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Lipid nutrition and settlement behaviour in American lobster *Homarus americanus*

Isabelle Thériault[1], Fabrice Pernet[2, 3,*]

[1]Département de Biologie, Université de Moncton, Moncton, Nouveau-Brunswick, Canada E1A 3E9
[2]Institut de Recherche sur les Zones Côtières, 232-B rue de l'Église, Shippagan, Nouveau-Brunswick, Canada E8S 1J2
[3]IFREMER, Laboratoire Environnement Ressources, Jean Monnet, BP 171, 34203 Sète Cedex, France

ABSTRACT: This study examines the effects of varying dietary lipids on growth, survival, lipid composition and behaviour in lobster *Homarus americanus* (Milne Edwards, 1837) postlarvae. Lobsters were fed one of 3 diets during their early ontogeny: (1) Artemac, a widely used *Artemia* replacement; (2) a ternary mixture consisting of Artemac, frozen *Artemia* adults and *Artemia* flakes; or (3) a mixture of live *A. salina* nauplii and adults. We evaluated the olfactory capacity (response to predator odour plume) and cryptic behaviour (ability to hide during settlement) of lobster postlarvae as a function of these diets and the lipid and the fatty acid composition of lobsters. Growth and survival of lobster postlarvae were highest in those fed live *Artemia*, followed by the ternary mix, then Artemac. The low performance of lobster postlarvae fed Artemac may be attributable to dietary deficiencies in phospholipids (PLs) or 20:4n-6 and coincided with low levels of endogenous triacylglycerols (TAG). Those lobsters fed live *Artemia* showed low levels of 22:6n-3 and 20:5n-3 compared to levels occurring in wild animals. Here we report the selective incorporation of 20:4n-6, 20:5n-3 and 22:6n-3 to polar lipids and, to a lesser extent, to neutral lipids in lobsters. Finally, lobster postlarvae fed the 3 experimental diets responded to a predator odour plume by reducing their activity levels and increasing the time spent swimming downstream. However, lobster settlement behaviour varied as a function of the diet: low-performing lobsters fed Artemac were less active than those fed the other diets, presumably due to low levels of endogenous TAG or changes in dietary long-chain PUFA.

KEY WORDS: Crustacean larvae · Fatty acid · Habitat selection · Lipid · Nutrition · Settlement behaviour

INTRODUCTION

Recruitment in natural populations as well as the successful aquaculture of benthic invertebrates depends to a large extent on the acquisition of energy reserves to support larval growth and transition through ontogenic stages. Most bivalve and crustacean larvae primarily rely on lipids to fulfil their energetic needs whereas protein catabolism occurs when the organism is short on lipid reserves (Holland & Spencer 1973, Anger 1998). Therefore, the triacylglycerol (TAG) content, normalised by some measure of body size such as the amount of sterol, protein or DNA, is often used as a proxy for physiological condition in bivalves and crustaceans (Fraser 1989, Harding & Fraser 1999, Miron et al. 1999, Ouellet & Allard 2002, Pernet et al. 2003, Tremblay et al. 2007).

Growth and survival of marine invertebrates also rely on the acquisition of essential fatty acids (EFAs) for incorporation into membrane phospholipids (PLs). EFA functions can be divided into 2 broad areas. On one hand, the n-3 polyunsaturated fatty acid (PUFA) docosahexaenoic acid (22:6n-3) is involved in maintaining the structural and functional integrity of biological membranes due to its unique structure, which facilitates rapid conformational changes in membrane proteins (Feller et al. 2002). On the other hand, C_{20} PUFAs, such as eicosapentaenoic acid (20:5n-3) and arachidonic acid (20:4n-6), represent precursors of eicosanoids, a group of highly biologically active hormones that includes prostaglandins, leukotrienes and hydroxyeicosatetraenoic acids (Smith & Murphy 2003). Eicosanoid production is associated very broadly with stressful situations, with excess eicosanoid production

*Corresponding author. Email: fabrice.pernet@ifremer.fr

having been recorded under pathological conditions in fish (Sargent et al. 2002).

A knowledge of settlement behaviour is of great importance to understanding the mortality, distribution and recruitment of most benthic invertebrate species (Kingsford et al. 2002). Several studies have suggested that the TAG content influences settlement behaviour or habitat selection in bivalves and crustaceans (Miron et al. 1999, Pernet et al. 2003, Tremblay et al. 2007). However, no studies have investigated the effect of EFAs on settlement behaviour in marine invertebrates, although it is generally accepted that dietary EFA levels affect cognitive functions in mammals, including humans (Fedorova & Salem 2006). It is therefore likely that EFA levels in the diet influence the settlement behaviour of invertebrate larvae.

American lobsters *Homarus americanus* (Milne Edwards, 1837) spend ca. 3 to 12 wk in the plankton, depending on temperature, and settle to the seabed at the 4th stage (Charmantier et al. 1991). Settlement is controlled by several biotic and abiotic factors (Butler et al. 2006, Phillips et al. 2006). For example, lobster postlarvae actively avoid predator odour plumes (Boudreau et al. 1993a) and preferentially settle in low-light shelters compared to microhabitats with higher light levels (Boudreau et al. 1990, 1993b). Indeed, olfactory capacities and cryptic behaviour are likely to increase post-settlement survival in lobsters by improving predator avoidance. Because *H. americanus* displays rather elaborate habitat selection behaviours at the time of settlement, and relies on TAG (Fraser 1989, Harding & Fraser 1999) and EFAs (Castell 1983, Nelson et al. 2006) for sustaining growth and survival, this species is an excellent study organism for investigating the relationship between lipid nutrition and settlement behaviour.

To this end, lobsters were fed 3 experimental diets with different lipid composition from Stage I to IV, to obtain animals with varying levels of TAG and EFAs. We then evaluated the olfactory capacities (response to predator odour plume) and cryptic behaviour (ability to hide during settlement) of lobster postlarvae as a function of these diets and the lipid composition of lobsters. As a secondary objective, we determined the effect of varying dietary lipid levels on the lipid composition of *H. americanus* postlarvae.

MATERIALS AND METHODS

Animal maintenance. Ovigerous specimens of lobster *Homarus americanus* were collected in Northumberland Strait (Wallace, Nova Scotia, Canada, 45° 48′ N, 63° 28′ W) between 22 and 26 May 2006, and were transported to the Coastal Zone Research Institute (CZRI, Shippagan, New Brunswick, Canada). Upon arrival at CZRI, lobsters were kept in a 1200 l tank equipped with a flow-through seawater system. The water flow was set at 5 l min^{-1}, and the volume was maintained constant with excess water exiting through an overflow. The seawater temperature was 11°C, salinity was 28 and the light:dark photoperiod was 15:9 h. Animals were fed to satiation on a diet of smelt *Osmarus mordax* 3 times per week.

Release of larvae was induced by thermal shock. Twenty ovigerous lobsters were randomly chosen and transferred to a 1200 l tank filled with 10 µm filtered seawater at 20°C. The hatching tanks were strongly aerated to keep newly hatched larvae near the surface (to avoid cannibalism) and to maintain oxygen at a sufficient level. Newly hatched larvae were collected from the surface of the hatching tank within 2 to 3 h. Approximately 2500 newly hatched lobsters were allocated to several 250 l circular tanks. Larvae and postlarvae were kept in 10 µm filtered seawater at 20°C; salinity 28, water flow set at 1 l min^{-1} and light:dark photoperiod at 16:8. In addition, strong aeration was provided to each tank so that larvae did not settle on the bottom, where cannibalism is most prevalent. Larvae were fed twice a day, at 09:00 and 17:00 h. The daily dry mass of the ration was ca. 15% of the mean dry mass of one larva, except for those fed live *Artemia* (see 'Experimental design', below).

Experimental design. Larvae were fed one of 3 diets: (1) Artemac, a widely used *Artemia* replacement (Aquafauna); (2) a ternary mixture consisting of Artemac, frozen adult *Artemia* and *Artemia* flakes (Aquatic Lifeline); or (3) a mixture of live nauplii and adult *A. salina* (strain *franciscana*). A ration of live *Artemia* consisted of forty newly hatched nauplii mixed with four 9 to 12 d old adults per larva. Live *Artemia* were used as a reference diet as traditionally, lobster hatcheries have primarily relied on this diet for lobster larvae (Fiore & Tlusty 2005). Live *Artemia* were reared in 900 l conical tanks at an initial density of ~10^4 nauplii l^{-1} and fed twice a day with *Artemia* Food (Northeast Brine Shrimp). The individual components of each diet were sampled twice during the experiment for lipid analysis.

An overview of the experiments is shown in Table 1. Five independent batches of lobster larvae were reared between July and August 2006 from Stage I to IV under the same conditions. Dry mass, survival and lipid composition of lobsters fed the 3 experimental diets described above (this section) were evaluated 2 d after larvae attained Stage IV, which coincided with the onset of the behavioural experiments. Unfortunately, unexpected mortality events or logistical limitations did not allow us to test each diet simultaneously (Table 1).

Table 1. Experimental design. Sampling dates of Stage IV lobsters for lipid analyses and behavioural assays as a function of the tested diets. Lobsters were sampled 2 d after moulting to Stage IV. L: lipid, B: behaviour, O: odour plume exposure, C: cryptic behaviour, +: complete mortality, nd: not done due to logistical limitations

Date	Block	Diets		
		Artemac	Ternary mix	Live *Artemia*
3–6 July	1	L, B (O, C)	nd	L, B (O)
12–14 July	2	L	L, B (O, C)	L, B (O, C)
19–22 July	3	L, B (O, C)	L, B (O)	L, B (O, C)
2 August	4	+	L, B (O, C)	L, B (O, C)
25 August	5	+	L, B (O, C)	nd

Behavioural assays. ***Exposure to predator odour plume:*** We determined the ability of lobster postlarvae fed the 3 diets to use predator odour plumes as a potential orientation mechanism during habitat selection at settlement. The chemotactic responses of lobster postlarvae were tested during the daylight portion of the light cycle in a black Plexiglas Y-maze similar to that described by Boudreau et al. (1993a). Briefly, each arm of the maze was supplied by gravity with either sterilised seawater in the control arm or the test solution in the experimental arm; rate: 150 ml min^{-1}. The test solution was made of 132 µm filtered seawater from a 250 l tank in which 5 mature cunners *Tautogolabrus adspersus*, a natural predator of *H. americanus*, were maintained for 24 h (hereafter termed 'experimental solution'). Alternatively, the test solution was made of the same water without fish as a control (hereafter termed 'control solution'). A minimum of 20 postlarvae $tank^{-1}$ were tested with each test solution over a 2 d period. The control and the experimental arms were switched every day. At this time, the Y-maze was cleaned with a non-toxic soap and rinsed thoroughly with control solution.

Each postlarva was gently introduced into the holding compartment located in the central arm of the maze (Boudreau et al. 1993a). After 2 min of acclimatization, the holding compartment was removed and the postlarva was observed for 3 min, during which it could remain in the central arm, move up the arms or down the maze to the drain. The position of the postlarva in the maze and its activity level were continuously monitored by 2 observers. The response variables were the proportion of observation time spent in each arm of the maze (arm selection) and the proportion of observation time spent swimming upstream or downstream or remaining inactive (activity budget). Postlarvae were used only once, with a total of 533 postlarvae for the experiments. Occasionally (ca. 4% of the time), postlarvae were completely inactive and were omitted from data analysis.

Examination of cryptic behaviour: We determined the ability of lobster postlarvae fed the 3 diets to hide during settlement. Forty shelters, circular black caps made of acrylonitrile butadiene styrene (3.5 × 1.5 cm, diameter × height) with one entrance of area 1 cm^2 (Boudreau et al. 1990), were placed on the bottom of a 200 l flow-through seawater aquarium containing a thin layer of coarse sand. Shelters and sand had been washed, rinsed, sterilised and conditioned in 10 µm filtered seawater prior to use.

At the beginning of each trial, 40 postlarvae of each diet per aquarium were gently released at the surface of the aquarium and left undisturbed for 10 h. Postlarva positions were recorded 5, 10, 15, 20, 80, 180, 300 and 450 min after being released during the daylight and also in the dark, after 600 min. Observations in the dark were conducted with a red light to avoid light disturbance (Boudreau et al. 1990). Postlarvae were recorded as swimming in the water column or unsheltered on the bottom. The number of sheltered animals was calculated by subtracting the number of swimmers and benthic unsheltered animals from the initial number released in the tank. The few mortalities were recorded and taken into account (<10% of the initial number of larvae). It was clear that these mortalities were not influenced by diet, since mortalities within each treatment were variable.

Lipid analysis. ***Sampling:*** Diets and lobster postlarvae were sampled for organic mass and lipid composition. Twenty lobsters were removed from each experimental tank, gently rinsed with distilled water and dried on a clean paper towel. Ten lobsters were placed in an aluminium dish, dried at 60°C for 48 h to obtain the dry mass, and then heated at 450°C for 24 h to determine the ash mass. The remaining animals were stored in lipid-clean amber glass vials with Teflon-lined caps flushed under nitrogen in 2 ml of dichloromethane at −80°C until lipid extraction. The same procedures were applied for diets except for live *Artemia*, which were first filtered onto 25 mm Whatman GF/C filters pre-combusted at 450°C.

Lipid extraction and lipid class analysis: Lipids were extracted following the method of Folch et al. (1957), spotted onto S-III Chromarods (Iatron), and separated into aliphatic hydrocarbons, sterol and wax esters, ketones, triglycerides, free fatty acids, free fatty alcohol, free sterols, diacylglycerols, acetone mobile polar lipids and PLs (Parrish 1999). Total lipid was obtained by the summation of all lipid classes. Chromarods were scanned by a flame ionization detection system (FID; Iatroscan Mark-VI, Iatron), and chromatograms were analysed using integration software (Peak Simple v3.2, SRI).

Neutral and polar lipid separations: Lipids were separated into neutral lipids (including triglycerides,

free fatty acids and sterols) and polar lipids (including mainly PLs and minor amounts of glycolipids) using column chromatography on silica gel hydrated with 6% water as previously described (Pernet et al. 2006). Briefly, the 100 mg columns were preconditioned with 1 ml of methanol and 1 ml of chloroform. Aliquots of 200 µl of lipid corresponding to ~1 mg of lipid were loaded onto the solid-phase extraction column. Samples were gently drawn into the solid phase with a slight vacuum. Columns were washed with 1 ml chloroform:methanol (98:2 v/v) to elute neutral lipids followed by 5 ml of methanol to elute polar lipids. The eluted fractions were collected in 7 ml tubes positioned in a vacuum manifold apparatus. The vacuum was adjusted to generate a flow rate of ~1 ml min^{-1}. The neutral fraction was further eluted on an activated silica gel microcolumn previously heated to 450°C and conditioned with 1 ml of isopropanol and 2 ml of hexane to eliminate the free sterols (ST).

Fatty acid analysis: Fatty acid methyl esters (FAMEs) from polar lipids were prepared using 2% H_2SO_4 in methanol following Lepage & Roy (1984). FAMEs were run on a Varian CP3900 gas chromatograph equipped with a ZB-wax fused silica capillary column (20 m × 0.18 mm internal diameter × 0.18 µm film thickness; Supelco). Helium was used as the carrier gas (flow velocity: 1 ml min^{-1}). FAMEs were injected at 250°C at a 1:10 split ratio. The temperature ramp was 140°C for 0.2 min, followed by an increase of 40°C min^{-1} to 170°C, followed by an increase of 4°C min^{-1} to 185°C, and finally by an increase of 2°C min^{-1} to 230°C. The detector was maintained at 260°C. FAMEs were identified by comparison of retention times with known standards (37-component FAME Mix, PUFA-3, and menhaden oil; Supelco) and quantified with nonadecanoic acid (19:0) as an internal standard. Chromatograms were analysed using the Galaxie Chromatography Data System (v. 1.9.3.2, Varian).

Data analysis. Differences in lipid classes and fatty acid composition among diets were investigated using 1-way multiple analyses of variance (MANOVA) on selected fatty acids or groups. Differences in performance characteristics (mass, growth and survival) among lobster postlarvae were investigated by separate 2-way ANOVAs, whereas differences in lipid classes and fatty acid composition of polar lipids in lobsters were investigated using 2-way MANOVAs. The 2 factors were block and diet and the interaction between these factors represented the error term in the analysis.

Differences among diet groups in the proportion of observation time spent in each arm of the maze (Arm Selection) and swimming upstream or downstream or remaining inactive (Activity Budget) were investigated using 2-way ANOVAs. Sources of variations were Block, Diet and Treatment (test solution) as the main effects and the interaction of Diet and Treatment.

Two-way split-plot ANOVAs were conducted to determine differences in the percentages of sheltered and swimming animals as a function of Diet and Time. The main plots were Block and Diet, and subplots were Sampling Times. Here we used a mixed linear model, which models not only the means of our data but their variances and covariances. The need for covariance parameters arose because the experimental units on which the variables were measured were grouped into clusters, and repeated measurements were taken on the same experimental unit. The repeated option was applied to the interaction term 'Time'. These terms were combined with the covariance structure of the matrix to take into account spatial and temporal dependence. The unit of replication in all statistical analysis was the tank in which the diet was applied.

Tukey multiple comparison tests were used when significant differences were detected. Residuals were screened for normality using the expected normal probability plot and further tested using the Shapiro-Wilks test. When necessary, data were log+1- or 1/square root(x)-transformed to achieve normality of residuals and homogeneity of variances. Homogeneity of the variance–covariance matrices was graphically assessed. These analyses were carried out using SPSS 14.0 and SAS 9.1.3.

RESULTS

Diet characteristics

Total lipid concentrations (mg g^{-1} dry mass) in the 3 experimental diets were comparable (Table 2). Triacylglycerol (TAG) and PL represented the major lipid classes (77 to 87% of total lipid) whereas sterol-esters and wax-esters (SE-WE), ST and acetone mobile polar lipids (AMPL) together accounted for <25%. Minor amounts of free fatty acid (<1%) were occasionally detected and are not discussed further. It is noteworthy that TAG levels in live *Artemia* were 6 to 7 times lower than that of other diets, which showed reduced PL levels.

Fatty acids were divided into 3 categories: saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. The emphasis of this study is focused on the long-chain PUFA (chain length ≥20 C). PUFA contributed >30% of the total fatty acids found in lobster diets (Table 2). Artemac had higher PUFA levels than the 2 other diets. More importantly, Artemac showed the highest level of 22:6n-3 (6.6%) followed by the ternary mix (2.8%) and live *Artemia*

Table 2. Lipid class and fatty acid composition of individual components of the diets used in this study. Mixed diet compositions were calculated from those of their individual constituents. Masses of total lipids and fatty acids are expressed as mg g^{-1} of dry mass. Ternary mix was composed of Artemac, frozen *Artemia* and *Artemia* flakes. Live *Artemia* consist of a mixture of live *A. salina* nauplii and adults. Data are mean ± SD (n = 2). Only fatty acids contributing >1% in at least one component are reported. Different letters (a,b,c) indicate significant differences among diets (1-way MANOVA, $p < 0.05$). **Bold values:** significant. AMPL: acetone mobile polar lipid; SFA, MUFA and PUFA: saturated, monounsaturated and polyunsaturated fatty acids, respectively

	Individual components (*Artemia*)				Diets			p
	Live adult	Live nauplii	Flakes	Frozen	Artemac	Ternary mix	Live *Artemia*	
Lipid class								
Sterol/wax esters	4.1 ± 7.0	4.9 ± 0.1	5.0 ± 0.4	12.4 ± 0.1	13.2 ± 1.9	11.3 ± 0.2	5.3 ± 3.1	0.069
Triacyglycerols	1.7 ± 1.5	6.1 ± 2.5	59.5 ± 0.5	17.3 ± 12.0	32.4^{a} ± 0.6	27.0^{a} ± 8.1	4.6^{b} ± 2.0	**0.021**
Sterols	3.3 ± 2.3	5.1 ± 0.6	1.2 ± 0.0	3.6 ± 0.7	6.2 ± 0.0	3.7 ± 0.4	4.7 ± 1.4	0.129
AMPL	6.3 ± 2.0	0.0 ± 0.0	1.5 ± 0.1	0.9 ± 0.5	3.2^{a} ± 0.0	1.4^{b} ± 0.3	2.1^{ab} ± 0.5	**0.025**
Phospholipids	83.8 ± 9.4	83.9 ± 3.1	32.5 ± 0.8	65.0 ± 10.6	44.8^{b} ± 1.4	56.0^{b} ± 6.9	83.0^{a} ± 5.7	**0.011**
Total lipids	98.0 ± 19.5	53.7 ± 8.3	136.9 ± 1.1	58.3 ± 7.2	99.0 ± 4.2	78.6 ± 5.3	71.1 ± 13.6	0.101
Fatty acid								
14:0	0.7 ± 0.1	0.6 ± 0.1	4.4 ± 0.0	2.0 ± 0.0	2.9 ± 0.0	2.6 ± 0.0	0.7 ± 0.1	
16:0	10.6 ± 1.0	9.9 ± 0.1	15.0 ± 0.0	12.0 ± 0.2	16.2 ± 0.3	13.2 ± 0.2	10.0 ± 0.5	
17:0	0.9 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	1.3 ± 0.0	0.2 ± 0.0	0.9 ± 0.0	0.7 ± 0.0	
18:0	8.7 ± 0.6	9.2 ± 0.2	3.2 ± 0.0	5.7 ± 0.0	5.5 ± 0.0	5.2 ± 0.0	9.1 ± 0.4	
Σ SFA	22.5 ± 0.4	21.9 ± 0.0	23.9 ± 0.1	22.4 ± 0.3	25.6^{a} ± 0.3	23.2^{b} ± 0.3	22.1^{c} ± 0.1	**0.002**
14:1n-5	1.0 ± 0.1	0.8 ± 0.0	0.2 ± 0.0	1.8 ± 0.0	0.1 ± 0.0	1.2 ± 0.0	0.8 ± 0.0	
16:1n-7	5.3 ± 0.4	1.5 ± 0.0	6.8 ± 0.0	17.3 ± 0.2	3.2 ± 0.0	13.1 ± 0.1	2.8 ± 0.1	
17:1	0.5 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	1.2 ± 0.0	0.3 ± 0.0	0.9 ± 0.0	0.5 ± 0.0	
18:1n-9	16.3 ± 1.2	19.0 ± 0.2	14.5 ± 0.0	13.1 ± 0.2	11.7 ± 0.2	13.1 ± 0.1	17.9 ± 0.4	
18:1n-7	12.5 ± 1.3	8.6 ± 0.1	3.2 ± 0.0	14.3 ± 0.0	2.3 ± 0.1	10.4 ± 0.0	10.1 ± 0.5	
20:1n-11	0.0 ± 0.1	0.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	
20:1n-9	0.4 ± 0.0	1.0 ± 0.0	5.2 ± 0.0	0.2 ± 0.0	6.4 ± 0.0	2.1 ± 0.0	0.7 ± 0.0	
22:1n-(11+13)	0.0 ± 0.0	0.0 ± 0.0	4.9 ± 0.0	0.0 ± 0.0	8.1 ± 0.1	2.2 ± 0.0	0.0 ± 0.0	
22:1n-9	0.0 ± 0.0	0.1 ± 0.0	0.9 ± 0.0	0.1 ± 0.0	1.1 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	
Σ MUFA	37.5 ± 0.2	32.6 ± 0.1	39.3 ± 0.0	50.2 ± 0.0	36.7^{b} ± 0.2	46.1^{a} ± 0.0	34.3^{c} ± 0.0	**<0.001**
16:2n-6	1.1 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	0.9 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	
18:2n-6	23.4 ± 0.9	5.1 ± 0.1	10.9 ± 0.0	3.8 ± 0.0	19.4^{a} ± 0.2	7.6^{c} ± 0.0	11.4^{b} ± 0.3	**<0.001**
18:3n-3	3.7 ± 0.4	24.2 ± 0.1	1.8 ± 0.0	3.0 ± 0.0	2.7^{b} ± 0.0	2.8^{b} ± 0.0	17.0^{a} ± 0.1	**<0.001**
20:3n-3	0.1 ± 0.0	1.4 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	1.0 ± 0.0	
18:4n-3	0.5 ± 0.1	4.4 ± 0.0	1.4 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.2 ± 0.0	3.0 ± 0.0	
20:4n-6	3.5 ± 1.0	1.1 ± 0.0	0.7 ± 0.0	1.8 ± 0.0	0.4^{b} ± 0.0	1.4^{ab} ± 0.0	2.0^{a} ± 0.4	**0.017**
20:5n-3	4.7 ± 0.4	3.1 ± 0.1	7.1 ± 0.0	10.5 ± 0.2	4.6^{b} ± 0.0	8.9^{a} ± 0.1	3.7^{c} ± 0.1	**<0.001**
22:5n-3	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 0.0	0.0 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	
22:6n-3	0.4 ± 0.0	0.2 ± 0.1	8.4 ± 0.0	0.3 ± 0.0	6.6^{a} ± 0.0	2.8^{b} ± 0.0	0.3^{c} ± 0.1	**<0.001**
Σ PUFA	40.0 ± 0.5	45.5 ± 0.1	36.7 ± 0.0	27.3 ± 0.3	37.7^{b} ± 0.1	30.7^{c} ± 0.2	43.6^{a} ± 0.1	**<0.001**
Σ n-6	29.4 ± 0.5	9.3 ± 0.0	13.0 ± 0.0	7.4 ± 0.1	20.5^{a} ± 0.1	10.5^{c} ± 0.0	16.4^{b} ± 0.2	**<0.001**
Σ n-3	9.5 ± 0.0	34.2 ± 0.3	21.8 ± 0.0	15.7 ± 0.3	16.4^{b} ± 0.1	16.8^{b} ± 0.2	25.6^{c} ± 0.2	**<0.001**
Σ n-3/Σ n-6	0.3 ± 0.0	3.7 ± 0.0	1.7 ± 0.0	2.1 ± 0.1	0.8^{c} ± 0.0	1.8^{b} ± 0.0	2.5^{c} ± 0.0	**<0.001**
20:4/20:5	0.7 ± 0.1	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1^{b} ± 0.0	0.1^{b} ± 0.0	0.5^{a} ± 0.1	**0.003**
22:6/20:5	0.1 ± 0.0	0.1 ± 0.0	1.2 ± 0.0	0.0 ± 0.0	1.4^{a} ± 0.0	0.5^{b} ± 0.0	0.1^{c} ± 0.0	**<0.001**
Total fatty acids	60.6 ± 29.7	41.1 ± 8.5	97.7 ± 8.8	55.9 ± 3.1	86.6^{a} ± 4.5	68.2^{ab} ± 4.3	46.2^{b} ± 12.5	**0.035**

(0.8%). In contrast, the level of 20:5n-3 was twice as high in the ternary mix compared to the other diets (Table 2). Finally, 20:4n-6 levels were generally low (<2%), with live *Artemia* > ternary mix > Artemac. It is noteworthy that high levels of 18:2n-6 and 18:3n-3 were characteristic of live *Artemia* and Artemac (18:2n-6 only), which generally lowered the relative contributions of the long-chain PUFA in these diets (Table 2).

Growth, survival and lipid composition of lobsters

The growth rate of lobsters clearly differed among the 3 diets, with animals fed live *Artemia* having the highest growth (0.7 mg dry mass d^{-1}; Table 3). The second highest growth was observed in lobsters fed the ternary mix; their growth rate was half that of those fed live *Artemia*. Finally, lobsters fed Artemac showed a growth rate of <0.2 mg d^{-1}. The live Artemia diet

Table 3. *Homarus americanus.* Characteristics of lobsters fed the 3 experimental diets for 11 to 14 d. Lobsters were sampled 2 d after moulting to Stage IV, which coincided with the onset of behavioural assays. Data are mean ± SD (n = 2 to 4 replicate tanks). Different letters (a,b,c) indicate significant differences among diets (1-way MANOVA, $p < 0.05$). **Bold values:** significant. Organic mass: ash-free dry mass

	Diets			p
	Artemac	Ternary mix	Live *Artemia*	
Performance characteristics				
Organic mass (mg larva^{-1})	2.0^{b} ± 0.9	3.4^{ab} ± 0.7	5.8^{a} ± 2.1	**0.031**
Growth (µg dry mass d^{-1})	194.5^{b} ± 57.9	379.2^{ab} ± 57.3	668.1^{a} ± 178.2	**0.042**
Survival (%)	1.3^{c} ± 0.7	9.1^{b} ± 2.8	30.0^{a} ± 6.7	**0.003**
Lipid content (µg larva^{-1})				
Triacylglycerols	0.6 ± 0.7	13.0 ± 11.4	15.2 ± 11.1	**0.018**
Sterols	5.5 ± 1.5	7.8 ± 1.4	12.9 ± 5.0	0.102
Phospholipids	64.1 ± 13.7	107.9 ± 21.7	147.8 ± 32.4	**0.032**
Total lipid	71.0 ± 15.7	131.5 ± 32.9	177.3 ± 43.9	**0.049**
Lipid proportion (% of total lipid)				
Triacylglycerols	0.7 ± 0.7	10.6 ± 5.4	7.7 ± 4.4	**0.014**
Sterols	7.8 ± 0.8	6.1 ± 0.9	7.1 ± 1.3	0.134
Phospholipids	90.5 ± 1.9	82.7 ± 4.6	84.4 ± 4.8	0.247

resulted in lobster postlarvae with the highest body mass, followed by those fed the ternary mix and finally the Artemac diet (see also Fig. 1). Interestingly, the fast-growing lobsters fed live *Artemia* also had the highest survival. In these animals, survival from Stage I to IV reached 30% and was 3 and 23 times higher compared to those fed the ternary mix and Artemac respectively (Table 3). Moulting to Stage IV in lobsters fed live *Artemia*, the ternary mix and Artemac occurred at 11, 13 and 14 d post-hatching, respectively. Although lobster fed the 3 different diets all reached Stage IV, animals fed Artemac were clearly less developed than those fed other diets (Fig. 1).

Lipid classes detected in lobster postlarvae were TAG, ST and PL (Table 3). Minor amounts of SE-WE, free fatty acids and AMPL (<1%) were occasionally detected and are not further discussed. The low-performing lobster fed Artemac showed absolute TAG concentrations 20 times lower than postlarvae fed other diets. Absolute concentrations of structural lipids (ST and PL) were positively correlated with the body mass of lobsters fed the 3 experimental diets. Relative TAG concentrations differed among the 3 diets in a way somewhat similar to those of the absolute concentrations, with live *Artemia* = ternary mix > Artemac. The patterns of %ST and %PL were essentially the inverse of that observed for %TAG, with Artemac > live *Artemia* = ternary mix.

The neutral lipid fatty acid composition in lobsters somewhat reflected that of the diets whereas polar lipids were much more strongly regulated: PUFA levels were high irrespective of diet (Table 4). Lobsters fed Artemac showed a marked increase in 22:6n-3 in both neutral and polar lipids compared to those fed other diets. In contrast, these animals showed a much reduced level of 20:4n-6 (only 2.2% in polar lipids) compared to those fed the ternary mix (4.7%) or live *Artemia* (7.2%). Levels of 20:5n-3 in neutral lipids were similar among lobsters fed the 3 experimental diets and varied moderately in the polar lipids, reflecting levels in the diets. Finally, differences in dietary levels of 18:2n-6 and 18:3n-3 in both neutral and polar lipids were observed.

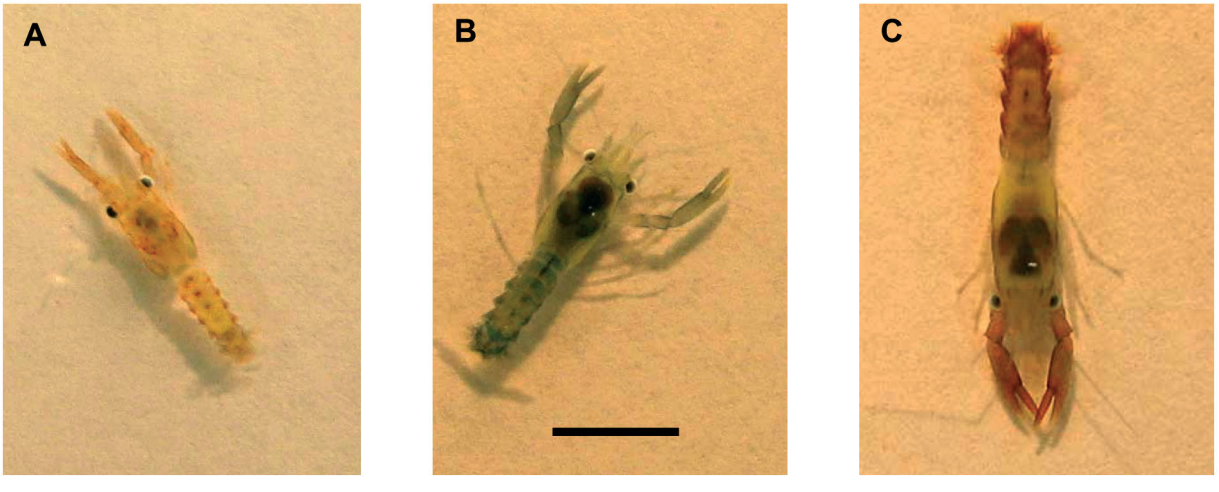

Fig. 1. *Homarus americanus.* Stage IV lobster after 11 to 14 d of exposure to various diet treatments (coloration also reflects diet): (A) Artemac, (B) ternary mix and (C) live *Artemia*. Scale bar = 5 mm (applies to all panels)

Table 4. *Homarus americanus.* Fatty acid composition (mass %) of neutral and polar lipids of lobsters fed the 3 experimental diets for 11 to 14 d. Lobsters were sampled 2 d after moulting to Stage IV, at the onset of behavioural assays. Data are mean ± SD (n = 2 to 4 replicate tanks). Different letters (a,b,c) indicate significant differences among diets (1-way MANOVA, $p < 0.05$). **Bold values:** significant

Fatty acid	Neutral lipids			p	Polar lipids			p
	Artemac	Ternary mix	Live *Artemia*		Artemac	Ternary mix	Live *Artemia*	
16:0	14.2 ± 1.0	13.5 ± 1.4	9.3 ± 0.1		15.0 ± 0.1	12.1 ± 0.3	10.6 ± 0.3	
17:0	1.0 ± 0.5	1.1 ± 0.2	1.0 ± 0.1		0.5 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	
18:0	7.9 ± 1.1	4.4 ± 1.0	7.1 ± 0.7		6.8 ± 0.5	7.0 ± 0.2	8.4 ± 0.3	
20:0	0.7 ± 0.3	0.3 ± 0.1	0.5 ± 0.1		1.1 ± 0.1	0.9 ± 0.0	1.0 ± 0.1	
22:0	0.7 ± 0.3	0.5 ± 0.1	0.6 ± 0.1		0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.0	
Σ SFA	26.7^{a} ± 2.4	22.9^{ab} ± 0.7	19.7^{b} ± 0.9	**0.015**	25.2^{a} ± 0.6	23.4^{b} ± 0.5	22.9^{b} ± 0.3	**0.006**
16:1n-7	2.2 ± 0.2	11.3 ± 0.9	3.2 ± 1.0		1.1 ± 0.0	3.9 ± 0.2	1.6 ± 0.2	
18:1n-9	10.2 ± 1.2	13.8 ± 0.5	15.5 ± 2.0		8.5 ± 0.2	9.9 ± 0.5	10.0 ± 0.4	
18:1n-7	4.6 ± 0.1	15.0 ± 1.0	13.5 ± 0.9		4.1 ± 0.2	11.7 ± 0.3	10.7 ± 0.3	
20:1n-9	4.7 ± 0.5	1.6 ± 0.3	0.8 ± 0.1		2.8 ± 0.2	1.2 ± 0.1	0.8 ± 0.1	
Σ MUFA	30.4^{c} ± 2.9	50.8^{a} ± 1.9	37.1^{b} ± 1.3	**0.002**	19.4^{c} ± 0.4	30.5^{a} ± 1.0	25.5^{b} ± 0.3	**<0.001**
18:2n-6	14.3^{ab} ± 2.3	7.1^{b} ± 1.2	16.6^{a} ± 3.3	**0.030**	13.3^{a} ± 1.2	6.2^{b} ± 1.6	14.5^{a} ± 2.2	**0.011**
20:2n-6	2.9 ± 0.3	0.6 ± 0.1	1.9 ± 0.2		2.9 ± 0.2	0.9 ± 0.1	1.9 ± 0.1	
18:3n-3	1.6 ± 0.3	2.2 ± 0.4	6.7 ± 2.8	**0.046**	1.2^{b} ± 0.1	1.8^{b} ± 0.2	5.3^{a} ± 0.7	**0.001**
20:4n-6	2.0^{b} ± 0.4	1.9^{b} ± 0.4	4.4^{a} ± 0.9	**0.032**	2.2^{c} ± 0.2	4.7^{b} ± 0.5	7.2^{a} ± 0.8	**0.001**
22:4n-6	0.4 ± 0.2	0.3 ± 0.1	0.9 ± 0.3		0.4 ± 0.1	0.7 ± 0.2	1.2 ± 0.2	
20:5n-3	9.0 ± 1.9	5.4 ± 1.4	5.9 ± 1.5	0.168	17.0^{ab} ± 1.1	20.0^{a} ± 1.5	13.9^{b} ± 1.6	**0.029**
22:6n-3	8.1^{a} ± 1.3	2.0^{b} ± 0.4	1.4^{b} ± 0.4	**0.001**	15.6^{a} ± 0.6	7.7^{b} ± 0.2	3.5^{c} ± 0.4	**<0.001**
Σ PUFA	42.9^{a} ± 3.8	26.3^{b} ± 2.6	43.2^{a} ± 1.1	**0.004**	55.3^{a} ± 0.3	46.0^{c} ± 0.8	51.6^{b} ± 0.6	**<0.001**
Σ n-6	20.1 ± 2.1	11.8 ± 0.9	25.6 ± 2.4		19.1 ± 0.7	13.8 ± 1.4	26.3 ± 1.3	
Σ n-3	21.3 ± 2.8	11.1 ± 2.3	16.4 ± 3.4		35.5 ± 0.5	31.0 ± 1.6	24.6 ± 1.8	
Σ n-3/Σ n-6	1.1 ± 0.2	0.9 ± 0.2	0.7 ± 0.2	0.129	1.9^{a} ± 0.1	2.3^{a} ± 0.3	0.9^{b} ± 0.1	**0.003**
20:4/20:5	0.2^{c} ± 0.0	0.4^{b} ± 0.0	0.8^{a} ± 0.1	**<0.001**	0.1^{c} ± 0.0	0.2^{b} ± 0.0	0.5^{a} ± 0.0	**<0.001**
22:6/20:5	0.9^{a} ± 0.1	0.4^{b} ± 0.0	0.2^{c} ± 0.0	**<0.001**	0.9^{a} ± 0.1	0.4^{b} ± 0.0	0.3^{c} ± 0.0	**<0.001**
Total fatty acids	11.9 ± 8.2	69.2 ± 22.4	72.5 ± 28.0	0.134	51.1^{b} ± 19.7	103.8^{ab} ± 11.0	153.2^{a} ± 26.4	**0.007**

The relative proportions of fatty acid in neutral or polar lipid of lobster larvae vs. that in experimental diets were used to evaluate the nutritional quality of the 3 experimental diets and to indicate whether a given dietary fatty acid is selectively incorporated or eliminated by larvae (Fig. 2). If the relative proportion of fatty acid in larvae/diet is equal to or <1, then the larval fatty acid requirement is presumably satisfied. In contrast, if the relative proportion of fatty acid in larvae/diet is higher than 1, then a fatty acid is selectively incorporated by larvae. Overall, lobsters fed the 3 experimental diets showed selective retention of 20:4n-6, 20:5n-3 and 22:6n-3 in their polar lipids. However, it is noteworthy that the level of dietary PUFA affected PUFA retention in lobsters. Indeed, lobsters fed the low-20:4n-6 Artemac showed the highest retention of 20:4n-6 in both neutral and polar lipids. In contrast, animals fed the low-22:6n-3 live *Artemia* showed a marked retention for this fatty acid, particularly in the polar lipids.

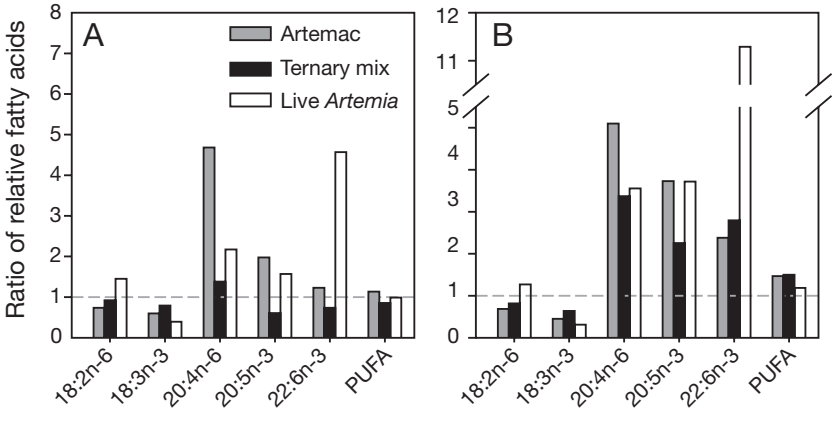

Fig. 2. *Homarus americanus.* Relative proportion of specific fatty acids in (A) neutral and (B) polar lipids of lobster postlarvae compared to dietary levels after 11 to 14 d of feeding on 3 experimental diets. The dashed line indicates the proportion of fatty acids in the larvae equal to those in the diet. PUFA: polyunsaturated fatty acid

Behavioural assays

Exposure to predator odour plume. The proportion of observation time spent in each arm of the maze (arm selection) and the proportion of observation time spent swimming upstream or downstream or remaining inactive (activity budget) both varied as a function of Diet and Treatment (Fig. 3, Table 5). The interactive effect of Diet × Treatment was not significant and is not further discussed. Lobsters fed Artemac spent a greater proportion of the observation time in the centre of the maze and not in the experimental arm (where the test solution was flowing) compared to animals fed other diets (Fig. 3A). Furthermore, lobsters fed Artemac were less active than those fed other diets, which spent a greater proportion of the observation time swimming upstream (Fig. 3B). Similarly, lobsters exposed to the experimental solution (in which fish had been maintained for 24 h) spent a greater proportion of observation time in the center of the maze (Fig. 3C), were less active, and spent a lower proportion of the observation time swimming upstream than those exposed to the control solution (Fig. 3D).

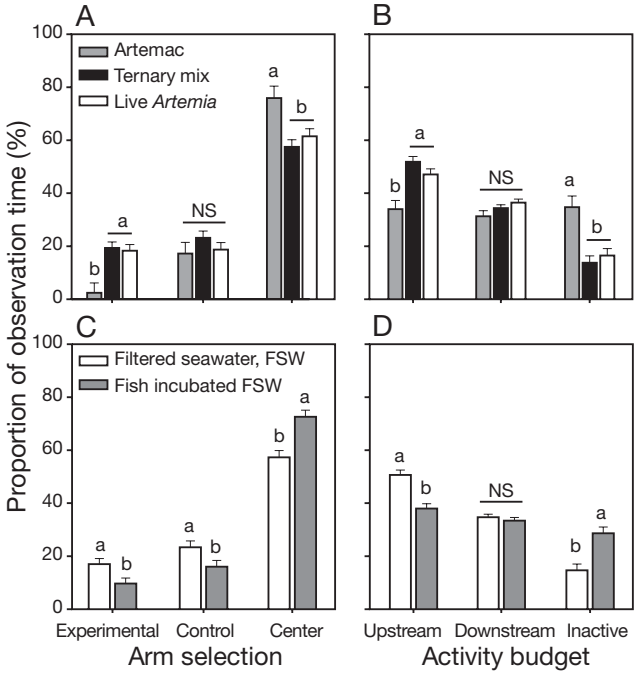

Fig. 3. *Homarus americanus.* (A,C) Proportion of observation time spent in each arm of the maze (arm selection) and (B,D) proportion of observation time spent swimming upstream or downstream or remaining inactive (activity budget) as a function of (A,B) diet and (C,D) test solutions. Data are mean ± SD (n = 2, or 4 replicate tanks, depending on diet). Different letters indicate significant differences. NS: not significant. See 'Materials and methods, Behavioural assays' for details of experiment

Table 5. Summary of the split-plot 2-way ANOVAs on the effect of Block (time of sampling), Diet and Treatment (test solutions: 132 µm filtered seawater, FSW, vs. fish-incubated FSW) as the main effects and the interaction of Diet and Treatment on the proportion of observation time spent by each lobster in each arm of the Y-maze (arm selection) and swimming upstream or downstream, or remaining inactive (activity budget). **Bold values:** significant

Dependent variable Sources of variation	df	MS	*F*	p
Arm selection				
Experimental				
Block	4	33.1	0.9	0.514
Diet	2	305.8	8.0	**0.006**
Treatment	1	255.3	6.7	**0.024**
Diet × Treatment	2	49.5	1.3	0.311
Error	12	38.3		
Control				
Block	4	49.0	1.0	0.454
Diet	2	39.8	0.8	0.474
Treatment	1	255.0	5.1	**0.043**
Diet × Treatment	2	41.7	0.8	0.458
Error	12	50.0		
Center				
Block	4	74.4	1.3	0.316
Diet	2	307.5	5.5	**0.020**
Treatment	1	1101.4	19.6	**0.001**
Diet × Treatment	2	60.5	1.1	0.372
Error	12	56.2		
Activity budget				
Upstream				
Block	4	12.1	0.4	0.805
Diet	2	280.3	9.3	**0.004**
Treatment	1	761.6	25.2	**<0.001**
Diet × Treatment	2	43.0	1.4	0.279
Error	12	30.2		
Downstream				
Block	4	11.9	1.0	0.458
Diet	2	30.9	2.5	0.123
Treatment	1	7.7	0.6	0.444
Diet × Treatment	2	11.0	0.9	0.435
Error	12	12.3		
Inactive				
Block	4	44.7	0.9	0.493
Diet	2	434.1	8.8	**0.004**
Treatment	1	922.4	18.6	**0.001**
Diet × Treatment	2	89.2	1.8	0.207
Error	12	49.5		

Examination of cryptic behaviour. Diet and time showed interactions in their effects on the percentages of benthic-sheltered (cryptic) and swimming lobsters (Table 6). Indeed, lobsters fed Artemac showed a constant percentage of sheltered animals (~70%) during the daylight compared to those fed other diets, where sheltering behaviour increased from ~25 to 40% during the first 300 min after release (Fig. 4). It is noteworthy that a higher percentage of lobsters fed Artemac sought shelter compared to lobsters fed the other diets

Table 6. Summary of the split-plot 2-way ANOVAs on the effect of Block (time of sampling), Diet and Time as the main effects and the interaction of Diet and Time on the proportion of observation time spent by each lobster in benthic shelters (cryptic) or swimming in the water column. **Bold values:** significant

Dependent variable Sources of variation	df	*F*	p
Cryptic behaviour			
Block	4	0.81	0.619
Diet	2	3.46	0.224
Error A	2		
Time	8	10.01	**<0.001**
Diet × Time	16	2.45	**0.009**
Error B	48		
Swimming behaviour			
Block	4	0.98	0.562
Diet	2	2.57	0.280
Error A	2		
Time	8	5.89	**<0.001**
Diet × Time	16	2.22	**0.017**
Error B	48		

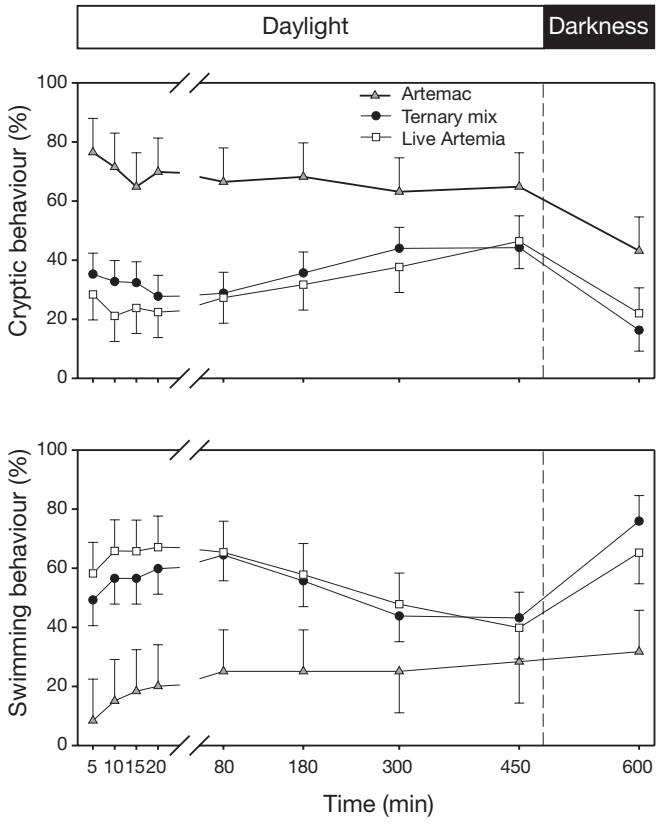

Fig. 4. *Homarus americanus.* Percentage of sheltered and swimming lobsters as a function of Diet and Time After Release. Data are mean ± SD (n = 2 or 3 replicate tanks, depending on diet; 40 lobsters were used in each replicate)

until 300 min after release, when percentages became similar. During the dark phase (600 min after release), the percentage of sheltered animals was half that observed during daylight, at 450 min after release ($p < 0.01$ for lobsters fed the 3 diets). Patterns of percentages of swimmers were essentially the inverse of those observed for percentages of sheltered lobsters. However, during the dark phase, the percentages of swimmers fed Artemac were similar to those observed during the daylight ($p = 0.676$), whereas it increased 2-fold in lobsters fed other diets ($p < 0.001$).

DISCUSSION

Live *Artemia* yielded the best survival and growth rates during the whole larval period of *Homarus americanus* compared to the other diets based on commercial *Artemia* replacements, as previously reported (Fiore & Tlusty 2005). Live *Artemia* showed the highest concentration of PL at the expense of TAG compared to the other diets tested. It is generally accepted that crustaceans have a limited capacity to biosynthesise PL and consequently cannot satisfy this nutritional requirement if PL is absent from their diet (Nelson et al. 2006). For example, the addition of phosphatidylcholine is essential for the survival of juvenile *H. americanus* (D'Abramo et al. 1981). Similarly, prawn *Penaeus japonicus* larvae did not metamorphose to postlarvae and died in 7 d when fed diets containing no PL, whereas growth and survival were improved by the addition of phosphatidylcholine to the diets (Kanazawa et al. 1985). PL may be superior to neutral lipids as a source of EFAs and energy for larvae due to their better digestibility, by improving the water stability of food particles, or by their action as antioxidants or feed attractants (Coutteau et al. 1997). It is therefore likely that the use of live *Artemia* met the PL requirements of lobsters whereas Artemac and the ternary mix were deficient in PL. Alternatively, Artemac and the ternary mix, 2 diets characterized by elevated TAG levels, may be less digestible than live *Artemia*, which are low in TAG.

The lowest performance found in lobsters fed Artemac coincided with a low dietary level of 20:4n-6 (0.4%), as previously reported in sea scallop *Placopecten magellanicus* larvae (Pernet & Tremblay 2004). Other studies showed that a diet enriched with 20:4n-6 resulted in higher survival of fish larvae and juveniles (e.g. Koven et al. 2001 and references therein). Therefore, the low larval growth and the high mortality of lobsters fed Artemac might be due to deficiency in 20:4n-6. It is noteworthy that 20:4n-6 is a precursor of eicosanoids, a group of highly biologically active hormones produced under stressful or pathological condi-

tions, so that a severe deficiency in this fatty acid may impair the overall immune system (Sargent et al. 2002).

Live *Artemia*, the diet that yielded the best survival and growth rate throughout the *Homarus americanus* larval period, showed very low levels of 22:6n-3 (<1%) and 20:5n-3 (3.7%). In contrast, planktonic and macroinvertebrate species living in Atlantic Canada showed constant high levels of n-3 long-chain PUFA, thus indicating the availability and the physiological importance of this group of fatty acids at low temperatures (Budge et al. 2002, Copeman & Parrish 2003). Levels of 22:6n-3 and 20:5n-3 in wild *H. americanus* harvested in the Gulf of St. Lawrence are respectively 7.7 and 17.0% of total fatty acids, TFA (Budge et al. 2002). We hypothesise that 22:6n-3 and 20:5n-3 are important fatty acids for optimising growth and survival of lobsters, as previously reported for juveniles (D'Abramo et al. 1980), but that the detrimental effect of dietary deficiencies in these EFAs was not visible under hatchery conditions. In our study, lobsters were maintained at a constant temperature (20°C), whereas 22:6n-3 and 20:5n-3 are particularly important when environmental temperature decreases, presumably to compensate for the ordering effect of temperature on membrane structure (Hochachka & Somero 2002). Alternatively, the potential effect of low dietary levels of 22:6n-3 and 20:5n-3 in live *Artemia* may have been counterbalanced by high levels of 18:3n-3, another essential PUFA for lobsters. However, the long-term effect of low levels of long-chain n-3 PUFA in lobster postlarvae needs further investigation as it may impair the animal's performance (e.g. Pernet et al. 2005 and references therein).

The effect of diet on lobster performance may be related to other unmeasured factors such as essential amino acids, cholesterol and other sterols, vitamins and minerals, attractants such as low molecular mass compounds (amino acids, amines, nucleotides and organic acids), and overall diet format (Nelson et al. 2006). For example, it was previously reported that live *Artemia* are usually more attractive than other commercial diets during the early ontogeny of *Homarus americanus* (Kurmaly et al. 1990). Therefore, differences in attractiveness may also contribute to the superior performance of lobster fed live *Artemia* in our study.

The top-performing lobsters fed live *Artemia* showed the highest absolute (µg larva^{-1}) and relative concentrations of TAG (% of total lipid or TAG:ST ratio) compared to that of low-performing lobsters fed the ternary mix or Artemac. Therefore, our results are in good agreement with the idea that TAG can be used as a proxy for the physiological condition of invertebrates (Fraser 1989, Harding & Fraser 1999, Miron et al. 1999, Pernet et al. 2003, Tremblay et al. 2007). Interestingly, absolute concentrations of TAG in lobsters fed live *Artemia* and the ternary mix were similar to those of wild animals collected near the Gulf of Maine, where TAG content varied from ca. 10 to 100 µg per larva from early Stage IV A to C (Harding & Fraser 1999). However, the absolute TAG concentration in lobsters fed Artemac was well below that of wild animals.

It is well established that, due to limitations in their ability to elongate and desaturate 18 C PUFA, the majority of crustaceans require pre-formed EFAs such as 20:4n-6, 20:5n-3 and 22:6n-3 in their diet to sustain growth and improve survival (Castell 1983, Nelson et al. 2006). Our investigation of the fatty acid partitioning between neutral and polar lipids provided useful and novel information concerning the regulation of EFA incorporation into membrane PL in *Homarus americanus*. To our knowledge, our study is the first to determine the relative responsiveness of the neutral and polar lipids to dietary change in lobster.

The proportion of 20:4n-6 recorded in lobsters (1.9 to 7.2%) was markedly higher than dietary values (0.4 to 2.0%), indicating the selective incorporation of this fatty acid into both lipid reserves and membranes. Our data are in good agreement with those on bivalves (Pernet & Tremblay 2004, Pernet et al. 2005) and marine finfish (e.g. Plante et al. 2007 and references therein), where the proportions of 20:4n-6 in animals were higher than that in their diets. In gilthead seabream *Sparus aurata*, the incorporation of 20:4n-6 was most efficient when dietary 20:4n-6 was less than 2% of TFA while it dropped considerably at higher levels (Fountoulaki et al. 2003). Therefore, it is possible that the low dietary proportions of 20:4n-6 recorded in our study (≤2%) may have encouraged the enhanced incorporation of 20:4n-6 in lobsters in a manner similar to that seen with seabream. However, it is generally believed that 20:4n-6 is deposited principally into the polar lipids, especially when present at lower dietary proportions (Fountoulaki et al. 2003). Hence, our finding of similar 20:4n-6 proportions in both neutral and polar lipids in lobsters fed Artemac appears somewhat paradoxical and should be investigated in more detail.

Like 20:4n-6, the level of 22:6n-3 in the polar lipids of lobsters (3.5 to 15.7%) largely exceeded the values found in diets (0.3 to 6.6%), particularly in animals fed live *Artemia,* which had levels 12 times higher. Similarly, spiny lobster *Jasus edwardsii* released from broodstock fed a diet low in 22:6n-3 (2.9%) showed elevated levels of this fatty acid (13.5%), presumably due to an overcompensation mechanism maximising the sequestering of essential 22:6n-3 (Smith et al. 2004) since de novo synthesis of 22:6n-3 by crustaceans is minimal (Kanazawa et al. 1979) or non-existent (Castell 1983, Nelson et al. 2006). Selective retention of 22:6n-3 was also reported in bivalves and marine finfish (e.g. Pernet et al. 2005, Plante et al. 2007 and references therein).

The proportions of 20:4n-6, 20:5n-3 and 22:6n-3 in the polar lipids of lobsters were higher than in the neutral lipids. This finding suggests that these long-chain PUFA were selectively incorporated into membrane PL at the expense of reserve lipids, most likely in response to the low dietary proportions. This hypothesis is supported by the fact that PLs in fish are generally higher in PUFA and more resistant to dietary change than neutral lipids (Sargent et al. 2002). More particularly, 22:6n-3 has been demonstrated to be a poor substrate for mitochondrial β-oxidation in rats, where its catabolism requires peroxisomal β-oxidation (Madsen et al. 1999). From a practical standpoint, these results suggest that the dietary proportions of long-chain PUFA recorded in the current study were insufficient to sustain growth and survival of lobsters without utilizing the PUFA located in the neutral lipid fraction.

Lobsters fed Artemac, which has low levels of TAG and 20:4n-6, were less active than those fed other diets; lobsters fed the other 2 diets spent a greater proportion of the observation time swimming upstream in the maze (predator odour plume experiment) or near the surface in aquaria (cryptic behaviour experiment). Although other unmeasured factors may have influenced the behaviour of lobster, we hypothesise that the low levels of TAG in lobster fed Artemac may have reduced their activity level. Indeed, TAG is the main energy reserve in several invertebrate species including *Homarus americanus* (Sasaki 1984). In support of this hypothesis, 40 d old sea scallop *Placopecten magellanicus* characterised by a high TAG:ST ratio (23.5) spent 12 to 15% of observation time exploring the substratum, whereas larvae characterised by low TAG:ST (8.0) did not explore the substratum (Pernet et al. 2003). It is therefore likely that the low TAG lobsters fed Artemac may not have enough energy reserves to make to a meticulous selection of settling site and therefore would settle more rapidly, whereas high TAG lobsters fed live *Artemia* or the ternary mix might swim to encounter better-quality settlement sites. The importance of TAG levels in habitat selection has previously been reported in barnacles (Miron et al. 1999, Tremblay et al. 2007).

Alternatively, the lower activity level recorded in lobsters fed Artemac may be attributable to a lower level of 20:4n-6 or a higher level of 22:6n-3 compared to those in lobsters fed the other diets. In contrast, fish larvae showed an increase in their swimming activity or a decrease in their erratic swimming behaviour when fed *Artemia* enriched with 22:6n-3 (Bransden et al. 2005 and references therein). However, many reports in rodents show an increase in locomotion in animals fed diets rich in n-6 PUFA or deficient in n-3 fatty acids (Fedorova & Salem 2006), thus supporting our results on lobsters. However, we must be cautious when interpreting our results in lobsters because the TAG levels and other unmeasured factors (see previous section) varied simultaneously with EFA levels.

Lobsters exposed to the predator odour plume spent more time in the center of the maze, were less active, and spent more time swimming downstream than those exposed to the control solution, which could be interpreted as a predator avoidance response. Indeed, postlarvae of the blue crab *Callinectes sapidus* either remained motionless or swam directly away from the target (Diaz et al. 1999). Additionally, lobster postlarvae submitted to cunner metabolites swim predominantly downstream (Boudreau et al. 1993a). However, Boudreau et al. (1993a) showed that postlarvae that swim upstream clearly avoid the experimental arm, which was not the case in our study. Discrepancies between our study and that of Boudreau et al. (1993a) may be due to differences in lobster ages: Boudreau et al. (1993a) used lobsters that had moulted to Stage IV at least 6 d earlier compared to the 2 to 4 d after moult that we used in our study.

Finally, lobsters fed the 3 diets spent more time outside the shelter during the night compared to the daylight. This observation is in good agreement with the fact that juvenile, adult and adolescent lobsters are more active during the night than during the day. Indeed, it is generally accepted that crypticity in lobsters protects them from visual predators (Butler et al. 2006, Phillips et al. 2006).

In summary, this study provides the first integrative view of the effect of dietary lipids on growth, survival, lipid composition and behaviour in lobster *Homarus americanus* postlarvae. Most notably, our study showed that lipid nutrition influenced settlement behaviour in *H. americanus*. From an ecological standpoint, this study suggests that lipids can be used as biochemical indicators of larval condition to gain mechanistic insight into the effects of nutrition on recruitment in economically important invertebrates. Also, this study has implications for considerations of phenotypic links or carry-over effects persisting from the larval into the postlarval phase of the complex life cycle of a marine invertebrate (Gimenez 2006). From a practical standpoint, we showed that lipid nutrition is of great importance to reliably produce good-quality postlarvae for stock enhancement or restoration of lobster fishing grounds.

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