grahl-Nielsen and Mjaavatten 1991), this approach shares the advantage of handling fatty acid profiles simultaneously. From our results it is clear that in terms of PUFA depletion (Martin et al. 1994) even the most conservative tissues (neural and visual) are clearly affected by diet (Figs. 4 and 5).

Viga and Grahl-Nielsen (1990) proposed that dietary effects on the fatty acid composition of fishes may result from the use of very different experimental diets and/or a lack of quantification of the fatty acid composition of the tissues affected. The former aspect is true for the present work, but not the latter. Viga and Grahl-Nielsen, citing the work of Muje et al. (1989) on the vendace *Coregonus albula* L., also suggested that metabolic control of the fatty acid composition develops with increasing age. This could be the case for herring (Navarro et al. 1993) and sea bass (present study), since a tendency for the structural lipids of the brain to maintain their typical fatty acid pattern irrespective of the dietary input has been suggested for adult sea bass (Pagliarani et al. 1986). In accordance with this, it is interesting to note that younger stages of herring possess surprisingly high amounts of neutral lipids in their brains and eyes (Mourente and Tocher 1992; Navarro et al. 1993) compared to other developmental stages. Since the neutral lipids are more directly affected by diet (Linko et al. 1985), one would expect a stronger dietary influence on the neural and visual lipids of the younger stages. The effect of dietary lipids on the fatty acid composition of the phospholipids of these tissues has also been clearly demonstrated (Navarro et al. 1993). This, together with the effect on the neutral lipids, would contribute to enhance the influence of dietary lipids on the fatty acid composition of the heads and eyes of the larvae.

Our results present sufficient evidence to suggest a tissue-related metabolic capacity parallel to the hypothetical age-related metabolic capacity mentioned above. This is clearly supported by the different fatty acid patterns obtained for the body zones analysed, irrespective of the diet (Fig. 2). More specifically, the ratio 22:6*n*-3:20:5*n*-3 in the supplemented *Artemia* sp. was transmitted unaltered to the larval bodies and heads. The eyes, however, had a much

higher 22:6*n*-3:20:5*n*-3 ratio than that in the larvae's supplemented naupliar diet. Therefore, there must be a selective discrimination for 22:6*n*-3 in the growing eyes. This raises the question of whether the 22:6*n*-3 in the naupliar diet is sufficient to fulfill the needs of the eyes or, indeed, of any other quantitatively minor but functionally very important tissue, including the brain. Are the high levels of 18:3*n*-3 in the heads a reflection of an under-efficient tissue-specific bioconversion pathway towards 22:6*n*-3? Linolenic acid is one of the most abundant fatty acids in *Artemia* sp. nauplii (Léger et al. 1986; Navarro et al. 1992a), and the larvae may well accumulate this fatty acid in the absence of sufficient amounts of other polyunsaturated fatty acids. In fact, the high content of 22:5*n*-3 mainly in the bodies and heads of the SS-fed fish may indicate a very significant capacity to elongate 20:5*n*-3 but a negligible capacity to perform delta-4 desaturation, as suggested by Fox (1990) and Navarro et al. (1993) for the herring.

The multivariate approach also differentiates among the "good" diets, a very difficult process when several "good" versus "bad" treatments are compared by means of univariate approaches (see for example the lack of significant differences in the levels of 22:6*n*-3 between the T- and SS-fed larvae in (Table 3).

The use of multivariate statistics, and discriminant analysis in particular, has a further advantage since it can be used as a reference framework to examine the results obtained in other studies with different foods and different species, and with the same species at different ages and from different origins, seasons, etc. To illustrate this, the T85 data were used to cross-validate the model presented here. The DA can be performed using data from the T80, SS and Y larvae only. The discriminant functions derived from this analysis can then be used to locate the T85 data in the graphs. In each case, the tested T85 points overlap with those of the similar T80 dietary group.

Integrated fatty acids are well discriminated by the model in terms of dietary group, body zone and dietary patterns within body zones, clearly illustrating the sensitivity of fish larvae to dietary fatty acid composition even during short-term nutrition experiments. This sensitivity is particularly apparent in the fatty acid pattern of the eyes of the fish from the various dietary groups, since the eyes are known to be a relatively conservative tissue in terms of fatty acid composition. Discriminant analysis establishes a reference framework that will help in the comparison of results of further nutrition studies using the same species with different diets, different species fed the same diets, fish of different ages, etc.

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