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THE USE OF LIPID EMULSIONS AS CARRIERS FOR ESSENTIAL FATTY ACIDS IN BIVALVES: A TEST CASE WITH JUVENILE *PLACOPECTEN MAGELLANICUS*

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ABSTRACT Although information on bivalve nutrition is still very scarce, several studies have demonstrated the importance of lipids, in particular triglycerides, as a source of energy and essential fatty acids in the early life stages. Experimental diets used so far to study bivalve nutrition either heavily pollute the water or are too complex to prepare in a hatchery. The potential use of lipid emulsions as off-the-shelf supplements was evaluated through the analytical verification of the ingestion and incorporation of n-3 highly unsaturated fatty acids (HUFA) by the juvenile sea scallop *Placopecten magellanicus* fed lipid emulsions of different fatty acid composition as a supplement to *Isochrysis* sp. (clone T-Iso). The average lipid content in the scallops fed the lipid supplements was 20% higher compared with that in the control fed algae only (3.29 ± 0.16 *versus* 2.75% of dry weight, respectively). Changes in the fatty acid composition, in particular of n-3 HUFA, were demonstrated in total lipids, polar lipids, and triglycerides of juvenile sea scallops supplemented with lipid emulsions on the basis of ethyl ester concentrates of n-3 HUFA and were dependent on the level and proportion of 20:5n-3 and 22:6n-3 present in the emulsion. The effective incorporation of essential fatty acids from lipid emulsions indicated that the supplementation of lipid emulsions to live algae may improve and standardize the dietary supply of lipids and fatty acids in hatchery production of bivalves.

KEY WORDS: Bivalve, lipid, fatty acid, algal supplement, *Placopecten magellanicus*

INTRODUCTION

Rearing bivalves in commercial systems has so far relied on the production and use of selected species of unicellular marine algae. Despite the growing knowledge of the effects of environmental conditions, disease, and genetic background, the success of commercial hatchery cultures remains highly unpredictable. Mortalities are often attributed to deficiencies in certain nutritionally important components. Although information on bivalve nutrition is still very scarce, several studies have demonstrated the importance of lipid quantity and quality, particularly triglycerides, in early life stages as a source of energy and essential fatty acids (Helm et al. 1973, Holland and Spencer, 1973, Waldock and Nascimento 1979, Gallager and Mann 1986, Gallager et al. 1986). A requirement for the n-3 highly unsaturated fatty acids (HUFA), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3), has been demonstrated for juvenile oysters (Langdon and Waldock 1981), and recent studies evaluating changes in fatty acid composition during larval development appear to confirm this for larval bivalves (Helm et al. 1991, Marty et al. 1992).

The importance of lipids for larval development has encouraged the development of artificial diets that provide specific lipid supplements during broodstock conditioning and larval rearing. Experimental diets used so far in bivalve nutrition studies, such as mixed diets (Trider and Castell 1980), liposomes (Parker and Selivonchick 1986) and microcapsules (Langdon and Waldock 1981), either heavily pollute the water or are too complex to prepare on a regular basis in a hatchery. Lipid microspheres that are easily prepared by sonication of an oil mixture with lecithin and vitamin E have been proposed as a nutritional supplement for oyster conditioning (Robinson 1992a, Robinson 1992b, Heras et al. 1994). Self-emulsifying concentrates of marine oils, which are widely used in fish hatcheries to enrich filter-feeding prey organisms like *Artemia* and rotifers with n-3 HUFA (Sorgeloos and Léger 1992), are off-the-shelf lipid supplements that may also be acceptable for bivalves. Previous work has demonstrated the potential use of these lipid emulsions as a supplement for the larval bivalves *Mercenaria mercenaria* and *Ostrea edulis* (Coutteau et al. 1994a). This study aimed at the analytical verification of the ingestion and incorporation of the fatty acids supplied through lipid emulsions of different n-3 HUFA content by juvenile *Placopecten magellanicus*.

MATERIALS AND METHODS

Juvenile sea scallops, *P. magellanicus*, were supplied by Fisheries Resource Development Ltd. (Sandy Cove, Nova Scotia) and André Mallet (Mallet Associates, Halifax, Nova Scotia). The seed originated from stocks kept in a field nursery. During a period of 2 d, the animals were acclimated gradually to the experimental temperature (14–15°C) and fed *Isochrysis* sp. (clone T-Iso). Initially, 3.5 g of scallops of approximately the same size (average live weight, 32.7 mg) were stocked per culture unit. The latter consisted of a small lantern net suspended in a bucket containing 25 l of filtered (using cartridge filters with pore sizes of 5 and 1 μm) seawater that was renewed daily. Each bucket was aerated with an airstone to prevent the food from settling. After 17 d of feeding on the experimental diets, scallops were starved overnight

in filtered seawater, rinsed with distilled water, and stored at −22°C for biochemical analysis.

Isochrysis sp. (clone T-Iso), which was selected as the algal control diet, was grown in 10-l batch culture with F/2 (Guillard) medium and harvested in the exponential phase. The scallops were fed an initial weight-specific daily ration of 0.88% (dry algae per initial wet seed biomass), which was administered over two feedings per day. The latter ration maintained algal concentration in the cultures above 15–20 cells μl^{-1}. Rations were adjusted and the biomass was restocked after 6 d of culture in order to feed approximately constant weight-specific daily rations throughout the experiment (Urban et al. 1983). Algal rations were based on a dry weight of 13.8 ± 0.6 pg $cell^{-1}$ (mean and standard deviation from analysis of four cultures) for *Isochrysis* sp. (clone T-Iso), determined according to the method described by Coutteau et al. (1994b). Lipid emulsions were added simultaneously with the algae to give 0.2% lipid supplementation per initial wet scallop biomass. The experimental lipid emulsions (prepared by INVE Aquaculture N.V.-S.A., Belgium) contained, on a wet weight basis, 50% lipid, liposoluble vitamins (0.013% vitamin D_3, 0.32% vitamin E, 0.08% ascorbyl palmitate, 0.18% vitamin A), emulsifiers, preservatives, antioxidants, and water. Lipid consisted of either coconut oil (Em0, control emulsion lacking HUFA) or ethyl ester concentrates of marine oils (Em50E and Em50D, approximately 50% Σ n-3HUFA, primarily EPA and DHA, respectively).

Lipids were extracted from whole animals (40–50 per extraction), algae (four independent samples in the course of the experiment), and emulsions with a mixture of chloroform:methanol (2:1 v/v) (Folch et al. 1957). Total lipid contents were determined gravimetrically after exhaustive removal of the solvent from the lipid extract. Lipid classes were separated by preparative thin-layer chromatography on silica gel plates with hexane:diethyl ether:acetic acid (85:15:1), and fatty acid methyl esters (FAME) were prepared from total lipids, total polar lipids, and triglycerides by transesterification with 7% BF_3-methanol:benzene (1:1 v/v) (Napolitano and Ackman 1993). Separation of the FAME was carried out on a Perkin-Elmer Model 8420 GC equipped with a flame ionization detector (FID) and an OMEGAWAX-10 flexible fused silica capillary column (30 m × 0.32 mm inner diameter) (Napolitano and Ackman, 1993). Relative areas were converted to weight percent amounts of fatty acids by correcting for the FAME FID responses (Ackman and Eaton 1978). Quantitative data (milligrams of fatty acid per gram of dry weight, mg/g DW) were obtained by adding 10% of 23:0 as internal standard before the transesterification of total lipids and by using the following equation:

$$mg_{FA} \cdot (g\ DW)^{-1} = \frac{area_{FA}}{area_{23:0}} \cdot \frac{mg_{23:0}}{L} \cdot \frac{TL}{CF \cdot 100}$$

with TL, total lipid sample (%DW); L, amount of lipid used for FAME preparation (g); CF, 1.04, correction factor for the conversion of fatty acids in FAME. Subsamples of scallops were dried at 70°C for 36 h to obtain the dry matter and then heated to 450°C for 4 h to obtain the ash weight. Organic matter was calculated from the difference between dry matter and ash.

RESULTS

Isochrysis sp. (clone T-Iso) exhibited an average n-3 HUFA content of 14.0% with a DHA/EPA ratio of 17.8. The emulsions Em50E and Em50D contained approximately 50% n-3 HUFA with a DHA/EPA ratio of, respectively, 0.7 and 5.8. The coconut-oil based emulsion Em0 contained 90% saturated fatty acids of which nearly 55% was 12:0 (Table 1).

Scallops that were starved for 17 d did not show any increase in wet weight and exhibited higher ash content and lower lipid content compared with the fed ones (Table 2). The average lipid content in the lipid-supplemented treatments was 20% higher compared with that in the control fed algae only (3.29 ± 0.16 *versus* 2.75% of dry weight, respectively), whereas dry weight and ash content for all fed treatments were similar (in the range of 44.9–47.8% and 82.2–85.7%, respectively).

The proportions of monoenoic fatty acids increased during starvation, mainly because of an increase of 20:1 (Table 3). A decrease of n-3 fatty acids could mainly be attributed to the decrease of 18:3n-3 and particularly 18:4n-3, which was abundant in the initial scallops. Starved scallops selectively retained n-3 HUFA with C > 20, in particular 22:6n-3, and n-6 HUFA, especially 20:4n-6, whereas the proportion of n-3 HUFA with 20 C, primar-

TABLE 1.

Fatty acid composition (weight % of total fatty acids) of the lipid emulsions and *Isochrysis* sp. (clone T-Iso).

Fatty acid	Em0	Em50E	Em50D	ISO*
12:0	54.5	—	0.2	0.1
TMTD	—	—	—	0.3
14:0	19.3	0.3	0.9	22.8
16:0	13.2	3.1	16.2	8.0
16:1n-9	—	1.3	1.8	0.3
16:1n-7	—	—	—	3.7
18:0	3.0	4.1	1.1	0.2
18:1n-9	7.8	11.2	16.4	9.4
18:1n-7	0.1	3.4	1.4	1.2
18:2n-6	1.9	1.2	2.4	6.4
18:3n-3	—	0.9	0.4	7.0
18:4n-3	—	2.4	0.6	13.4
20:1n-11	—	—	—	3.0
20:1n-9	0.1	3.4	0.3	—
20:1n-5	—	—	—	—
20:2n-6	—	3.7	4.1	0.1
20:4n-6	—	1.8	0.3	0.2
20:4n-3	—	1.5	0.1	—
20:5n-3	—	25.9	6.4	0.7
21:5n-3	—	1.6	0.9	0.5
22:2NMID	—	—	—	1.4
22:1n-11 + n-13	—	1.6	—	—
22:5n-6	—	0.9	1.3	2.9
22:5n-3	—	4.0	2.9	0.7
22:6n-3	—	19.3	37.5	12.1
24:1n-9	—	1.0	2.5	—
Σ saturated	90.0	8.9	18.3	32.9
Σ monoenoic	8.1	24.1	22.2	17.9
Σ polyenoic	1.9	66.8	59.3	47.5
Σ n-3HUFA**	0	52.8	47.9	14.0
DHA/EPA	—	0.7	5.8	17.8

Minor components identified (<1%) and not included in the table are Iso-15:0; Ant-15:0; 15:0; Iso-16:0; Ant-16:0; 7-methyl-hexadecanoic acid; 16:1n-5; 16:2n-6; 16:2n-4; 16:3n-4; 16:3n-3; 16:4n-1; 17:0 + phytanic (3,7,11,14-tetramethylhexadecanoic) acid; 18:2n-4; 18:3n-6; 18:3n-4; 18:4n-1; 20:0; 20:1n-7; 20:2NMID; 20:3n-6; 20:3n-4; 20:3n-3; 22:0; 22:1n-9; 22:1n-7; 22:1n-5; 22:4n-6; 22:4n-3.

* Average values from analysis of four cultures.

** ≥20:3n-3.

TABLE 2.

Average individual wet weight (WW) and composition of juvenile *P. magellanicus* at the start of the experiment and after 17 d of starvation or feeding on various diets.

Treatment	Average WW (mg ind^{-1})	Dry Weight (% WW)	Ash (% DW)	Total Lipid (% DW)
Initial	32.7	45.7	ND	2.01
After 17 d of culture				
Starved	33.0	44.0	88.5	1.23
ISO	47.5	46.1	82.9	2.75
ISO + Em0	54.1	45.7	85.7	3.46
ISO + Em50E	53.9	47.8	82.2	3.14
ISO + Em50D	57.0	44.9	82.7	3.27

DW, dry weight; ND, not determined.

ily 20:5n-3, decreased. Compared with the initial fatty acid composition, scallops fed *Isochrysis* showed an increased proportion of certain monoenoic fatty acids that were also abundant in the alga, *i.e.*, 16:1n-7 and 18:1n-9, whereas the relatively high 20:1n-11 content in the algae did not affect that of the scallops (Table 3). The change of the HUFA content in total lipids after the adaptation to the *Isochrysis* diet reflected the HUFA composition of the alga, *i.e.*, a predominance of 22:6n-3 and a strongly reduced content in 20:5n-3.

The supplementation of the emulsion Em0, despite its high content in 12:0, resulted in the presence of only 1% of 12:0 in the total lipids (Table 3). The proportion of total n-3 HUFA decreased slightly as the result of the Em0 supplementation. The effect of the supplementation of the Em50 emulsions on the fatty acid composition of total lipids was mainly restricted to an increase of the dominant n-3 HUFA, either EPA (from 3.3 to 5.6% for Em50E) or DHA (from 17.9 to 19.4% for Em50D). As a result, the supplementation of lipids with a lower DHA/EPA ratio than that of the algae resulted in a decrease of the DHA/EPA ratio in the scallop lipids from 5.5 in the control diet to 3.0 and 4.7 for the emulsions Em50E and Em50D, respectively (Fig. 1A). In accordance with the increase of the total lipid content and the proportion of n-3 HUFA, the absolute concentration of EPA and DHA was considerably higher in the scallops receiving the Em50 emulsions (respectively, 1.13 and 3.44 mg/g DW for ISO + Em50E, and 0.91 and 4.29 mg/g DW for ISO + Em50D) compared with the control fed algae only (respectively, 0.54 and 3.00 mg/g DW) (Fig. 1A). The supplementation of the Em0 emulsion resulted in an increase of approximately 15% in the concentration of EPA and DHA.

The above changes in the fatty acid composition of the total lipids due to lipid supplementation were amplified in the triglycerides (Fig. 1B) and attenuated in total polar lipids (Fig. 1C). In this way, the DHA/EPA ratio in the various dietary treatments was in the range of 3.2–8.1 and 2.8–3.7 for triglycerides and total polar lipids, respectively. Equivalent values for the total n-3 HUFA content were 15.5–23.0% and 34.7–36.6%, respectively.

DISCUSSION

Research on bivalve nutrition, including the study of lipid requirements, has been seriously hampered by the lack of suitable experimental diets. In this regard, lipid vesicles may have several advantages over other synthetic microparticles, *e.g.*, their near-neutral buoyancy, suitable size range for efficient filtration by bivalves, and composition of nontoxic, digestible materials. Parker and Selivonchick (1986) demonstrated that juvenile *Crassostrea gigas* were able to metabolize the phosphatidylcholine and cholesterol present in the lamellae of liposomes. The protection from leaching of entrapped compounds makes liposomes particularly interesting as carriers for the delivery of water-soluble nutrients to filter-feeding organisms, whereas lipid microspheres consisting of emulsified lipid droplets provide a maximal amount of lipid per particle and may constitute effective carriers for lipid-soluble nutrients. Robinson (1992a) prepared microspheres of a lipid mixture of menhaden oil, egg phosphatidylcholine, and a partially hydrogenated vegetable oil with polyvinyl alcohol as an emulsifier. Although the latter author demonstrated the potential use of the lipid microspheres either as a supplement to or as a substitute for algae in the brookstock conditioning of the Pacific oyster, a similar lipid content and fatty acid composition was reported for nonfed oysters and oysters fed only the lipid microspheres (Robinson 1992b). Recently, Heras et al. (1994) improved the formulation of the emulsion of Robinson (1992a) by adding vitamin E and replacing the menhaden oil with a concentrate of n-3 fatty acid ethyl esters, thereby increasing the proportion of n-3 HUFA in the lipid supplement from 12.6 to 44.3%. Although it has been demonstrated with fluorescent beads that lipid microspheres are ingested and disintegrated by adult *O. edulis* (Heras et al. 1994), the latter does not prove the effective assimilation of nutrients from the lipid supplement. This work showed that essential fatty acids supplied as an emulsion of ethyl esters are assimilated and incorporated into the triglycerides and the polar lipids of juvenile sea scallops fed the lipid emulsion as a supplement to live algae. Similar work with *O. edulis* confirmed this for larval stages (Coutteau et al. 1994a), which may imply that lipid emulsions could be used to provide dietary lipids to the various life stages and species of bivalve molluscs.

The similar growth of scallops receiving a dietary supplement based on coconut oil and those fed a supplement rich in n-3 HUFA indicated that the algal control diet in this study, consisting of the DHA-rich *Isochrysis*, may have satisfied the requirement for n-3 HUFA in juvenile *P. magellanicus*. Nevertheless, the supplementary dietary lipid may have provided additional energy, which was at least partially stored as lipid reserves in the tissues. The very limited accumulation of 12:0 in the total lipids (1% 12:0 in the tissue *versus* 54% in the emulsion), despite the increase of the total lipid content in the scallops fed the emulsion Em0, may indicate a rapid oxidation of a large part of the lipid supplement based on

TABLE 3.

Selected fatty acid composition of total lipids of juvenile *P. magellanicus* at the start of the experiment and after 17 d of starvation or feeding on various diets (weight percent of total fatty acids).

Fatty Acid	Initial	Starved	ISO	ISO + Em0	ISO + Em50E	ISO + Em50D
12:0	0.2	0.2	0.1	1.1	0.1	0.1
TMTD	2.7	—	—	—	—	—
14:0	2.9	1.5	8.3	8.9	8.3	8.0
15:0	0.5	1.1	0.4	0.3	0.3	0.3
16:0	15.2	14.3	11.8	11.8	11.9	11.8
16:1n-7	1.4	1.1	4.8	5.2	4.6	4.8
16:1n-5	0.6	1.0	0.1	0.1	0.1	0.2
16:3n-3	0.3	0.7	0.5	0.3	0.3	1.4
16:4n-1	0.3	1.9	0.9	1.3	1.2	0.1
17:0*	0.6	0.9	0.2	—	0.3	0.2
18:0	3.7	6.6	1.8	2.0	2.1	1.8
18:1n-9	3.4	3.5	8.0	8.4	8.1	8.1
18:1n-7	2.5	2.7	4.1	4.1	4.1	4.0
18:2n-6	2.9	1.3	5.1	5.3	5.0	4.9
18:3n-3	3.5	0.7	5.7	5.8	5.6	5.4
18:4n-3	12.6	1.9	13.5	13.5	13.2	13.2
20:1n-11	1.1	3.4	1.0	1.1	1.0	0.9
20:1n-9	1.1	1.8	0.9	0.9	1.0	1.0
20:1n-7	0.7	1.4	0.4	0.5	0.4	0.3
20:1n-5	0.4	1.2	0.6	0.6	0.5	0.6
20:2n-6	2.0	1.8	1.3	1.5	1.2	1.3
20:3n-3	1.3	0.9	0.8	0.8	0.7	0.7
20:4n-6	1.1	3.4	1.0	1.1	1.0	1.1
20:4n-3	0.8	0.7	0.4	0.4	0.4	0.4
20:5n-3	13.0	10.1	3.3	3.0	5.5	4.1
21:5n-3	1.1	1.3	1.0	0.8	0.9	0.8
22:5n-6	0.6	1.2	2.7	2.2	2.2	2.5
22:5n-3	0.6	0.9	0.6	0.3	0.8	0.6
22:6n-3	19.0	26.3	17.9	16.3	16.7	19.4
Σ saturated	23.7	25.1	23.1	24.1	22.9	22.3
Σ monoenoic	11.7	17.1	20.5	21.6	20.5	20.4
Σ polyenoic	61.5	57.2	56.2	54.0	56.3	57.1
Σ n-3HUFA**	35.8	40.3	23.8	21.6	25.0	26.0
DHA/EPA	1.5	2.6	5.5	5.4	3.0	4.7

Minor components identified (<1%) and not included in the table are Iso-16:0; 7-methyl-hexadecanoic acid; 16:1n-9; 16:2n-6(n-4?); 16:3n-4; 16:4n-3; 20:0; 20:2NMID; 22:2NMID; 22:4n-6; 24:0.

* Includes phytanic (3,7,11,14-tetramethylhexadecanoic) acid.

** ≥20:3n-3.

coconut oil. Similarly, small proportions of 12:0 have been observed in the muscle and liver lipids of sunshine bass fed coconut oil as main dietary lipid (Nematipour and Gatlin 1993). A low accumulation of medium chain triglycerides (MCT) observed in ayu fish fed MCT (mainly 8:0) as a supplement to pollack liver oil has been attributed to the ability of fish to use MCT as a direct source of energy (Nematipour et al. 1989). The use of the coconut oil as a caloric supplement may have had a sparing effect on the catabolism of essential fatty acids provided through the algae, as indicated by the 15% increase of the concentration of EPA and DHA in the scallops fed the emulsion Em0 as a supplement (Fig. 1A).

The conservative nature of the fatty acid composition of the polar lipid fraction compared with the triglycerides is widely accepted for fish (Sargent et al. 1993) and confirmed for various stages of bivalves (Waldock and Nascimento 1979, Langdon and Waldock 1981, Delaunay et al. 1993, Napolitano and Ackman 1993). The conservation of 20:1 fatty acids, in particular 20:1n-11, observed in the starved and *Isochrysis*-fed scallops is in agreement with its possible structural role in the gill membranes (Napolitano and Ackman 1993). The selective retention of 22:6n-3 and 20:4n-6 in the starved scallops, contrary to the decrease of 20:5n-3, indicated the greater importance of the former fatty acids. Similar observations on the relative importance of 20:4n-6 and 22:6n-3 *versus* 20:5n-3 can be deduced from other bivalve studies (Helm et al. 1991, Delaunay et al. 1993), and particularly, DHA may play an important role during larval development and metamorphosis in the scallop *Pecten maximus* (Marty et al. 1992, Delaunay et al. 1993).

For bivalve larvae, it has been suggested that triglycerides may play a double role, first, as a storage of large amounts of saturated and monoethylenic fatty acids for energy purposes and, second, as a temporary reservoir of PUFA that could be transferred to structural polar lipids and specific metabolic pathways during metamorphosis (Napolitano et al. 1988). This is in agreement with several studies showing the importance of the content as well as the fatty acid composition of lipids, particularly triglycerides, for the early life stages of bivalves (Helm et al. 1973, Holland and

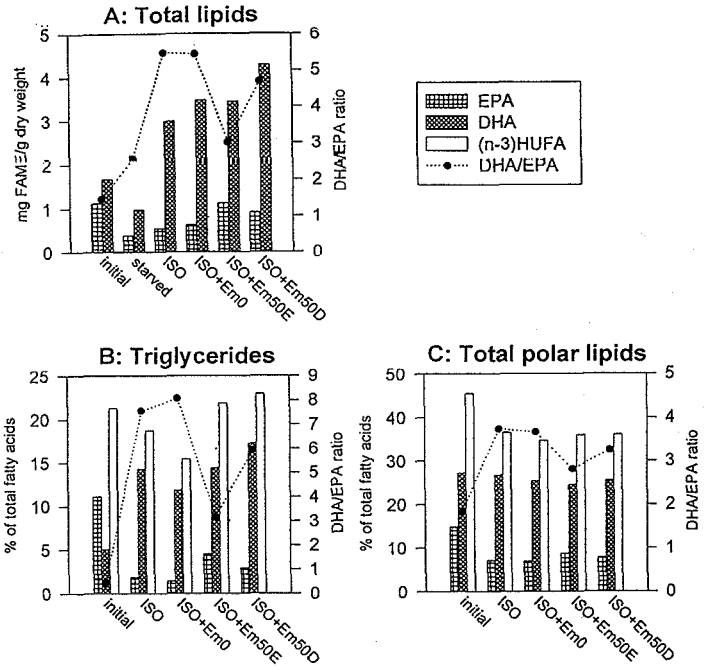

Figure 1. **Effect of starvation and supplementation of various lipid emulsions to *Isochrysis* sp. (clone T-Iso) on the content of EPA, DHA, and n-3 HUFA in total lipids [(A) mg fatty acid/g of dry weight] and triglycerides and total polar lipids [(B and C) weight percent of total fatty acids] of juvenile *P. magellanicus*.**

Spencer 1973, Gallager and Mann 1986, Gallager et al. 1986, Helm et al. 1991, Utting and Doyou 1992). Lipid emulsions may be a convenient artificial diet to study lipid and fatty acid requirements during critical stages of bivalve culture, *i.e.*, broodstock conditioning and larval rearing. Furthermore, the ability of manipulating both the quantity and the fatty acid composition of bivalve lipids, in particular of triglycerides, through the supplementation of off-the-shelf lipid supplements holds promise for application in hatchery rearing. Hatchery operators currently rely on live algae to supply essential fatty acids, and the fatty acid composition of algae may strongly vary with culture conditions (Pohl and Zurheide 1979). In this study, EPA and DHA concentrations increased by >100 and >40%, respectively, in juveniles fed lipid emulsions as a supplement to *Isochrysis* compared with the milligram/gram dry weight values in the control fed algae only. In particular, this accumulation of essential fatty acids through specific diet supplements could be used to standardize the dietary supply of lipids and essential fatty acids in broodstock conditioning and larval rearing.

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