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The use of silages prepared from fish neural tissues as enrichers for rotifers (*Brachionus plicatilis*) and *Artemia* in the nutrition of larval marine fish

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Abstract

Marine fish larvae have a requirement for large quantities of $n-3$ highly unsaturated fatty acids (HUFA), particularly docosahexaenoic acid (DHA; 22:6$n-3$), and dietary deficiencies of these induce a range of pathologies in fish including behavioural abnormalities. Therefore, there is a growing demand for natural lipids highly enriched in DHA as nutritional supplements during larval development. In the present study, DHA-rich products were obtained from fish neural tissues by a procedure involving ensiling in organic acids followed by neutralisation, drying, milling and sieving to produce the final particulate, crumbed products. Lipid content varied in the neural tissue silages from 5.5% to 46.5% but all lipid contained high percentages of DHA and high DHA:EPA (eicosapentaenoic acid; 20:5$n-3$) ratios ranging from 3.5 to 5.4. Tuna eye silage was a high lipid, high triacylglycerol (90.6% of total lipid) product and was particularly effective in increasing DHA content, outperforming a commercial product, in rotifer (*Brachionus plicatilis*) enrichment. Cod brain/eye silage had an intermediate content (18.2%) of lipid, which contained almost 50% as phospholipid, and proved as effective as a commercial enricher in increasing DHA content in *Artemia* nauplii. Moreover, *Artemia* nauplii, enriched with the cod brain/eye silage were more effective than nauplii enriched with a commercial enricher in increasing the DHA content of larval turbot brain. In conclusion, tuna eye and cod brain/eye silages are useful as enrichers of *Artemia* nauplii and rotifers for feeding marine fish larvae prior to weaning onto pelleted formulated diets.

Keywords: *Artemia*; Rotifers; Docosahexaenoic acid; Lipids; Silage; Enrichment

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1. Introduction

The neural tissues of all vertebrates, including fish, are rich in docosahexaenoic acid (22:6*n*–3; DHA) (Sastry, 1985). It has long been known that dietary *n*–3 polyunsaturated fatty acids (PUFA) are essential for the optimal growth and development of fish (Henderson and Tocher, 1987), but more recently, it has been established in mammals, including man, that the crucial aspect under-pinning the essentiality of *n*–3 PUFA is the role of DHA in neural tissues (Neuringer et al., 1988; Bazan, 1990). The essentiality of DHA is focused during the early developmental stages when neural tissue is increasing greatly, which in fish includes the free-living larval and early juvenile stages (Sargent et al., 1993). Consistent with this, it has been shown that DHA specifically accumulates in the brain of fish during early development (Mourente et al., 1991; Mourente and Tocher, 1992). Recently, it was shown that juvenile herring reared on diets deficient in DHA have impaired visual and/or neural functioning compared with fish reared on diets supplemented with DHA as evinced by impaired predation responses (Bell et al., 1995a). Therefore, marine fish larvae have a requirement for large quantities of *n*–3 PUFA, particularly DHA, and dietary deficiencies of these induce a range of pathologies in fish including behavioural abnormalities (Henderson and Tocher, 1987; Sargent et al., 1989).

The small size of marine fish larvae has dictated that conventional pelleted diets are initially unsuitable and other techniques such as microencapsulation have not fully resolved the problem of feeding larvae prior to weaning onto pelleted diets (Watanabe and Kiron, 1994). As a result, a succession of live prey species going through algae, rotifers such as *Brachionis plicatilis* and *Artemia* nauplii is still the predominant feeding strategy up to weaning (Watanabe and Kiron, 1994). However, these two live prey species, particularly *Artemia*, are deficient in long-chain *n*–3 highly unsaturated fatty acids (HUFA, $\geq C_{20}$ with three or more double bonds), especially DHA (Leger et al., 1986, 1987; Navarro et al., 1993). Therefore, the *n*–3 HUFA and DHA content of the live prey has to be increased prior to being offered to the larvae, essentially by pre-feeding with *n*–3 HUFA-rich materials/products (Scott and Middleton, 1979; Watanabe et al., 1983; Leger et al., 1985, 1987). As a result, many different enrichment diets have been used including microalgae, HUFA-modified yeasts, compound diets, coated microparticles, oil-based emulsions and microencapsulated preparations (Leger et al., 1986; Walford and Lam, 1987; Dhert et al., 1990; Støttrup and Attramadal, 1992; Rimmer et al., 1994; Southgate and Lou, 1995).

Due to the above there is a growing demand for natural lipids highly enriched in DHA as nutritional supplements during larval development. This demand cannot be easily met by conventional commercial fish oils because their content of DHA seldom exceeds 15% (Sargent, 1976; Ackman, 1980). An obvious source of natural lipids rich in DHA is the brain and retinal tissue contained in abundant fish head by-products of commercial fisheries (Tocher and Harvie, 1988). In the present study, a DHA-rich product was prepared from fish neural tissues by a procedure involving ensiling in organic acids followed by neutralisation, drying, milling and sieving to produce the final particulate, crumbed products. The efficacy of these products in increasing the DHA content of *Artemia* nauplii and rotifers was assessed in enrichment trials in comparison with widely used commercial enricher products using standard techniques. The

dietary/nutritional suitability of fish neural silage-enriched *Artemia* nauplii for fish larvae was also investigated. The trials showed that crumbed fish neural tissue silage is a useful nutritional supplement for mariculture.

2. Materials and methods

2.1. Materials

In Scotland, cod (*Gadus morhua*) and whiting (*Merlangus merlangus*) heads were obtained from Golden Sea Produce (GSP) Ltd., Hunterston, Ayrshire (via Ayr fish market) on the day the fish were landed. In Spain, tuna (*Thunnus thunnus*) and blue shark (*Prionance glauca*) eyes were obtained from Algeciras fish market, Andalucia, Spain. Ensiling fluid was a commercial product, ''Addsafe'', which was simply an ammoniated solution of 75% formic acid (BP Chemicals, Sunbury-on-Thames, Middlesex). High-performance thin-layer chromatography (HPTLC) plates ($10 \times 10 \times 0.15$ mm), pre-coated with silica gel 60 were obtained from Merck (Darmstadt, Germany). All solvents were of HPLC grade and were obtained from Rathburn Chemicals Ltd. (Walkerburn, UK). Pepsin, butylated hydroxyanisole, butylated hydroxytoluene (BHT), propyl gallate, citric acid, propylene glycol and sodium hydroxide were purchased from Sigma Chemical Co. Ltd., Poole, Dorset.

2.2. Preparation of silages

Fish brains and/or eyes were removed from the fish heads on ice and weighed. Ensiling fluid up to 3.5% by weight was added and the resultant mixture blended using a Waring blender. When using whole heads, the weighed heads were minced prior to the addition of ensiling fluid. Crude pepsin was added to the mixture at 0.1% (1 g per kg) followed by 0.1% of an antioxidant mixture (containing 60 mg ml^{-1} butylated hydroxyanisole, 60 mg ml^{-1} propyl gallate and 40 mg ml^{-1} citric acid) dissolved in propylene glycol. The proteolytic enzyme, pepsin, was added to the ensiling mixture due to the anticipated low levels of endogenous acid-catalysed hydrolytic enzymes present in brain and eye tissue (Gildberg, 1993). The reaction vessel was placed in a water bath at 25°C and slowly mixed with an overhead stirrer. The reaction was continued for 16 h by which time the ensiling process was complete.

2.3. Processing of silages

Liquid silages were neutralised by the addition of solid sodium hydroxide pellets. The amount of sodium hydroxide being determined by calculation based on the known amount and concentration of acid (ensiling fluid) added. The addition of solid sodium hydroxide meant that no additional water was added which would have increased the volume for freeze-drying. The pH of the neutralised silage was determined prior to freeze-drying to ensure neutrality was achieved. Freeze-drying was carried out using an Edwards freeze-drying apparatus.

The dried silages were ground in a Waring blender to produce a basic crumble. Particle size could be reduced by increasing blending time and additional grinding/milling in a mortar and pestle. The crumbed product was finally sieved through fine mesh grids to optimise the size and improve the uniformity of the crumble. Final particle size was approximately 20 ± 10 μm.

2.4. *Use of silages as enricher feeds for* Artemia *and rotifers*

The crumbed silages were used as enricher feeds for *Artemia* using two methods. In laboratory-based trials, a protocol similar to that recommended for use with a commercial enricher Protein Selco (INVE Aquaculture NV, Baasrode, Belgium) was followed. Protein Selco was the most suitable product for comparison as it was also a dry diet, although it is only specifically recommended for rotifer enrichment. *Artemia* (200 000 l^{-1}) were enriched over 24 h at 28°C in artificial seawater with aeration in excess of 4.5 ppm oxygen. Protein Selco (0.6 g l^{-1}) and crumbed silages were added in equivalent doses at 0 and 12 h. *Artemia* were collected after 24 h for lipid and fatty acid analyses as described above. Rotifers, strain S1 of *Brachionus plicatilis*, were enriched with tuna eye silage and Protein Selco using a protocol similar to that recommended by the manufacturer of Protein Selco as described in more detail in the Table legends. Other details of enrichment procedures are given in the legends to the tables or figures. In 'on-site' trials, the procedures routinely used by a commercial mariculture company were followed with parallel enrichments under identical conditions for the silages and commercially available enrichers.

2.5. *Larval fish feeding trials*

Silages were used as feeds for larval turbot (*Scophthalmus maximus*) indirectly via enriched-*Artemia*. For this, the silage-enriched *Artemia* produced as described above and in the figure legends replaced commercially enriched *Artemia* in the diets of the larval fish at a commercial fish farm.

2.6. *Lipid extraction and content of silages*

Total lipid contents of the silages, rotifers, *Artemia* and fish tissues were determined gravimetrically after extraction with chloroform/methanol (2:1, v/v) essentially by the method of Folch et al. (1957). All solvents contained 0.01% BHT as antioxidant.

2.7. *Lipid class composition analyses*

Lipid classes were separated by one-dimensional, double-development HPTLC using methyl acetate/propan-2-ol/chloroform/methanol/0.25% aqueous KCl (25:25:25:10:9, by volume) and hexane/diethyl ether/acetic acid (85:15;1.5, by volume) as developing solvents as described previously (Tocher and Harvie, 1988). Lipid classes were quantified by charring followed by calibrated scanning densitometry using a Shimadzu CS-9000 dual wavelength flying spot scanner and DR-2 recorder in Scotland and a

Shimadzu CS-9001PC dual wavelength flying spot scanner in Spain (Olsen and Henderson, 1989).

2.8. Fatty acid analyses

Fatty acid methyl esters were prepared from total lipid by acid-catalysed transmethylation at 50°C for 16 h (Christie, 1982) and were extracted and purified as described previously (Tocher and Harvie, 1988). In Scotland, methyl esters were analysed in a Carlo Erba Vega GC6000 gas chromatograph (Carlo Erba Instrumentazone, Milan, Italy) equipped with a CP Wax 51 fused silica capillary column (50 m × 0.32 mm i.d., Chrompack U.K. Ltd., London), with on-column injection using hydrogen as carrier gas and a biphasic thermal gradient from 50°C to 225°C. Individual fatty acid methyl esters were identified by comparison with known standards and a well-characterised fish oil and by reference to published data as described previously (Tocher and Harvie, 1988) and were quantified using a Carlo Erba DP800 data processor. All solvents contained 0.01% BHT as an antioxidant.

Identical methods were used in Spain except that analysis was performed using a Hewlett-Packard 5890A gas chromatograph equipped with a chemically bonded (PEG) Omegawax-320 fused silica wall-coated capillary column (30 m × 0.32 mm i.d., Supelco Inc., Bellefonte, USA) and using on-column injection. Hydrogen was used as

Table 1
Lipid content and class compositions of fish neural tissue silages. Results are means ± s.d. of three silage preparations except for the data without errors which are the means of two silages

Lipid	Silages				
	Whiting head	Whiting brain/eye	Cod brain/eye	Tuna eye	Shark eye
Lipid content (% dry weight)	39.0	5.5 ± 0.5	18.2 ± 1.4	46.5 ± 4.8	5.7
Lipid class composition (% of total lipid)					
Phosphatidylcholine	4.3	19.0 ± 1.8	15.6 ± 0.8	1.6 ± 0.7	9.0
Phosphatidylethanolamine	2.5	15.2 ± 2.3	15.0 ± 0.7	0.8 ± 0.5	6.6
Phosphatidylserine	1.4	6.0 ± 1.0	5.1 ± 0.3	0.8 ± 0.2	5.3
Phosphatidylinositol	0.6	2.1 ± 0.3	2.1 ± 0.1	0.5 ± 0.4	1.6
Cardiolipin/phosphatidic acid	0.2	2.6 ± 0.2	1.1 ± 0.1	n.d.	0.4
Sphingomyelin	2.3	1.0 ± 0.1	1.8 ± 0.2	0.5 ± 0.2	3.2
Lyso-phospholipid	1.1	0.7 ± 0.4	2.3 ± 0.5	n.d.	n.d.
Cerebroside/sulphatide	0.9	5.5 ± 0.4	5.1 ± 0.3	n.d.	1.7
Total polar lipids	13.3	52.1 ± 3.3	48.1 ± 1.9	4.4 ± 1.6	27.8
Pigment	t	1.3 ± 0.6	1.5 ± 0.3	t	t
Total neutral lipids	86.7	46.6 ± 3.3	50.4 ± 1.9	95.6 ± 1.6	72.2
Triacylglycerol	59.0	5.6 ± 0.5	4.0 ± 0.2	90.6 ± 1.1	44.9
Cholesterol	12.6	18.6 ± 1.9	23.6 ± 2.0	3.5 ± 1.1	17.7
Free fatty acids	8.8	15.0 ± 1.1	16.1 ± 0.8	1.4 ± 0.5	9.6
Steryl ester	6.3	7.4 ± 0.8	6.7 ± 0.4	t	t

n.d., not detected; t, trace (< 0.05%).

carrier gas with an oven thermal gradient from an initial 50°C to 180°C at 25°C min^{-1} and then to a final temperature of 235°C at 3°C min^{-1}. The final temperature was maintained for 10 min. Separated fatty acid methyl esters were identified as above and quantified by means of a PC linked to the GC and utilising Hewlett-Packard 3365 ChemStation software.

3. Results

The silages from whole whiting head and tuna eye had the highest lipid contents at around 40% or above with the cod brain/eye silage containing 18.2% of the dry weight as lipid (Table 1). In contrast the whiting brain/eye and shark eye silages had relatively

Table 2
Fatty acid compositions (% of total fatty acids) of fish neural tissue silages. Results are presented as percentages of weight and are means ± s.d. of three silage preparations except for the data without errors which are the means of two silages. Totals include minor fatty acids such as 15:0, 17:0, 20:0, 18:3*n*−6, 20:3*n*−6, 22:4*n*−6, 22:5*n*−6, 18:4*n*−3, 20:4*n*−3 and C_{16} PUFA generally present at < 0.2%

Fatty acid	Silages				
	Whiting head	Whiting brain/eye	Cod brain/eye	Tuna eye	Shark eye
14:0	5.6	0.8 ± 0.4	0.6 ± 0.1	2.9 ± 0.1	2.8
16:0	17.3	13.3 ± 0.2	12.3 ± 1.0	17.2 ± 0.3	17.4
18:0	2.3	7.7 ± 0.4	8.7 ± 0.2	4.1 ± 0.7	5.3
Total saturates	25.6	22.1 ± 0.9	22.0 ± 1.2	26.8 ± 0.5	29.3
16:1*n*−7	6.7	2.5 ± 0.5	2.8 ± 0.3	6.0 ± 0.1	5.9
18:1*n*−9	12.6	18.0 ± 0.9	20.4 ± 1.8	18.1 ± 0.7	16.7
18:1*n*−7	3.1	2.6 ± 0.2	2.8 ± 0.3	2.5 ± 0.1	2.0
20:1*n*−9	8.3	3.2 ± 0.1	3.5 ± 0.3	2.4 ± 0.4	3.0
22:1	14.1	1.7 ± 0.1	1.5 ± 0.1	0.8 ± 0.1	n.d.
24:1	0.5	3.5 ± 0.3	5.0 ± 0.2	0.9 ± 0.0	n.d.
Total moncenes	44.7	31.5 ± 1.3	36.0 ± 2.3	30.9 ± 0.3	27.6
18:2*n*−6	1.1	1.1 ± 0.2	0.4 ± 0.1	1.1 ± 0.1	1.3
20:2*n*−6	0.2	0.1 ± 0.0	0.1 ± 0.0	0.7 ± 0.3	2.7
20:4*n*−6	0.9	1.6 ± 0.3	1.6 ± 0.2	1.5 ± 0.3	1.9
Total *n*−6 PUFA	2.6	3.3 ± 0.5	2.6 ± 0.2	6.1 ± 0.7	6.9
18:3*n*−3	0.9	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	0.7
20:5*n*−3	8.3	5.5 ± 0.4	6.6 ± 0.5	5.7 ± 0.5	5.2
22:5*n*−3	0.9	1.0 ± 0.1	1.0 ± 0.1	1.8 ± 0.1	1.7
22:6*n*−3	9.5	29.6 ± 1.5	23.1 ± 1.4	21.3 ± 0.6	19.5
Total *n*−3 PUFA	21.9	36.8 ± 1.7	31.5 ± 1.6	32.7 ± 1.2	28.4
Total *n*−3 HUFA	20.4	36.4 ± 1.7	31.1 ± 1.6	30.0 ± 1.1	27.1
Total PUFA	26.1	40.4 ± 1.8	34.2 ± 1.7	38.8 ± 1.6	36.2
22:6*n*−3/20:5*n*−3	1.1	5.4	3.5	3.7	3.8
Total dimethylacetals	0.3	4.3 ± 1.3	4.5 ± 1.2	t	t

HUFA, highly unsaturated fatty acids; PUFA, polyunsaturated fatty acids; t, trace (< 0.05%).

Table 3
Absolute fatty acid contents (μg fatty acid mg^{-1} dry weight of silage) of the tuna and blue shark eye silages

Fatty acid	Silage	
	Tuna eye	Blue shark eye
14:0	13.2 ± 1.3	1.3
15:0	4.0 ± 0.4	1.3
16:0	77.1 ± 9.2	8.2
16:1*n*–7	27.0 ± 3.1	2.7
C16PUFA	10.2 ± 1.3	0.8
17:0	3.4 ± 0.6	0.3
18:0	18.2 ± 1.7	2.5
18:1	80.6 ± 6.8	8.8
18:2*n*–6	5.2 ± 1.1	0.6
18:3*n*–3	2.8 ± 0.9	0.3
18:4*n*–3	3.8 ± 0.8	0.4
20:0	1.6 ± 0.5	0.2
20:1	11.2 ± 3.3	1.3
20:2*n*–6	3.7 ± 0.2	1.3
20:3*n*–6	1.0 ± 0.7	0.1
20:4*n*–6	6.7 ± 0.6	0.9
20:3*n*–3	1.2 ± 0.4	0.0
20:4*n*–3	2.8 ± 0.6	0.2
20:5*n*–3	25.9 ± 5.9	2.4
22:1	3.6 ± 0.9	0.0
22:5*n*–6	4.4 ± 0.4	0.3
22:5*n*–3	8.2 ± 1.6	0.8
22:6*n*–3	95.9 ± 15.1	9.2

PUFA, polyunsaturated fatty acid.

low lipid contents. The total lipid of the gadoid brain/eye silages contained approximately 50% polar lipids, primarily glycerophospholipids, with the main neutral lipids being cholesterol and free fatty acids (Table 1). The lipid of the other silages was dominated by triacylglycerol (TAG), particularly the lipid in the tuna eye silage which was 90.6% TAG. The highest DHA content (almost 30%), *n*–3 HUFA and DHA:EPA (eicosapentaenoic acid; 20:5*n*–3) ratio were found in the lipid of the whiting brain/eye silage whereas lipid from whole whiting head silage had the lowest levels of these fatty acids and ratio (Table 2). The lipids in cod brain/eye, tuna eye and shark eye silages contained similar proportions of DHA (19.5–23.1%) and *n*–3 HUFA (27.1–31.1%) and similar DHA:EPA ratios (3.5–3.8). The influence of lipid contents of the silages on their absolute levels of DHA is clear from the data in Table 3, where the high lipid tuna eye silage supplied over ten-fold more DHA than the low lipid shark eye silage per unit dry weight of silage.

The moisture and lipid contents and the lipid class compositions of *Artemia* nauplii were not significantly affected by enrichment with either a commercial enricher or the cod brain/eye silage (Table 4). However, both the commercial enricher and the cod brain/eye silage significantly increased the percentages of EPA, DHA and total *n*–3 HUFA in total lipid of *Artemia* nauplii but the values were not significantly different

Table 4
Moisture and lipid contents and lipid class compositions of unenriched *Artemia* nauplii and nauplii enriched with a commercial enricher and cod brain/eye silage. Results are means ± s.d. ($n = 3$ for unenriched, 5 for commercial enricher and 4 for cod brain/eye silage). Data were subjected to one-way ANOVA. There were no significant differences between values within a row

Parameter	Enricher		
	Unenriched	Commercial enricher	Cod brain/eye silage
Moisture content (%)	91.5 ± 0.1	90.5 ± 1.6	91.5 ± 2.4
Lipid content (% dry weight)	16.6 ± 1.9	17.0 ± 2.2	20.4 ± 7.9
Lipid class composition (% of total lipid)			
Phosphatidylcholine	12.8 ± 0.5	11.6 ± 0.9	12.3 ± 0.8
Phosphatidylethanolamine	9.4 ± 0.2	7.9 ± 1.0	8.1 ± 0.9
Phosphatidylserine	2.8 ± 0.6	2.1 ± 0.4	2.1 ± 0.4
Phosphatidylinositol	4.0 ± 0.7	3.1 ± 0.8	3.0 ± 0.6
Cardiolipin/phosphatidic acid	2.8 ± 0.6	2.3 ± 0.5	2.1 ± 0.5
Sphingomyelin	0.7 ± 0.1	0.5 ± 0.0	0.6 ± 0.3
Unknown	0.6 ± 0.5	0.6 ± 0.3	0.5 ± 0.4
Total polar lipids	33.0 ± 3.5	28.1 ± 3.7	27.5 ± 4.3
Pigment	1.8 ± 0.0	1.5 ± 0.6	1.5 ± 0.2
Total neutral lipids	65.2 ± 3.5	70.4 ± 3.3	70.0 ± 3.0
Triacylglycerol	46.7 ± 2.6	48.5 ± 2.9	47.7 ± 2.0
Cholesterol	9.6 ± 1.3	9.3 ± 1.1	11.0 ± 1.2
Free fatty acids	6.8 ± 0.4	6.9 ± 0.8	7.5 ± 0.4
Steryl ester	2.2 ± 0.2	5.8 ± 2.4	3.9 ± 0.3

between the two enrichers (Fig. 1). The DHA:EPA ratio of the lipid was also significantly increased by both enrichers, but the ratio was significantly greater in cod brain/eye silage-enriched *Artemia* compared with those enriched with the commercial product.

The effect of dose level in the enrichment procedure was investigated with the tuna eye silage. Without supplementation, the nauplii contained no DHA at all (Fig. 2). Supplementation with the dried tuna eye silage at the same dose level of 0.6 g l^{-1} as a commonly used powdered commercial product, Protein Selco, resulted in a level of DHA comparable with Protein Selco. Increasing the dosage of the dried tuna eye silage crumble three-fold significantly increased the level of DHA in the *Artemia* nauplii. In a second trial, a greater range of dose levels of tuna eye silage, both above and below the concentration normally recommended for use with the commercial enricher, and a slightly modified enrichment procedure were used (Table 5). Again, using tuna eye silage at the same concentration as the commercial enricher, the levels of DHA in the nauplii were very similar both as percentages of total fatty acids in total lipid and in terms of μg DHA mg^{-1} dry weight of *Artemia*. However, the highest levels of DHA mg^{-1} dry weight of *Artemia* nauplii were achieved with dosages of tuna eye silage of twice and, surprisingly, half that of the commercial enricher. A dose of tuna eye silage of four times the commercial enricher concentration did not increase the μg DHA mg^{-1} dry weight of *Artemia* above that observed with the commercial enricher. The DHA:EPA

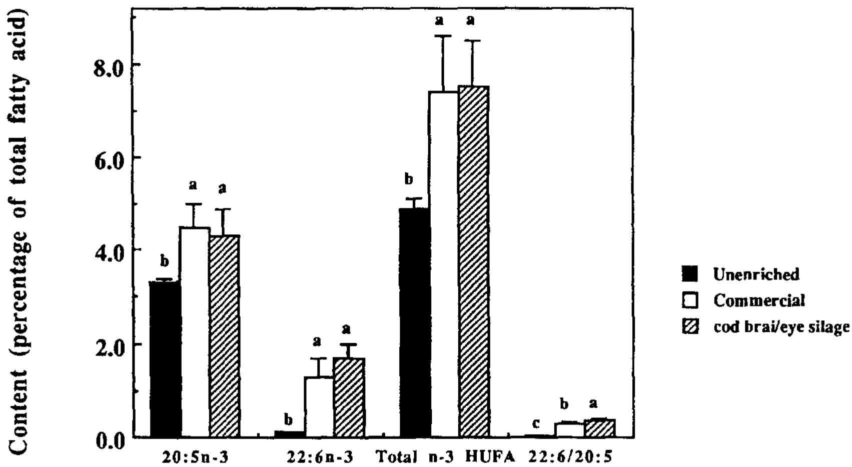

Fig. 1. Effects of enriching *Artemia* nauplii with a commercial enricher product (□) and codbrain/eye silage (▨) on the content of 20:5n−3, 22:6n−3, total (n−3) HUFA and the DHA:EPA (22:6/20:5) ratio in comparison with unenriched nauplii (■). The enrichment procedure was carried out 'on-site' at a commercial mariculture facility as described in Materials and methods. Results (except the DHA:EPA ratio) are presented as percentages of total fatty acids (by weight) and are means ± s.d. ($n = 3$ for unenriched, 5 for commercial enricher and 4 for the cod brain/eye silage). The data were subjected to one-way ANOVA and Tukey's multiple comparison test. Values within a group with different superscript letters were significantly different ($P < 0.05$).

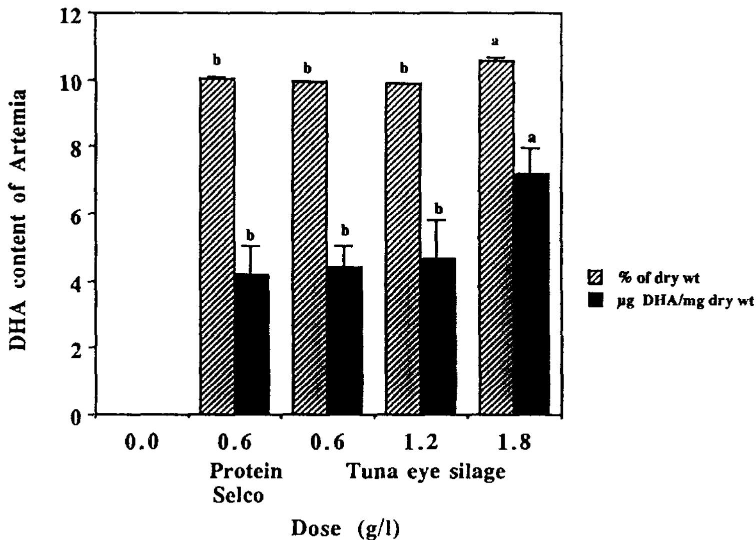

Fig. 2. Effects on DHA content of supplementing newly hatched *Artemia* nauplii with the dried tuna eye silage in comparison with a commercial enricher product (Protein Selco; INVE Aquaculture NV, Bassrode, Belgium). *Artemia* nauplii at a concentration of approximately 200000 l^{-1} were enriched with the enrichers at the doses indicated as described in Materials and methods. Results are presented both as a percentage of dry weight (▨) and in μg DHA mg^{-1} dry weight of *Artemia* (■) and are means ± s.d. ($n = 3$). The data were subjected to one-way ANOVA and Tukey's multiple comparison test. Values within each group (percentage or μg DHA) with different superscript letters were significantly different ($P < 0.05$).

Table 5
Effects on n−3 highly unsaturated fatty acid (HUFA) content in newly hatched *Artemia* nauplii of enrichment with graded doses of tuna eye silage in comparison with the commercial enricher, Protein Selco. *Artemia* nauplii at a concentration of 150000–200000 l^{-1} were enriched with the enrichers above at the dosages detailed. Half of the enrichment dose was supplied at time 0, with the second half added 10 h later in a total enrichment period of 16 h. Data were subjected to one-way ANOVA and Tukey's multiple comparison test

Fatty acid content	Enricher doses (mg l^{-1})					
	Protein Selco	Tuna silage				
	300	15	150	300	600	1200
% of weight						
20:5n−3	6.7±0.3a	3.6±0.2b	3.6±0.3b	3.1±0.2b	3.4±0.2b	3.2±0.3b
22:5n−3	0.6±0.0a	0.6±0.1a	0.5±0.1a	0.4±0.0b	0.6±0.0a	0.6±0.0a
22:6n−3	2.7±0.2ab	2.2±0.3b	3.3±0.4a	2.9±0.2ab	3.2±0.2a	2.8±0.3ab
Σn−3 HUFA	10.0±0.4a	6.4±0.3c	7.4±0.4b	6.4±0.3c	7.2±0.3bc	6.6±0.4bc
22:6/20:5	0.4±0.0c	0.6±0.1b	0.9±0.1a	0.9±0.0a	0.9±0.0a	0.9±0.0a
μg mg^{-1} dry weight						
20:5n−3	8.7±0.4a	4.6±0.2b	4.6±0.2b	4.1±0.2b	4.6±0.3b	4.0±0.3b
22:5n−3	0.8±0.1a	0.8±0.0a	0.6±0.0b	0.5±0.0b	0.9±0.0a	0.9±0.0a
22:6n−3	3.6±0.3b	2.8±0.2c	4.2±0.3ab	3.8±0.3ab	4.4±0.3a	3.5±0.3bc
Σn−3 HUFA	13.1±0.7a	8.2±0.5c	9.4±0.6bc	8.6±0.3bc	9.9±0.4b	8.2±0.4c
Σn−3 HUFA (% of dry weight)	1.3±0.1a	0.8±0.0c	0.9±0.0bc	0.8±0.0c	1.0±0.0b	0.8±0.0c

Values within a row with different letters were significantly different ($P < 0.05$).

Table 6
Effects on the n−3 highly unsaturated fatty acid (HUFA) content in the rotifer *Brachionis plicatilis* of enrichment with graded doses of tuna eye silage in comparison with the commercial enricher, Protein Selco. The rotifer, *Brachionus plicatilis* S1 strain, was initially reared on bakers yeast. Rotifers at a concentration of 500000 l^{-1} were enriched with the enrichers above at the dosages detailed. Half of the enrichment dose was supplied at time 0, with the second half added 3 h later in a total enrichment period of 8 h. Data were subjected to one-way ANOVA and Tukey's multiple comparison test

Fatty acid content	Enricher doses (mg l^{-1})					
	Protein Selco	Tuna silage				
	750	250	500	750	1500	3000
% of weight						
20:5n−3	14.2±0.4a	15.1±1.2a	14.9±0.9a	11.9±0.2b	10.2±0.0bc	9.3±0.1c
22:5n−3	4.3±0.4a	4.4±0.7a	4.0±0.3ab	4.1±0.5a	3.6±0.2ab	2.9±0.1b
22:6n−3	6.6±0.1d	2.3±0.4f	3.6±0.5e	10.3±0.2c	14.3±0.2b	16.7±0.3a
Σn−3 HUFA	25.1±0.4b	21.8±1.5c	22.5±1.2c	26.4±0.7ab	28.0±0.9a	28.9±0.4a
22:6/20:5	0.4±0.0d	0.2±0.0d	0.2±0.1d	0.8±0.0c	1.4±0.1b	1.9±0.2a
μg mg^{-1} dry weight						
20:5n−3	8.9±0.1b	7.4±0.4c	7.9±0.3bc	8.4±0.2bc	10.8±0.1a	11.2±0.1a
22:5n−3	2.7±0.3c	2.7±0.3c	2.1±0.2c	2.8±0.4bc	3.7±0.1a	3.5±0.1ab
22:6n−3	4.1±0.4d	1.4±0.2e	1.9±0.1e	7.2±0.1c	15.1±0.1b	19.9±0.3a
Σn−3 HUFA	15.7±0.2d	11.5±0.8e	11.9±0.7e	18.5±0.3c	29.6±0.2b	34.6±0.2a
Σn−3 HUFA (% of dry weight)	1.6±0.2b	1.2±0.2b	1.2±0.1b	1.9±0.2b	3.0±0.3a	3.5±0.5a

Values within a row with different letters were significantly different ($P < 0.05$).

ratio in total lipid was significantly greater (up to 0.9) in the tuna eye silage-enriched *Artemia* compared with the Protein Selco-enriched *Artemia* (0.6) (Table 5).

The effects of various doses of tuna eye silage on the n–3 HUFA content of rotifers are shown in Table 6. There was a very clear and consistent dose effect with the levels of DHA and total n–3 HUFA in the total lipid of rotifers increasing with increasing dose of silage. Using the tuna eye silage at the same concentration as the commercial enricher, the level of DHA in the silage-fed rotifers was significantly higher than the level found in Protein Selco-enriched rotifers when expressed both as percentages of total fatty acids and in terms of μg DHA mg^{-1} dry weight of rotifers. Further increases in the dose of tuna eye silage to two- and four-times the dose of Protein Selco resulted in further significant increases in the DHA content of the rotifers. In absolute terms (μg DHA mg^{-1} dry weight), a silage dose of twice the commercial enricher dose resulted in a level of DHA 3.7-fold higher, and a dose four-times the commercial enricher dose resulted in the content of DHA in the rotifers being 4.9-fold higher (Table 6). Furthermore, unlike the *Artemia* nauplii, the DHA:EPA ratio increased in the rotifers with increasing dose of silage (Table 6).

Table 7
Effects of feeding cod brain/eye silage-enriched *Artemia* on lipid content (% of brain dry weight) and lipid class composition (% of total lipid) of young juvenile turbot brain. Results are means ± s.d. ($n = 3$). Data were subjected to one-way ANOVA and Tukey's multiple comparison test

Lipid class	*Artemia* enricher		
	Commercial enricher [a]	Commercial enricher then weaned [b]	Cod brain/eye silage [c]
Total lipid content	0.76 ± 0.05b	0.97 ± 0.05a	0.94 ± 0.07a
Phosphatidylcholine	22.9 ± 0.7b	25.7 ± 0.2a	23.5 ± 0.4b
Phosphatidylethanolamine	21.0 ± 0.7b	20.9 ± 0.3b	23.0 ± 0.5a
Phosphatidylserine	9.0 ± 0.2	9.3 ± 0.1	9.9 ± 0.2
Phosphatidylinositol	2.3 ± 0.1	2.0 ± 0.1	3.0 ± 0.5
Cardiolipin/phosphatidic acid	2.7 ± 0.3	2.8 ± 0.1	3.0 ± 0.3
Cerebroside/sulphatide	3.0 ± 0.7	3.3 ± 0.2	4.1 ± 0.5
Sphingomyelin	0.6 ± 0.1	0.6 ± 0.1	1.0 ± 0.3
Total polar lipids	62.8 ± 0.6b	64.7 ± 0.7b	67.7 ± 1.1a
Total neutral lipids	37.2 ± 0.6a	35.3 ± 0.7a	32.3 ± 1.1b
Cholesterol	31.2 ± 0.6a	31.0 ± 0.5a	24.4 ± 1.0b
Triacylglycerol	2.1 ± 0.7b	1.3 ± 0.2b	4.5 ± 0.5a
Steryl esters	3.0 ± 2.2	2.8 ± 0.6	1.9 ± 0.3
Free fatty acids	0.9 ± 0.4	0.3 ± 0.2	1.5 ± 0.6

[a] Turbot (approx. 50 days old) reared to the point of weaning using the normal commercial procedure and enrichers used at the farm. Data taken from Mourente and Tocher (1992).
[b] Turbot as above, but weaned onto a dry pelleted diet approximately on Day 43 and fed this for 1 week prior to sampling and analysis. Data taken from Mourente and Tocher (1992).
[c] Turbot (approx. 45 days old) reared under the normal commercial procedure except that they were fed cod brain/eye silage-enriched *Artemia* for 20 days prior to sampling and analysis.
Values within a row with different letters were significantly different ($P < 0.05$).

Artemia nauplii, enriched with either commercial enricher or the cod brain/eye silage, were fed to juvenile turbot prior to weaning. The brains of these turbot and others that had been weaned on to a pelleted diet 1 week prior to sampling, were then analysed

Table 8
Effects of feeding cod brain/eye silage-supplemented *Artemia* on fatty acid composition (weight percentage of total fatty acids) of total lipid from young juvenile turbot brain. Results are means ± s.d. ($n = 3$). Data were subjected to one-way ANOVA and Tukey's multiple comparison test

Fatty acid	*Artemia* enricher		
	Commercial enricher [a]	Commercial enricher then weaned [b]	Cod brain/eye silage [c]
14:0	0.7 ± 0.1b	1.9 ± 0.9a	0.5 ± 0.1b
15:0	0.3 ± 0.1	0.4 ± 0.0	0.2 ± 0.1
16:0	13.3 ± 1.6	14.9 ± 1.0	17.6 ± 1.4
18:0	7.8 ± 0.7b	6.6 ± 0.8b	14.9 ± 1.5a
20:0	0.6 ± 0.3	0.5 ± 0.3	0.1 ± 0.1
Total saturates	23.4 ± 1.8b	24.7 ± 1.0b	33.3 ± 1.9a
16:1*n*−7	1.4 ± 0.1b	3.6 ± 1.4a	2.4 ± 0.7ab
18:1*n*−9	15.7 ± 1.8	13.5 ± 3.3	12.6 ± 2.5
18:1*n*−7	4.1 ± 0.5ab	2.9 ± 0.7b	4.9 ± 0.6a
20:1*n*−9	1.4 ± 0.7ab	2.8 ± 0.7a	0.8 ± 0.3b
20:1*n*−7	0.4 ± 0.2	0.4 ± 0.1	0.4 ± 0.1
22:1	1.0 ± 0.7a	2.5 ± 1.6a	0.3 ± 0.1b
24:1*n*−9	0.9 ± 0.1	1.0 ± 0.0	1.3 ± 0.2
Total monoenes	26.6 ± 2.0	27.3 ± 5.9	22.7 ± 3.5
18:2*n*−6	6.7 ± 2.4a	1.7 ± 0.2b	3.1 ± 0.9ab
20:2*n*−6	0.4 ± 0.2	0.5 ± 0.2	0.2 ± 0.1
20:3*n*−6	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
20:4*n*−6	1.4 ± 0.3b	1.4 ± 0.3b	3.2 ± 0.5a
22:4*n*−6	tr	tr	0.2 ± 0.1
22:5*n*−6	0.1 ± 0.1	0.2 ± 0.0	0.3 ± 0.1
Total *n*−6 PUFA	9.7 ± 2.2a	5.2 ± 0.3b	7.2 ± 1.1a
18:3*n*−3	2.1 ± 0.3b	0.8 ± 0.1c	4.4 ± 0.4a
18:4*n*−3	0.7 ± 0.2	1.2 ± 0.7	0.4 ± 0.2
20:3*n*−3	1.1 ± 0.2a	0.4 ± 0.1b	1.8 ± 0.3a
20:4*n*−3	0.5 ± 0.1	0.5 ± 0.9	0.8 ± 0.2
20:5*n*−3	6.3 ± 0.8	6.0 ± 2.6	7.1 ± 1.1
22:5*n*−3	2.1 ± 0.5	1.7 ± 0.4	2.2 ± 0.3
22:6*n*−3	5.2 ± 0.8b	10.9 ± 1.9a	10.1 ± 1.2a
Total *n*−3 PUFA	19.7 ± 2.6	22.9 ± 4.7	26.8 ± 2.1
Total PUFA	29.4 ± 2.8	28.1 ± 4.8	34.0 ± 2.9
Total HUFA	15.2 ± 1.5b	19.5 ± 2.3ab	22.0 ± 1.4a
22:6*n*−3/20:5*n*−3	0.8 ± 0.2b	1.8 ± 0.3a	1.5 ± 0.2a
Total dimethylacetals	2.9 ± 0.9	2.2 ± 0.6	2.8 ± 0.4

[a,b,c] As described in Table 7.
Values within a row with different letters were significantly different ($P < 0.05$).
HUFA, highly unsaturated fatty acids; PUFA, polyunsaturated fatty acids; tr, trace (< 0.05%).

and lipid and fatty acid composition determined. Total lipid of brains from turbot fed for 20 days prior to sampling on cod brain/eye silage contained a significantly greater percentage of structural phospholipids than turbot grown entirely on *Artemia* enriched with the commercial enricher and turbot that had been weaned on to a high n–3 PUFA fish oil-containing pelleted diet for 1 week (Table 7). Phosphatidylethanolamine was the only phospholipid class that showed a significantly increased proportion in the brains of turbot fed silage-enriched *Artemia*. Brain total lipid of turbot reared for 20 days prior to sampling on cod brain/eye silage had a DHA content almost double that of brain total lipid from turbot reared entirely on *Artemia* enriched with the commercial product (Table 8). The percentage of DHA in the total lipid of brain from turbot reared on the silage was comparable to the percentage of DHA found in the brain total lipid from turbot fed for 1 week on a high n–3 PUFA-containing pellet (Table 8). Interestingly, the percentage of arachidonic acid (20:4n–6) was also significantly increased in total lipid from brain of turbot fed the silage-enriched *Artemia* nauplii compared with turbot reared on Artemia enriched with the commercial product or weaned fish (Table 8).

4. Discussion

Ensiling was the method chosen to process the fish neural tissues since silages prepared from fishery by-products have been used in the past in fish diets and are currently still under investigation (Heras et al., 1994). As the fish neural tissue silages were prepared specifically for use with larval stages of marine fish, which may have only limited digestive capabilities, the fact that ensiling results in considerable hydrolysis of proteins to smaller peptides and amino acids (Raa and Gildberg, 1976) may have specific benefits. The use of silages, prepared from fish waste, in the formulation of diets for use in aquaculture has been the subject of several investigations (Hardy et al., 1984; Jackson et al., 1984; Espe et al., 1992; Fagbenro and Jauncey, 1993; Heras et al., 1994). In the earlier studies, the silages were generally produced from whole fish or fish offal and were dried for use primarily as a fish meal replacement in normal pelleted diets for ongrowing fish such as rainbow trout (Hardy et al., 1984), Atlantic salmon (Jackson et al., 1984; Espe et al., 1992) and various Asiatic clariid catfish (Fagbenro and Jauncey, 1993). The present study was a progression from the generalised situation in that specific tissues (brain and eyes) were used to prepare a silage product specifically designed to meet the lipid requirements of marine fish larvae at first feeding, a time when there is a high requirement for DHA for the rapidly developing nervous system. Recently, the use of neural tissue itself as a source of dietary supplements rich in the DHA required for neural development was investigated in mammals (Bourre et al., 1993).

The techniques for the preparation of the neural silage were relatively simple but tailored initially to the equipment and apparatus available in the laboratory. In a commercial procedure, spray-drying would most likely be a more viable technique for the drying stage rather than freeze-drying. In this respect, neutralisation, if essential, by ammonium bicarbonate may be ideal as the salts formed are more volatile than those formed with sodium hydroxide.

The whiting head silage had a high lipid content but the lipid contained only 9.5%

DHA and so it was decided not to pursue whole head silages any further. The highest level of DHA as a percentage of fatty acids in total lipid was found in the whiting brain/eye silage, but the lower lipid contents of whiting brain/eye and shark eye silages meant they actually delivered less DHA per unit dry weight compared with the silages with high (tuna eye) and intermediate (cod brain/eye) lipid levels. The tuna eye silage with its high lipid content with a high percentage of DHA will potentially deliver the greatest amount of DHA per unit weight of silage. The availability of cod, reasonable size of the brain and eyes and their relatively high total lipid and DHA contents indicated that the cod brain/eye silage would also provide sufficient material with approximately 25% DHA in its lipid. However, the lipid class compositions of the silages indicated that there was a major difference between these two silages. As expected, the cod brain/eye silage, like the whiting brain/eye silage, was relatively rich in polar lipids, particularly glycerophospholipids. This had been a consideration in our rationale for using neural tissues as a source of DHA-rich lipid as DHA is normally found in higher concentration in polar lipids (Henderson and Tocher, 1987). Furthermore, several studies had indicated that glycerophospholipids were superior to triacylglycerols in promoting growth of larval and juvenile fish (Kanazawa, 1985; Hung et al., 1987; Olsen et al., 1991). Surprisingly, though, the tuna eye silage lipid was almost totally TAG. It is known that the intra-orbital fat in tuna is a unique source of DHA-rich oil (Sawada et al., 1993), but as we had intended producing polar lipid-rich silages, the orbital fat had been trimmed as much as possible from the tuna eyes. Although orbital fat may have contributed to the high TAG of the tuna eye silage, it appears that tuna eyes themselves contain DHA-rich TAG. Previously we had shown that trout retinal total lipid contained 30% TAG although it only contained 12% DHA (Tocher and Harvie, 1988). The tuna eye silage, with its high lipid content and high percentage of triacylglycerol (like commercial enrichers) combined with its high DHA level suggested it may be the best silage as an enricher for live prey. However, the cod brain/eye silage was also studied to investigate the effect of high polar lipid on its efficacy as an enricher.

The above points on the suitability of the silages in the nutrition of marine fish larvae are not negated by using the silages as enrichers for live prey. The enrichment process, in *Artemia* at least, is generally regarded as a 'bioencapsulation' process whereby the *Artemia* ingest the enricher particles until the gut is full. Little biotransformation of the enricher product occurs in the gut of the *Artemia*. This means that in subsequent feeding of the *Artemia* to fish larvae, the larvae must digest the lipid of the enricher largely in the chemical form that the lipid exists in the enricher, i.e. if the enricher is TAG-rich, as in Protein Selco and the tuna eye silage, then the fish larvae must digest TAG. Due to the above, the HUFA content of the enriched prey is proportional to the HUFA content of the enriching diet (Walford and Lam, 1987; Lemm and Lemarie, 1991; Støttrup and Attramadal, 1992). Emulsion-type enrichers have an advantage over dried enrichers as they have very high lipid and HUFA contents (Dhert et al., 1990; Støttrup and Attramadal, 1992). However, oil emulsions can be very difficult to remove from rotifers/*Artemia* following enrichment and this can result in transfer of oil to larval rearing tanks with consequent loss of water quality and associated problems of larval viability (Foscarini, 1988).

In the comparative trials in which the silages were used in exactly the same concentration and with the same protocols as with commercial enrichers, both the cod brain/eye and tuna eye silages increased the DHA levels in the *Artemia* nauplii to the same extent as the commercial products. There was evidence from one trial with tuna silage that increasing the dosage of silage could further increase the DHA content of the *Artemia*, but this was not confirmed in a second trial. However, the lower EPA content of the silages in comparison with commercial enrichers (Leger et al., 1987; Lavens et al., 1994) meant that, although total $n-3$ HUFA in the silage-enriched *Artemia* could be lower, not the case with the cod brain/eye silage, the DHA:EPA ratios in the silage-enriched *Artemia* were significantly higher. The DHA:EPA ratio in larval diets has been coming under increasing attention recently (Mourente et al., 1993; Lavens et al., 1994; Reitan et al., 1994) and has led to the an increasing demand for enricher products containing higher levels of DHA.

The tuna eye silage was even more effective as an enricher for rotifers. In this case not only did the tuna silage give significantly higher DHA than the commercial enricher at equivalent doses, but also there was a very significant dose effect. The total $n-3$ HUFA and DHA:EPA ratio was also significantly greater with the silage at the equivalent dose to the commercial enricher and also showed significant dose effects. It should be noted that the increasing DHA:EPA ratio with increasing dose of silage in the rotifer does not indicate metabolism of the ingested silage or specific retention of DHA in comparison to other $n-3$ PUFA in the rotifer, as the DHA:EPA ratio in the rotifer is initially low whereas in tuna silage it is 3.4 and so an increasing DHA:EPA ratio only reflects the greater dilution of the original rotifer lipid with silage lipid. The reason for the tuna eye silage being so effective in enriching rotifers in comparison with *Artemia* was unclear. It may simply be due to greater ingestion of the silage particles by rotifers but whether this was due to physical factors such as size or chemical factors such as 'taste' is not known.

As mentioned above, the process of enriching *Artemia* is basically a 'bioencapsulation' process with little biotransformation of the enricher in the gut of the *Artemia*. The results of the trial in which turbot larvae were reared on *Artemia* enriched with cod brain/eye silage showed that the lipids and fatty acids in the cod brain/eye silage were digested and assimilated very effectively by the fish larvae. Furthermore, the results demonstrated that the DHA in *Artemia* enriched with the cod brain/eye silage was apparently more readily incorporated and/or retained in the brains of juvenile turbot fed the silage-enriched *Artemia*, on an identical weight for weight basis, compared with the DHA in *Artemia* fed on the commercial enricher. Previously we had shown that weaning turbot on to a pelleted diet dramatically increased brain DHA within 1 week (Mourente et al., 1991; Mourente and Tocher, 1992). In the present study, it was noteworthy that feeding the turbot larvae on the cod brain/eye silage-enriched *Artemia* resulted in the turbot having a level of DHA in the brain identical to that of fish weaned for 1 week on to the pelleted diet. It is interesting to speculate on why DHA esterified mainly to phospholipid was more effectively incorporated into the larval turbot brain. It is possible that phospholipids are more readily digested in the larval gut compared with TAG but there is no evidence to suggest that phospholipids are more readily hydrolysed than TAG in the digestive tract of juvenile turbot (Koven et al., 1994). Furthermore,

immunoreactive phospholipase A_2-like protein was not detected in the intestine of red sea bream larvae until 85 days post-hatch (Uematsu et al., 1992). If exogenous lipases (i.e. from the *Artemia*) are involved it is possible that phospholipases were more efficient, but there may be a less specific reason related to the inherent emulsification and antioxidant properties of phospholipids. Govoni et al. (1986) have suggested that pinocytosis of food particles in the gut is an important route for the 'digestion' and uptake of nutrients in larval fish. Although their study focused on protein sources, it has been speculated that pinocytosis may also play a role in the uptake of lipids in fish larvae (Olsen et al., 1991). However, this mechanism is poorly understood and it is unclear whether this route would favour phospholipid uptake.

It was also interesting that phosphatidylethanolamine was the only phospholipid class that showed a significantly increased proportion in the brains of turbot fed silage-enriched *Artemia*, as this class has been implicated as having a special role in neural tissues due to its high content in neural tissues and the high levels of DHA it contains (Tocher and Harvie, 1988). The increased percentage of arachidonic acid in total lipid from brain of turbot fed the silage-enriched *Artemia* nauplii compared with turbot reared on *Artemia* enriched with the commercial product or weaned fish may also be important. Arachidonic acid is accumulated in mammalian brain during early development (Sinclair and Crawford, 1972) and is incorporated into turbot brain lipid in amounts proportional to dietary input (Bell et al., 1995b). Furthermore, we reported growth-promoting activity of arachidonic acid that provided strong support for the contention that dietary 20:4n–6 is essential for juvenile turbot (Castell et al., 1994).

5. Conclusions

The results of the present study have demonstrated that silages prepared from fish neural tissue (obtained from a common waste product of many commercial fisheries, namely fish heads) can be either phospholipid-rich or TAG-rich depending on the species and tissue. Lipid content also varied but they all contained high levels of DHA and high DHA:EPA ratios. Tuna eye silage was particularly effective in increasing DHA content and outperformed a commercial product in rotifer enrichment. The cod brain/eye silage was as effective as the commercial enricher in increasing DHA in *Artemia* nauplii but *Artemia* enriched with the silage were more effective than nauplii enriched with the commercial product in increasing larval turbot brain DHA content. Therefore, tuna eye and cod brain/eye silages may be useful as enrichers of *Artemia* nauplii and rotifers, commonly used as live foods at the onset of independent feeding of marine fish larvae prior to weaning on to pelleted formulated diets.

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