Changes in gut content and composition of juvenile *Artemia* after oil enrichment and during starvation

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Abstract

Some predators, such as spiny lobster phyllosoma larvae, tear *Artemia* to pieces before ingestion. This results in the loss of gut content, which may partly negate the benefits of enrichment with essential fatty acids (EFA). Therefore, the influence of gut content on the lipid composition of juvenile *Artemia* (5 day old) was examined by starvation alone or starvation with forced gut evacuation using 20-30 µm plastic beads. *Artemia* gut content at 3 h and 6 h after the completion of feeding did not contribute significantly to the total lipid or fatty acid profiles of the *Artemia*. *Artemia* subjected to starvation alone (without beads) failed to evacuate their gut over the 6 h starvation period suggesting they require the intake of suitable sized particulate matter to undertake gut evacuation. To assess the uptake of EFA in nauplii (day 2) and juveniles, an enrichment diet containing high levels of arachidonic [AA, 20:4(n-6)] and eicosapentaenoic acid [EPA, 20:5(n-3)] was compared to a basal oat-based diet and a commercial oil emulsion high in docosahexaenoic acid [DHA (22:6(n-3)]. Both AA and EPA were increased in juvenile *Artemia* within a 24 h enrichment period at a rate proportional to their inclusion in the enrichment, while DHA was incorporated to a lesser degree. For all three EFA, the percentage loss during 6h starvation was small, but was greater for DHA than EPA or AA. Juvenile *Artemia*, a life stage seldom used in feeding regimes, have the ability to assume the AA and EPA profile of their dietary source. The ability to produce a larger food source with a non-traditional fatty acid profile may be valuable for a number of larval crustacean and fish species.
Keywords: juvenile *Artemia*, gut evacuation, arachidonic acid, eicosapentaenoic acid, starvation, Jasus edwardsii, phyllosoma

Introduction

Many enrichment protocols for *Artemia* in the past have centered on nauplii, and in particular, on how to achieve and maintain high levels of docosahexaenoic acid [DHA, 22:6(n-3)], eicosapentaenoic acid [EPA, 20:5(n-3)], and increase the DHA:EPA ratio (Watanabe et al., 1982; Rees et al., 1994; Rasowo et al., 1995; Narciso et al., 1999). While this is a suitable line of investigation for many cultured marine finfish and crustacean species, it may not be suited to phyllosoma larvae of the spiny lobster *Jasus edwardsii* (Phleger et al., 2001), a species under investigation for culture in Australia, New Zealand and Japan. In the wild, phyllosoma larvae of spiny lobsters prey on a variety of invertebrates (Shojima, 1963; Thomas, 1963). However it has been shown, that they will

reach metamorphosis to puerulus on a diet consisting solely of *Artemia* (Kittaka et al., 1988, Tong et al., 1997). Optimal growth and survival of phyllosoma are obtained when feeding juvenile *Artemia* (5 day old) of at least 1.5 mm in length (Tong et al. 1997; Ritar, 2001). It is also likely that there will be a requirement for an enrichment product with a greater emphasis on arachidonic acid (AA, 20:4(n-6)) and in particular the EPA:AA ratio (Smith, 1999; Phleger et al., 2001). This is in contrast to current enrichment products which are targeted primarily towards the uptake of DHA and EPA in *Artemia* nauplii (Evjemo et al., 1997; McEvoy and Sargent, 1998; Narciso et al., 1999).

An understanding of the rate of incorporation and loss of essential fatty acids (EFA) in *Artemia* nauplii during enrichment and subsequent starvation has assisted in the development of feeding regimes targeted towards finfish (Estevez et al., 1998; Evjemo et al., 2001). However, these processes in juvenile *Artemia* remain poorly understood. The use of enrichment diets with juvenile *Artemia* provides the opportunity to alter their fatty acid profiles to more closely match suggested phyllosoma lipid requirements (Smith, 1999). As limited research has been conducted on enrichment of juvenile *Artemia,* in particular with emphasis on AA, this work provides an opportunity to enhance existing knowledge based primarily on *Artemia* nauplii and metanauplii (Rasowo et al., 1995; Evjemo et al., 1997; Sorgeloos et al., 1998; Han et al., 2000; Evjemo et al., 2001).

Phyllosoma feed on large prey organisms such as ongrown *Artemia* by tearing them into smaller pieces before ingestion (Nelson and Cox, pers com), which may result in significant release and loss of material, particularly gut contents, into the surrounding aqueous environment. The benefits of short-term enrichment where a proportion of the enrichment may reside in the gut of large *Artemia* with little incorporation into cellular tissue could be negated with this type of feeding. Our observations (unpublished) suggested that ongrown *Artemia* starved for up to 24 h failed to fully evacuate their gut cavity. The food was observed as a distinctive coloration, but whether the gut content contributed significantly to the lipid and fatty acid content of the *Artemia* after this period of time was unknown.

The purpose of this study was to determine whether gut content after enrichment was an important part of the overall composition of *Artemia*, and to approximate the fatty acid profile of newly hatched *J. edwardsii* phyllosoma, in particular AA, in short-term enriched juvenile *Artemia*. A novel method is described whereby the food in the gut is evacuated and replaced by inert plastic beads. The target lipid enrichment profiles for *Artemia* were drawn from studies by Smith (1999) and Phleger et al. (2001) on the lipid class and fatty acid profiles of *J. edwardsii* phyllosoma.

Materials and Methods

Artemia ongrowing

Decapsulated *Artemia* cysts (INVE, Great Salt Lake Prime Gold) were hatched at 28 ± 1°C in 50 l white fibreglass cones in 0.2 µm filtered brackish water (27 ± 1‰). At 24 h, newly-hatched *Artemia* nauplii were removed from the hatching containers, rinsed in freshwater for 2 min and transferred to 1000 l conical tanks containing 200 l of filtered seawater (0.2 µm filtered, 34 ± 1‰, 28 ± 1°C). This volume was progressively increased to 1000 l over a 5 day ongrowing period providing a final *Artemia* density of 4 ml^{-1} with a total length of 1.5 ± 0.23mm (mean ± *s.e.m.*). During ongrowing for the gut evacuation trial, *Artemia* were fed with an "oat bran diet" consisting (w/w) of 75% oat bran, 12% wheat germ, 7% lecithin and 6% fish oil (Fishaphos, Felton Grimwade and Bickford Pty Ltd, Victoria). The fish oil contained (v/v) 17% EPA and 11% DHA. *Artemia* used for the

latter enrichment trial received only, oat bran: wheat germ: lecithin (OWL, 50:6:4) during the ongrowing period. *Artemia* diet was added to the water in the 1000 l tanks three times daily at a rate to maintain a Secchi depth of 25–30 cm.

Gut evacuation trial

Artemia in 1000 l batch cultures were harvested after ongrowing for 5 days, they did not undergo a period of enrichment. An initial sample (0 h) of 32 000 *Artemia* was removed, rinsed and partitioned into 4 subsamples (fresh and lyophilized samples in duplicate). A further 80 000 *Artemia* were partitioned into either seawater alone without beads (w/o beads - starvation) or seawater with beads (w/beads – forced gut evacuation) at a density of 8 ml^{-1}. The experimental treatments were conducted in white plastic containers holding *Artemia* in 2.5 l of 0.2 μm filtered seawater (28°C and 34 ‰) in duplicate. Individual plastic beads (20–30 µm diameter; Ionics Inc, USA) were suspended in seawater at a density of 1.0 x 10^6 l^{-1} (beads weighed 4.74 ± 0.19 ng $bead^{-1}$). Counting of beads in the water was undertaken at 0 h, 3 h and 6 h.

24 h Artemia enrichment experiment

Three enrichments were used to examine the uptake of essential fatty acids (EFA) in juvenile *Artemia*, and consisted (w/w) of:

1. OWL - oat bran: wheat germ: lecithin (50:6:4)
2. OWL+OIL - oat bran: wheat germ: lecithin: oil (50:6:4:40). The oil consisted (v/v) of a 4.5:1 ratio of Max EPA (containing 18% EPA and 12% DHA, Martek Biosgenes, USA) and ARASCO (containing 40% AA; Martek Biosgenes, USA).
3. A1 DHA Selco (INVE Group, Belgium).

The ratio and level of oil inclusion in enrichment 2 were estimated from examination of oil incorporation in the gut evacuation study. The oat-based diets were prepared daily by blending ingredients suspended in 500 ml of seawater in a household blender (high speed 15 min, Sunbeam, Australia) and large oat particles were removed by sieving (63 µm screen). A1 DHA Selco was prepared according to the manufacturer's directions. All enrichments were applied at a rate of 0.6g l^{-1}.

As for the gut evacuation trial, the 24 h enrichment experiment utilized *Artemia* ongrown in 1000 l batch culture systems. *Artemia* were harvested on Days 1 and 4 of ongrowing and partitioned into 1 l beakers at a density of 30 000 *Artemia* per beaker and 10000 *Artemia* per beaker, respectively. Twenty-seven beakers were utilized for each *Artemia* age class; these were divided equally into three groups and enriched with 1 of 3 diets for 24 h. After 24 h, triplicate samples for each age class and enrichment were rinsed and stored for analysis (9 beakers per age class). *Artemia* in the remaining beakers were rinsed and resuspended in either seawater alone without beads (w/o beads) or seawater with beads (w/beads) for a further 6 h. After 6 h, all treatments were terminated, rinsed and stored for analyses.

Lipid class and fatty acid analysis

In general, *Artemia* and feed samples were filtered through 4.7 cm Whatman glass filters (GF/F) and rinsed with 0.5 M ammonium formate. Filters were lyophilized overnight prior to analysis. Dry weight of the samples ranged from 5.6–262.5 mg. Additionally, the method of sample preparation (fresh or lyophilized) was examined to determine whether there was any loss or modification in fatty acid and lipid class composition. At 0 h in the ‘gut evacuation trial’, duplicate fresh samples were extracted

immediately with solvent followed by analysis while another set of duplicate samples were frozen and lyophilized before extraction.

Samples were quantitatively extracted using a modified Bligh and Dyer (1959) one-phase methanol/chloroform/water extraction (2:1:0.8, by vol); each sample was extracted overnight and the phases were separated the following day by addition of chloroform and water (final solvent ratio, 1:1:0.9, v/v/v, methanol/chloroform/water). The total solvent extract was concentrated (i.e. solvents removed *in vacuo*) using rotary evaporation at 40°C and lipid content determined gravimetrically; weights of lipid were 0.8–49.7 mg. Lipid class analyses were conducted within three days with samples stored in a known volume of chloroform.

An aliquot of the total solvent extract was analyzed using an Iatroscan MK V TH10 thin-layer chromatography–flame-ionization detector (TLC–FID) analyzer (Tokyo, Japan) to determine the abundance of individual lipid classes (Volkman and Nichols, 1991). Samples were applied in duplicate or triplicate to silica gel SIII Chromarods (5 μm particle size) using 1 μl disposable micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper. The solvent system used for the lipid separation was hexane/diethyl ether/acetic acid (60:17:0.2, by vol), a mobile phase resolving non-polar compounds such as wax ester (WE), triacylglycerol (TAG), free fatty acid (FFA) and sterol (ST). A second non-polar solvent system of hexane/diethyl ether (96:4 vol/vol) was also used for selected samples to separate hydrocarbon from WE. After development, the chromarods were oven-dried and analyzed immediately to minimize adsorption of atmospheric contaminants. The FID was calibrated for each compound class [phosphatidylcholine, cholesterol, cholesteryl ester, oleic acid, squalene, WE (derived from fish oil) and TAG (derived from fish oil); 0.1–10 μg range]. Peaks were quantified on an IBM compatible computer using DAPA software (Kalamunda, Western Australia). Iatroscan results are generally reproducible to ± 5% or better for individual classes (Bakes et al., 1995).

Fatty acid methyl esters (FAME) were extracted from an aliquot of the total solvent extract treated with methanol/hydrochloric acid/chloroform (10:1:1, by vol; 80°C, 2 h). FAME were extracted into hexane/chloroform (4:1, v/v, 3 x 1.5 ml) and then treated with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA, 50 μl, 60°C, 1 h) to convert sterols to their corresponding TMSi (trimethylsilyl) ethers.

Gas chromatographic (GC) analyses of FAME and sterols were performed with a Hewlett Packard 5890A GC (Avondale, PA) equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm i.d.), an FID, a split/splitless injector and an HP 7673A auto sampler. Hydrogen was the carrier gas. Following addition of methyl tricosanoate internal standard, samples were injected in splitless mode at an oven temperature of 50°C. After 1 minute, the oven temperature was raised to 150°C at 30°C min^{-1}, then to 250°C at 2°C min^{-1} and finally to 300°C at 5°C min^{-1}. Peaks were quantified with Waters Millennium software (Milford, MA, USA). Individual components were identified using weight spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC results are subject to an error of ± 5% of individual component abundance.

GC–weight spectrometric (GC–MS) analyses were performed on a Thermoquest GCQ GC-weight spectrometer (Austin, TX, USA) fitted with an on-column injector. The GC was fitted with a capillary column similar to that described above.

Statistics

Statistical analyses were conducted using one way analysis of variance with Tukey-Kramer HSD tests used for post-hoc comparison (Sokal and Rohlf, 1995). Arcsin√ transforms were performed on percentage data. $P < 0.05$ were considered significantly different. Data are presented as mean ± *s.d.*, unless stated. Statistics were executed using JMP version 3.2.1. (SAS Institute Inc.).

Results

Gut evacuation trial

Gut evacuation of Artemia with inert plastic beads

Artemia readily consume plastic beads giving the appearance of a gut cavity full of beads (Fig.1). The mean number of beads counted in the gut cavity of a 1.5mm, 5-day old juvenile *Artemia* after 6 h exposure to beads was 67 ± 6.

At 0 h, there was 1.0 x 10^6 beads l^{-1} present in the water column. After 3 h, the number of beads suspended in the water column was reduced to 0.533 x 10^6 ± 0.012 beads l^{-1}, which did not differ significantly from the count at 6 h (0.535 x 10^6 ± 0.028 beads l^{-1}). Uptake of beads into the *Artemia* was therefore approximately 50% of the available beads in the water column at 3 h and 6 h. More than 50% of the beads in the water column at 3 h were fouled with faecal material indicating the passage of beads through the *Artemia* took less than 3 h. The distinctive brown-yellow colouration previously evident in the gut of all *Artemia* was reduced to negligible levels (visual observation) in the w/ beads treatment after 6 h, but not at 3 h. In *Artemia* in the w/o beads treatment, there was no visible reduction in the gut colouration at 3 h or 6 h compared to 0 h. Only a minor amount of faecal material was detected in the water column during the 6 h starvation period (*Artemia* w/o beads) indicating that only minimal voiding of the gut contents into the water column had occurred in this treatment.

Juvenile Artemia - lipid class and fatty acid composition

The method of sample preparation (freshly prepared or lyophilized) did not significantly alter the lipid content nor the % of lipid class extracted (9.4 ± 2.1 mg g^{-1} wet weight (ww) compared to 7.7 ± 1.5 mg g^{-1}ww, respectively, Table 1). Due to the method of sample preparation where freshly prepared samples are not dried before extraction, only wet weight is presented in this section. Although a decrease in lipid content was observed in *Artemia* between 0 h, 3 h and 6 h, this was not significant. There was no difference in the lipid class composition in the treatments, with or without beads at different sampling times. Polar lipid (PL) was the major lipid class (LC), comprising 53-58% of total lipid, followed by (TAG) with 39-44% of total lipid. All samples contained low levels of sterol (ST) (1.8-2.1%), and free fatty acid (FFA) (0.5-1.1%).

There were only minor significant differences between the fatty acid profiles of freshly extracted and lyophilized *Artemia*, and did not include the EFAs (Table 1). There were no significant differences in the fatty acid profiles of any of the lyophilized samples across all sampling times either w/beads or w/o beads. The essential fatty acids, AA, EPA and DHA, were present as minor components (0.6-3.1%) of the total fatty acid profile.

24 h Artemia enrichment experiment

Artemia size

There was a significant increase in *Artemia* length following 24 h enrichment for each age class, but there was no significant difference between enrichment treatments. *Artemia* total length at day 1 and 4 was 0.72 ± 0.01 and 1.38 ± 0.01 mm, respectively, and following 24 h enrichment total length increased to 0.82 ± 0.02 and 1.49 ± 0.03 mm, respectively (n = 15 for all *Artemia* measures).

Dietary lipid and fatty acid composition

The major lipid classes in the enrichment diets were TAG and PL (33-88% and 11-65% of total lipid, respectively; Table 2). ST and FFA were approximately 1% or less, and wax ester (WE) was only detected in the A1 DHA Selco diet (0.2%). The OWL+ OIL and A1 DHA Selco diets were high in lipid (288 and 806 mg g^{-1} dry weight (dw), respectively) dominated by TAG (85 and 88%, respectively), in contrast to the PL-dominated (65%) OWL enrichment containing 165 mg g^{-1} dw of lipid. The dominant fatty acids in the OWL enrichment diet (Table 3), were 18:1(n-9), 18:2(n-6) and 16:0, while AA and EPA made substantial contributions to the OWL+ OIL diet (8.9% and 11.7% respectively, Table 4), as did DHA to the A1 DHA Selco diet (20.7%, Table 5). The latter two diets contained substantially lower levels of 18:2(n-6) and 18:1(n-9) compared to that found in the OWL diet. The n-3/n-6 ratio of the 3 enrichment diets differed substantially. The OWL diet was dominated by high levels of n-6 fatty acids (n-3/n-6 ratio of < 0.1), whereas equal amounts of n-3 and n-6 fatty acids were present in the OWL+ OIL diet (1.1), and the n-3 class dominated the A1 DHA Selco diet (n-3/n-6 ratio of 3.1). The ratios of the n-3/n-6 fatty acids in the different diets was mirrored by their respective EPA:AA ratios.

Enriched Artemia - lipid and fatty acid composition

There were no significant differences across all enrichment treatments and *Artemia* age in the lipid class profiles for animals starved w/beads or w/o beads, so data from both starved treatments was pooled (n = 6) for comparisons with 24 h enrichment (Table 2). Lipid data for Stage I *J. edwardsii* phyllosoma (obtained from wild caught ovigerous females), the target predator species, are also included for comparison. As was seen in the enrichment profiles, TAG and PL in 24 h enriched *Artemia* (Day 2 and 5) were the dominant lipid classes. There were significant increases in the relative level of TAG in *Artemia* exposed to all enrichments after 24 h. The greatest increases were present in *Artemia* fed the OWL+ OIL (38% and 73% Day 2 and 5, respectively) and A1 DHA Selco (41% and 76% Day 2 and 5, respectively) diets. As a result of the increased TAG, the percentage contribution of PL was significantly reduced in all 24 h enriched *Artemia*. All Day 5 *Artemia* contained between 7-18% more TAG than Day 2 *Artemia*.

After 24 h enrichment and with 6 h starvation, Day 2 OWL enriched *Artemia* demonstrated a further significant increase in TAG while PL decreased. No such changes were evident in TAG or PL in any of the other treatments at Day 2 or Day 5. A number of small but significant shifts in the minor lipid components (WE, FFA and ST) occurred in all three enrichment treatments after 6 h starvation. However, this was confined to Day 2 *Artemia*, and the FFA levels of A1 DHA Selco enriched Day 5 *Artemia.*

In Day 2 *Artemia*, the amount of lipid accumulated expressed as mg g^{-1} dw was significantly greater in animals enriched for 24 h with A1 DHA Selco, while in Day 5 *Artemia* there were significant increases in lipid dry weight in both OWL+ OIL and A1

DHA Selco enriched animals. There was no significant change in lipid dry weight across any treatment or *Artemia* age with starvation.

The major fatty acids in Day 1 and Day 4 *Artemia* were 18:1(n-9), 18:2(n-6), 16:0, 18:1(n-7), 18:0 (Tables 3-5). A number of these fatty acids were also prominent in the diets and subsequently in the 24 h enriched Day 2 and Day 5 *Artemia*. In particular, 18:2(n-6) made a large contribution to the total fatty acid profile of the OWL diet (44.4%) with subsequent transfer to both Day 2 and 5 *Artemia*. The OWL+ OIL and A1 DHA Selco diets contained lower levels of 18:2(n-6) (10.0 and 5.4%, respectively). However, in both cases 18:2(n-6) still assumed major prominence in 24 h enriched *Artemia* (range of 8.9-10.3% in Day 2 *Artemia* and 13.2-16.9% in Day 5 *Artemia*).

OWL+ OIL and A1 DHA Selco enriched *Artemia* incorporated increased proportions of EFA largely reflecting the profiles of their diets. There was a greater uptake of AA and EPA seen in Day 5 *Artemia* compared to Day 2 *Artemia*, while the uptake of DHA was similar for both Day 2 and 5 *Artemia*. Where these three EFA were present as minor enrichment components, such as in the OWL diet (0.1%), enrichment resulted in EFA depletion in Day 5 enriched *Artemia*. When the dietary contribution of AA, EPA and DHA to Day 5 *Artemia* was prominent (OWL+ OIL - 8.9, 11.7, 7.5%, respectively; A1 DHA Selco 1.7, 5.3, 20.7%, respectively), starvation caused a reduction in their percentage contribution, and in particular, the contribution of the essential fatty acid DHA.

The quantitative changes in the EFA (Fig 2 & 3) largely mirrored the results of the percentage data (Tables 3-5). An exception was evident in the essential fatty acid DHA in the A1 DHA Selco treatment, where Day 2 and Day 5 starved *Artemia* lost 22% ($P < 0.05$, Fig 2) and 20% ($P = 0.07$, Fig 3) respectively, compared to pre-starvation levels.

Other fatty acids reduced by starvation (Tables 3-5) included the saturated fatty acids (SFA) 14:0, 16:0 and 18:0, and the monounsaturates (MUFA), 18:1(n-7) and 18:2(n-6). While 18:2(n-6) was reduced with starvation, its large contribution to the diets and subsequently to the *Artemia* is reflected in low (n-3)/(n-6) ratios. In particular, this ratio in the OWL and OWL+ OIL fed animals was in the range of 0.1-0.7 and 0.3-1.0, respectively, compared to the target phyllosoma ratio of 2.7.

Discussion

Gut evacuation trial

Gut evacuation

Even though enrichment of *Artemia* is widely used in aquaculture, it has to date been unclear what proportion of the enrichment is incorporated into body tissue and how much remains resident in the gut. The gut evacuation trial demonstrated that the gut content of juvenile *Artemia* (5 day old) at 3 h and 6 h made only a minor contribution to the total lipid and fatty acid profiles of the *Artemia*. While minimal gut retention times were not monitored in this trial, a similar study has demonstrated that the gut retention time of actively feeding juvenile *Artemia* is approximately 7 min (Smith, unpub). This is intermediate to retention times previously recorded for nauplii and adult *Artemia* of 10 and 3 min, respectively (Dobbeleir et al., 1980). Gut coloration in *Artemia* offered beads was reduced considerably at 3 h and was negligible by 6 h. The reduction in gut coloration was thought to be due to sequential loss of bulk food content and then associated pigmentation from the gut lining. In the w/o beads *Artemia* samples, there was no visible difference between the gut content prior to and after 6 h starvation with minimal faeces present in the water column. *Artemia* are a continuous, non-selective filter feeder (Provasoli and

Shiraishi, 1959; Sorgeloos et al., 1998), so it appears that without the intake of suitable sized particulate matter the normal processes to stimulate gut evacuation do not occur. Material is maintained in the gut for at least 6 h when *Artemia* are held at 28ºC in clear water with no particulate matter present.

Juvenile Artemia - lipid and fatty acid composition

Artemia in the w/o beads treatment still had food remaining in their gut for at least 6 h after the commencement of the trial, i.e., after feeding was ceased. It is probable that this prolonged gut resident time would allow the extraction of additional lipid from the food than otherwise would have occurred in actively feeding *Artemia*. As there was no difference in the amount of lipid between evacuated *Artemia* (w/beads) and starved *Artemia* (w/o beads) at 3 h, we propose that the majority of the lipid was removed by 3 h with resident material in the gut devoid of lipids. It is likely that the time required to extract the majority of lipid was much less than 3 h, although a further trial is needed to confirm this. Therefore, any loss of gut content through predator feeding behaviour, as often occurs when *Artemia* are fed to spiny lobster phyllosoma, should only alter the total *Artemia* lipid profile within a short period of the commencement of feeding, ie. < 3 h, after which time the remaining lipid in the gut content can be considered as largely not contributing to the lipid profile of the *Artemia*. In juvenile *Artemia* which have not been enriched, the essential fatty acids, AA, EPA and DHA, only made a minor contribution to the total fatty acid profile (see Table 1). This finding highlights the need for a suitable source of enrichment if *Artemia* were to match the observed lipid profiles for *J. edwardsii* phyllosoma from a wild origin (Smith, 1999; Phleger et al., 2001).

The differences in the total fatty acid profiles were relatively minor when comparing either fresh or lyophilized samples, and in particular did not extend to the EFA. It was therefore considered that either method was appropriate for subsequent analyses, lyophilized samples were used thereafter.

24 h Artemia enrichment experiment

Diets and Artemia - lipid and fatty acid composition

The 24 h enrichment trial reinforced the minor role that the gut content had on the total lipid or fatty acid profile of Day 5 *Artemia*. The result of minimal influence of gut content on lipid or fatty acid profiles was confirmed for Day 2 *Artemia* when sampled 6 h later. The enrichment diets used in the 24 h enrichment trial differed substantially from each other in both the level and type of lipid inclusion. Prior to enrichment, PL was the dominant lipid class in both Day 1 and Day 4 *Artemia*. However, after 24 h enrichment, TAG became the major lipid class, even in *Artemia* fed a PL rich diet (OWL). This is probably due to *Artemia* taking up lipid in excess to requirements and storing it as TAG, a readily available energy source (Wickins et al., 1995). The efficiency at which lipids were assimilated varied greatly between diets. A1 DHA Selco is composed of 806 mg g^{-1} lipid dw, 4.9 and 2.8 times greater than the lipid content of the OWL and OWL+ OIL diets, respectively. While A1 DHA Selco provided significantly greater lipid inclusion to Day 2 *Artemia* than did the enrichments of OWL and OWL+ OIL, this pattern was not repeated for larger juvenile *Artemia*. There was no significant difference between the lipid dw of Day 5 *Artemia* enriched with either A1 DHA Selco or OWL+ OIL regardless of whether the enrichment contained a high lipid content (A1 DHA Selco, 81% lipid) compared to one with a moderate level of lipid (OWL+ OIL, 29% lipid). This absence of a difference suggests that juvenile *Artemia* have a maximum lipid uptake during the 24 h enrichment

period. In juvenile *Artemia*, the low uptake rate of enrichment products that are high in lipid suggests that increasing the density of *Artemia* in the enrichment diet, or reducing the application rate of the lipid emulsion would not appreciably alter lipid uptake.

Starvation for 6 h at 28ºC in this study resulted in no observable difference in lipid content in 2 or 5 day old *Artemia*, although there were significant changes in fatty acids. This contrasts with the findings of Evjemo et al., (2001) who found that *Artemia* nauplii lost 34% of their lipid content during 24 h starvation at 26ºC, while at 12ºC lipid losses during starvation were reduced to 11%. As it is the intention to feed enriched juvenile *Artemia* to the predator *J. edwardsii* phyllosoma at 18ºC (Ritar, 2001) the loss of lipids in enriched juvenile *Artemia* during a 20 h tank resident time would be low. Our results suggest that while there would be a decline in the essential fatty acid DHA over time in juvenile *Artemia*, it would occur at a marginally slower rate than if Day 2 *Artemia* (this trial) or nauplii (Evjemo et al., 2001) were used. The large contribution of 18:2(n-6) to the total fatty acid profile of both diet and enriched *Artemia* resulted in a low n-3/n-6 ratio, particularly in the OWL and to a lesser extent the OWL+ OIL fed animals. We suggest that feeding these *Artemia* may result in the transfer of a low n-3/n-6 ratio to the predator species. It has been postulated, particularly in fish, that n-3/n-6 ratios less than 1, as occurs in the OWL and OWL+ OIL enriched *Artemia*, may increase larval susceptibility to stress (Sargent, 1995). By reducing the level of 18:2(n-6) in the *Artemia* diet (hence in *Artemia*), the n-3/n-6 ratio potentially could be increased. This would be at the expense of the potential benefits of 18:2(n-6) being available as an energy source (D'Souza, 1998) or precursor for AA production (Sargent, 1995). However, this should not be considered a negative factor, as many species are unable to produce a sufficient amount of AA by elongation of 18:2(n-6), and even if they do, often numerous other unwanted C_{20} and C_{22} by-products may be formed (Sargent, 1995).

The specific inclusion of AA as a dietary EFA was targeted at the putative requirements of *J. edwardsi* phyllosoma. Analysis of phyllosoma hatched from wildcaught ovigerous broodstock, those subjected to starvation trials (Smith, 1999) as well as wild caught larvae (Phleger et al., 2001) demonstrated that AA was a major fatty acid, contributing up to 8% to the total fatty acid profile of *J. edwardsii* phyllosoma. While it is unusual for temperate and polar marine species to contain such high levels of AA (Sinclair et al., 1986), this is not so for benthic species (Nichols et al., 1998a,b; Dunstan et al., 1999). Recent research on larval penaeids (*Penaeus japonicus*, *P. semisulcatus* and *P. monodon*) (D'Souza and Loneragan, 1999), suggests that there is a need for the inclusion of AA into diets for some larval crustaceans. The inclusion of AA at levels approaching those observed in phyllosoma from a wild origin was obtained in *Artemia* enriched with OWL+ OIL. However, the percentage inclusion of EPA may still be insufficient in *Artemia* enriched with this diet. It is the balance of n-3/n-6 fatty acids and in particular of EPA:AA ratio which has been signaled as important in this and other larval crustaceans (Sargent, 1995; D'Souza and Loneragan, 1999; Smith, 1999; Phleger et al., 2001). In a number of other species, AA has been suggested as having a major role as a precursor of eicosanoids, the highly biologically active molecules linked to moulting and stress response (Lytle et al., 1990; Sargent, 1995). Eicosanoid production from AA (n-6 fatty acid) is modulated by EPA (n-3 fatty acid), and failure to supply these two EFAs in the appropriate balance may result in adverse biochemical responses when fed to the predator organisms (Sargent, 1995).

It is the ability of 24 h enriched *Artemia*, in particular at Day 5, to resemble the EFA profile of their dietary source, that shows promise for further manipulation to meet the needs of host crustacean predators. Within a 24 h period, Day 5 *Artemia* fed the OWL+

OIL and A1 DHA Selco enrichments mirrored the percentage dietary inclusion levels of both AA and EPA. The incorporation of DHA into *Artemia*, at Day 5 was markedly less than its level in the enrichment, however greater losses occurred in younger (Day 2) *Artemia*. Estevez et al. (1998) found that, of the EFAs, the incorporation rate of DHA was less during *Artemia* nauplii enrichment and the loss highest during starvation, a situation analogous to that found in Day 2 and 5 *Artemia* in this study. Lower DHA incorporation in juvenile *Artemia* compared to dietary inclusion levels may be associated with the inability of juveniles to preferentially assimilate DHA during 24 h enrichment. We consider that DHA did not undergo significant retroconversion to EPA, as often occurs in *Artemia* nauplii (Navarro et al., 1999; Evjemo et al., 2001), because EPA was incorporated at a rate equivalent to inclusion levels in both OWL+ OIL and A1 DHA Selco diets and appeared to increase independently of DHA.

The uptake and maintenance efficiency of various fatty acids in *Artemia* during 24 h enrichment to a large degree dictates the level that they should be included in the enrichments to attain a desired profile. Both AA and EPA were incorporated and effectively maintained in juvenile *Artemia* at levels similar to their inclusion in the enrichments. However, to achieve a fatty acid profile similar to the desired target species (*J. edwardsii* phyllosoma), the percentage inclusion of EPA would need to be increased in the OWL+ OIL enrichment.

In conclusion, it appears that juvenile *Artemia* require the intake of suitably sized particulate matter to enable gut evacuation to occur. While gut content was not noticeably voided within 6 h of the cessation of enrichment, we also found that at 3 h and 6 h the gut content did not make a significant contribution to the total lipid content or fatty acid profiles of juvenile *Artemia*. Therefore, any loss of gut content in juvenile *Artemia* as a result of predator feeding behaviour is insignificant in terms of the lipid content or fatty acid composition shortly after the cessation of enrichment. Juvenile *Artemia*, a life stage seldom used in feeding regimes, demonstrated the ability to assume the EFA profile of their dietary source, in particular the AA and EPA profiles and to a lesser degree, DHA. This is a trait that has considerable potential for use in species that have diverse and perhaps non-traditional EFA requirements. Further research on optimizing *Artemia* EPA:AA ratios, decreasing the level of 18:2(n-6) and increasing the n-3/n-6 ratio to reflect the composition of *J. edwardsii* phyllosoma, is being conducted.

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Table 1. Lipid class composition (percentage of total lipids), lipid content (wet weight) and percentage composition of major (>2%) and essential fatty acids in juvenile *Artemia* (1.5mm long). At time 0 h freshly prepared and lyophilized samples were analyzed. Lyophilized *Artemia* samples were used thereafter at 3 h and 6 h, during forced gut evacuation with beads (w/beads) or starvation without beads (w/o beads). Data are presented as mean ± *s.d.*; $n = 2$.

	0 h		3 h		6 h	
	Fresh	Lyophilized	Lyophilized		Lyophilized	
Lipid composition			w/ beads	w/o beads	w/ beads	w/o beads
Triacylglycerol	43.8 ± 0.4	44.4 ± 2.6	40.8 ± 1.5	40.9 ± 4.8	43.1 ± 2.1	39.0 ± 2.4
Free fatty acid	1.1 ± 0.2	0.5 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.9 ± 0.2	0.9 ± 0.2
Sterol	1.9 ± 0.2	1.9 ± 0.0	1.8 ± 0.7	2.1 ± 0.2	2.1 ± 0.7	1.9 ± 0.2
Polar lipid	53.1 ± 0.8	53.2 ± 2.8	56.7 ± 0.7	56.4 ± 4.4	53.9 ± 3.0	58.2 ± 2.4
Lipid mg g^{-1} (wet weight)	9.4 ± 2.1	7.7 ± 1.5	6.1 ± 1.1	6.1 ± 0.8	5.5 ± 0.1	4.2 ± 1.5
Fatty acid composition[1]						
16:0	12.3 ± 0.2*	11.8 ± 0.0	12.3 ± 0.1	12.2 ± 0.2	12.6 ± 1.0	12.2 ± 0.1
18:2(n-6)	32.3 ± 0.4*	33.8 ± 0.1	31.6 ± 1.6	32.4 ± 0.2	31.1 ± 0.5	32.5 ± 0.3
18:1(n-9)c/18:3(n-3)[2]	27.2 ± 0.5	28.0 ± 0.4	27.0 ± 1.0	27.7 ± 0.3	27.0 ± 0.6	28.0 ± 0.2
18:1(n-7)c	4.3 ± 0.2	4.4 ± 0.1	4.1 ± 0.3	4.3 ± 0.1	3.9 ± 0.5	4.0 ± 0.3
18:0	6.3 ± 0.1*	5.8 ± 0.1	6.4 ± 0.2	6.4 ± 0.1	7.1 ± 0.5	6.6 ± 0.1
20:4(n-6) AA	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.1	0.6 ± 0.0
20:5(n-3) EPA	3.0 ± 0.0	3.1 ± 0.1	3.0 ± 0.0	3.0 ± 0.1	3.1 ± 0.1	3.1 ± 0.2
22:6(n-3) DHA	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.0
Other	13.1	13.1	14.1	12.6	13.8	12.2
Σ SFA[3]	21.7 ± 1.0	20.7 ± 0.1	21.5 ± 1.2	21.3 ± 0.8	22.4 ± 1.4	21.1 ± 0.0
Σ MUFA	34.8 ± 0.5	35.5 ± 0.3	34.3 ± 1.4	35.0 ± 0.3	33.8 ± 1.2	34.9 ± 0.2
Σ PUFA	39.8 ± 0.5*	41.4 ± 0.3	40.4 ± 0.0	40.0 ± 0.7	39.6 ± 1.4	40.3 ± 0.4
Σ (n-3)	5.2 ± 0.3	4.9 ± 0.1	5.3 ± 0.2	4.9 ± 0.3	5.4 ± 0.5	5.4 ± 0.5
Σ (n-6)	34.2 ± 0.6*	35.9 ± 0.0	33.8 ± 1.4	34.4 ± 0.4	33.3 ± 1.0	34.2 ± 0.1
Ratio (n-3)/(n-6)	0.2	0.1	0.2	0.1	0.2	0.2
Ratio EPA/AA	5.1	5.5	5.4	5.2	5.5	5.3
Ratio DHA/EPA	0.3	0.3	0.3	0.3	0.3	0.3

[1]Other fatty acids at <2%:14:0, i15:0, 16:1(n-9)c, 16:1(n-7)c, C_{16} PUFA, i17:0, 17:0, 18:3(n-6), 18:4(n-3), 20:1(n-11)c, 22:0, 22:5(n-3) and C_{22} PUFA.
[2] Under GC these two components coeluted. GC-MS analysis showed that 18:1 (n-9)c was the predominant component.
[3]SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.
* Signifies a significant difference between freshly prepared or lyophilized samples at time 0.

Table 2. Lipid class composition (percentage of total lipids) and lipid content of enrichments and *Artemia* at Day 1 and Day 4, after 24 h enrichment (Day 2 and Day 5, respectively), and further 6h starvation. Newly-hatched *Jasus edwardsii* phyllosoma profiles (target predator species) are included for comparison (Smith,1999). Data are presented as mean ± *s.d.* (n = 3, except for starved 6 h, where n = 6).

	Wax ester	Triacylglycerol	Free fatty acid	Sterol	Polar lipid	Lipid as mg g^{-1} (dw^3)
Jasus edwardsii phyllosoma[1]	3.3	2.5	1.3	8.7	81.8	104.6
Enrichments[2]						
OWL	0.0 ± 0.0	33.4 ± 2.3	1.2 ± 0.3	0.7 ± 0.1	64.7 ± 2.5	164.5 ± 1.0
OWL+ OIL	0.0 ± 0.0	87.8 ± 1.4	0.0 ± 0.0	1.1 ± 0.2	11.2 ± 1.6	287.8 ± 6.7
A1 DHA Selco	0.2 ± 0.0	85.3 ± 0.2	0.5 ± 0.0	0.5 ± 0.0	13.6 ± 0.1	806.1 ± 38.8
Day 1 *Artemia*	0.6 ± 0.2	[a] 45.0 ± 1.4	0.8 ± 0.2	[a] 2.3 ± 0.4	[a] 51.3 ± 1.9	[a] 149.6 ± 5.5
Day 2 *Artemia*						
24h OWL enriched	0.4 ± 0.1 [a]	[a] 50.8 ± 3.6 [a]	1.7 ± 0.7	[ab] 2.8 ± 0.4	[b] 44.3 ± 3.4 [a]	[a] 202.9 ± 37.4
starved 6h	0.0 ± 0.0 [b]	57.4 ± 2.6 [b]	4.1 ± 2.0	2.8 ± 0.6	35.8 ± 2.8 [b]	202.2 ± 24.1
Day 2 *Artemia*						
24h OWL+ OIL enriched	0.5 ± 0.1[a]	[b] 62.1 ± 3.4	3.8 ± 3.2	[a] 2.2 ± 0.1	[c] 31.5 ± 0.3	[a] 203.6 ± 15.1
starved 6h	0.2 ± 0.1[b]	66.6 ± 3.8	2.1 ± 1.5	2.1 ± 0.4	28.9 ± 3.2	264.3 ± 91.8
Day 2 *Artemia*						
24h DHA enriched	0.7 ± 0.1 [a]	[b] 65.3 ± 1.5	5.0 ± 0.3 [a]	[b] 3.1 ± 0.2 [a]	[c] 26.0 ± 1.5	[b] 264.7 ± 17.3
starved 6h	0.2 ± 0.2 [b]	73.2 ± 9.4	1.5 ± 0.4 [b]	1.6 ± 0.6 [b]	23.4 ± 8.4	255.2 ± 28.8
Day 4 *Artemia*	0.3 ± 0.2	[a] 40.7 ± 1.7	[a] 1.2 ± 0.5	[a] 3.4 ± 0.3	[a] 54.5 ± 2.2	[a] 182.1 ± 34.3
Day 5 *Artemia*						
24h OWL enriched	0.2 ± 0.2	[b] 68.4 ± 13.6	[a] 1.6 ± 1.0	[a] 1.9 ± 0.9	[b] 27.9 ± 11.7	[a] 186.4 ± 7.3
starved 6h	0.1 ± 0.1	46.6 ± 19.3	2.8 ± 0.9	3.0 ± 0.6	47.5 ± 18.7	151.0 ± 20.1
Day 5 *Artemia*						
24h OWL+ OIL enriched	0.1 ± 0.2	[b] 70.6 ± 1.9	[ab] 1.8 ± 0.7	[ab] 2.2 ± 0.4	[b] 25.3 ± 1.8	[b] 258.9 ± 5.8
starved 6h	0.1 ± 0.1	69.2 ± 2.6	3.4 ± 2.3	1.8 ± 0.2	25.6 ± 0.8	242.6 ± 74.7
Day 5 *Artemia*						
24h DHA enriched	0.0 ± 0.0	[b] 71.8 ± 3.3	[b] 4.4 ± 1.5 [a]	[b] 1.6 ± 0.5	[b] 22.3 ± 1.3	[b] 296.5 ± 31.7
starved 6h	0.0 ± 0.0	74.8 ± 2.7	1.9 ± 0.7 [b]	1.2 ± 0.1	22.1 ± 2.7	283.8 ± 34.8

[1]In phyllosoma samples, wax ester also contains hydrocarbon; sterol contains diacylglycerol.
[2]Enrichment composition: OWL - oat bran: wheat germ: lecithin (50:6:4); OWL+ OIL - oat bran: wheat germ: lecithin: oil (50:6:4:40). Oil composed of Max EPA:ARASCO - 4.5:1; A1 DHA Selco (INVE Group, Belgium).
[3]dw = dry weight.
Different superscript preceding data values denote significant differences between Day 1 or Day 4 ongrown *Artemia* and *Artemia* after 24h enrichment.
Different superscripts following data values denote significant differences between 24 h enrichment and 6 h starvation within a dietary group.

Table 3. Percentage composition of major (>2%) and essential fatty acids in OWL enrichment, *Artemia* at Day 1 and Day 4, after 24 h enrichment with OWL (Day 2 and Day 5, respectively) and 6h starvation. Newly-hatched *Jasus edwardsii* phyllosoma profiles (target predator species) are included for comparison (Smith 1999). Data are presented as mean ± *s.d.* (n = 3, except for starved 6 h, where n = 6). Enrichment composition: OWL - oat bran: wheat germ: lecithin (50:6:4).

	Jasus edwardsii phyllosoma	OWL enrichment	Ongrown Day 1 *Artemia*	24h enrichment (Day 2 *Artemia*)	6h starved (Day 2 *Artemia*)	Ongrown Day 4 *Artemia*	24h enrichment (Day 5 *Artemia*)	6 h starved (Day 5 *Artemia)*
14:0	0.9	0.1 ± 0.0	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.2	1.8 ± 0.0 [a]	1.0 ± 0.1 [b]	1.2 ± 0.2 [b]
16:1(n-7)c	2.3	0.1 ± 0.0	3.2 ± 0.2 [a]	2.2 ± 0.0 [b]	2.3 ± 0.2 [b]	2.8 ± 0.0 [a]	1.5 ± 0.1 [c]	2.0 ± 0.1 [b]
16:0	13.1	19.1 ± 0.1	11.6 ± 0.1 [b]	11.0 ± 0.4 [b]	12.3 ± 0.5 [a]	12.3 ± 0.3 [a]	11.5 ± 0.2 [b]	12.5 ± 0.2 [a]
18:4(n-3)	0.0	0.0 ± 0.0	3.7 ± 0.1 [a]	1.7 ± 0.1 [b]	1.5 ± 0.2 [b]	3.5 ± 0.2 [a]	1.3 ± 0.0 [c]	1.7 ± 0.1 [b]
18:2(n-6)	1.8	44.4 ± 0.5	10.3 ± 0.3 [b]	25.2 ± 0.4 [a]	25.0 ± 1.3 [a]	22.8 ± 0.1 [c]	35.2 ± 0.7 [a]	30.6 ± 0.8 [b]
18:1(n-9)c/18:3(n-3) [1]	13.2	30.1 ± 0.3	42.9 ± 0.6 [a]	36.5 ± 1.1 [b]	35.6 ± 1.3 [b]	29.5 ± 0.4 [b]	31.2 ± 0.8 [ab]	29.8 ± 1.2 [b]
18:1(n-7)c	4.8	1.2 ± 0.0	8.4 ± 0.2 [a]	6.3 ± 0.2 [b]	5.7 ± 0.4 [c]	5.9 ± 0.1 [a]	3.7 ± 0.1 [b]	4.3 ± 0.2 [b]
18:0	7.2	2.6 ± 0.0	5.8 ± 0.1 [b]	6.1 ± 0.4 [a]	6.2 ± 0.2 [a]	6.9 ± 0.0 [a]	5.5 ± 0.0 [b]	6.6 ± 0.2 [a]
20:4(n-6), AA	8.2	0.1 ± 0.0	0.6 ± 0.0 [b]	0.6 ± 0.1 [ab]	1.1 ± 0.8 [a]	1.2 ± 0.0 [a]	0.7 ± 0.0 [b]	1.2 ± 0.2 [b]
20:5(n-3), EPA	20.3	0.1 ± 0.0	3.1 ± 0.0 [a]	2.0 ± 0.0 [b]	2.5 ± 0.9 [b]	2.8 ± 0.0 [a]	1.6 ± 0.1 [b]	2.5 ± 0.3 [a]
22:6(n-3), DHA	11.3	0.0 ± 0.0	0.0 ± 0.0 [b]	0.3 ± 0.2 [a]	0.6 ± 0.5 [a]	1.2 ± 0.0 [a]	0.7 ± 0.1 [b]	1.1 ± 0.2 [ab]
Other[2]	16.9	2.2	9.6	7.3	6.4	9.3	6.1	6.5
Sum (n-3)	33.1	0.1 ± 0.0	7.5 ± 0.1 [a]	4.3 ± 0.2 [b]	4.8 ± 1.6 [b]	8.0 ± 0.3 [a]	3.8 ± 0.1 [c]	5.5 ± 0.6 [b]
Sum (n-6)	12.2	44.6 ± 0.4	11.5 ± 0.2 [b]	26.3 ± 0.6 [a]	26.6 ± 0.9 [a]	25.2 ± 0.1 [c]	36.6 ± 0.8 [a]	32.7 ± 0.6 [b]
Sum (n-3)/(n-6)	2.7	<0.1	0.7 [a]	0.2 [b]	0.2 [b]	0.3 [a]	0.1 [c]	0.2 [b]
Ratio EPA/AA	2.5	0.7	5.0 [a]	3.6 [b]	2.9 [b]	2.3 [a]	2.2 [b]	2.0 [b]
Ratio DHA/EPA	0.6	0.5	0.0 [b]	0.1 [a]	0.2 [a]	0.4	0.5	0.4
Total fatty acids (mg/g)			144.3 ± 10.6	167.5 ± 7.0	150.8 ± 19.4	138.4 ± 7.4	165.0 ± 14.0	139.5 ± 21.3

Different superscript denotes a significant difference between Day 1 or Day 5 *Artemia*, 24h enrichment and 6h starvation.
[1]Under GC, these two components coeluted. GC-MS analysis showed that 18:1 (n-9)c was the predominant component.
[2]Other fatty acids include: i15:0, 16:1(n-9)c, C_{16} PUFA, i17:0, 17:0, 18:3(n-6), 20:1(n-11)c, 22:0, 22:5(n-3) and C_{22} PUFA.

Table 4. Percentage composition of major (>2%) and essential fatty acids in OWL+ OIL enrichment, *Artemia* at Day 1 and Day 4, after 24 h enrichment with OWL+ OIL (Day 2 and Day 5, respectively) and 6h starvation. Newly-hatched *Jasus edwardsii* phyllosoma profiles (target predator species) are included for comparison (Smith 1999). Data are presented as mean ± *s.d.* (n = 3, except for starved 6 h, where n = 6). Enrichment composition: OWL+ OIL - oat bran: wheat germ: lecithin: oil (50:6:4:40). Oil composed of Max EPA: ARASCO - 4.5:1.

	Jasus edwardsii phyllosoma	OWL+ OIL enrichment	Ongrown Day 1 *Artemia*	24h enrichment (Day 2 *Artemia*)	6h starved (Day 2 *Artemia*)	Ongrown Day 4 *Artemia*	24h enrichment (Day 5 *Artemia*)	6 h starved (Day 5 *Artemia*)
14:0	0.9	5.6 ± 0.2	0.8 ± 0.0 [b]	2.1 ± 0.0 [a]	2.0 ± 0.5 [a]	1.8 ± 0.0 [b]	2.2 ± 0.0 [a]	2.3 ± 0.2 [a]
16:1(n-7)c	2.3	6.6 ± 0.6	3.2 ± 0.2 [b]	4.3 ± 0.3 [a]	4.6 ± 0.5 [a]	2.8 ± 0.0 [b]	4.8 ± 0.1 [a]	4.8 ± 0.2 [a]
16:0	13.1	17.1 ± 0.4	11.6 ± 0.1	11.7 ± 0.4	11.5 ± 1.6	12.3 ± 0.3 [a]	10.3 ± 0.1 [b]	11.9 ± 0.4 [a]
18:4(n-3)	0.0	0.0 ± 0.0	3.7 ± 0.1 [a]	1.9 ± 0.1 [b]	2.1 ± 0.2 [b]	3.5 ± 0.2 [a]	2.1 ± 0.1 [b]	2.0 ± 0.2 [b]
18:2(n-6)	1.8	10.0 ± 0.3	10.3 ± 0.3	10.3 ± 0.6	11.8 ± 1.1	22.8 ± 0.1 [a]	16.7 ± 0.2 [b]	16.9 ± 1.1 [b]
18:1(n-9)c/18:3(n-3) [1]	13.2	14.2 ± 0.2	42.9 ± 0.6 [a]	26.8 ± 0.3 [b]	28.3 ± 1.0 [b]	29.5 ± 0.4 [a]	22.2 ± 0.3 [b]	21.7 ± 4.1 [b]
18:1(n-7)c	4.8	2.8 ± 0.0	8.4 ± 0.2 [a]	6.0 ± 0.2 [b]	6.3 ± 0.2 [b]	5.9 ± 0.1 [a]	4.1 ± 0.1 [c]	5.0 ± 0.3 [b]
18:0	7.2	4.4 ± 0.1	5.8 ± 0.1 [a]	5.3 ± 0.3 [b]	5.3 ± 0.4 [a]	6.9 ± 0.0 [a]	4.1 ± 0.0 [c]	5.3 ± 0.3 [b]
20:4(n-6), AA	8.2	8.9 ± 0.0	0.6 ± 0.0 [b]	7.4 ± 0.6 [a]	6.0 ± 2.5 [a]	1.2 ± 0.0 [c]	8.9 ± 0.2 [a]	7.5 ± 0.9 [b]
20:5(n-3), EPA	20.3	11.7 ± 0.3	3.1 ± 0.0 [b]	9.9 ± 0.3 [a]	8.1 ± 3.2 [a]	2.8 ± 0.0 [c]	11.3 ± 0.1 [a]	9.5 ± 0.8 [b]
22:6(n-3), DHA	11.3	7.5 ± 0.0	0.0 ± 0.0 [b]	5.2 ± 0.2 [a]	4.5 ± 1.1 [a]	1.2 ± 0.0 [c]	5.3 ± 0.4 [a]	4.3 ± 0.6 [b]
Other[2]	16.9	11.2	9.6	9.1	9.5	9.3	8.0	8.8
Sum (n-3)	33.1	23.0 ± 0.4	7.5 ± 0.1 [b]	18.7 ± 0.4 [a]	16.3 ± 2.2 [a]	8.0 ± 0.3 [c]	20.3 ± 0.5 [a]	17.2 ± 1.7 [b]
Sum (n-6)	12.2	20.5 ± 0.5	11.5 ± 0.2 [b]	19.0 ± 0.2 [a]	19.2 ± 2.2 [a]	25.2 ± 0.1	27.2 ± 0.2	25.9 ± 1.6
Sum (n-3)/(n-6)	2.7	1.1	0.7 [b]	1.0 [a]	0.8 [b]	0.3 [c]	0.7 [a]	0.7 [b]
Ratio EPA/AA	2.5	1.3	5.0 [a]	1.3 [b]	1.4 [b]	2.3 [a]	1.3 [b]	1.3 [b]
Ratio DHA/EPA	0.6	0.6	0.0 [b]	0.5 [a]	1.1 [a]	0.4	0.5	0.4
Total fatty acids (mg/g)			144.3 ± 10.6[a]	197.7 ± 10.2[b]	214.5 ± 12.2 [b]	138.4 ± 7.4 [a]	231.8 ± 26.0 [b]	139.5 ± 21.3 [a]

See Table 3 for additional information.

Table 5. Percentage composition of major (>2%) and essential fatty acids in A1 DHA Selco enrichment, *Artemia* at Day 1 and Day 4, after 24 h enrichment with A1 DHA Selco (Day 2 and Day 5, respectively) and 6h starvation. Newly- hatched *Jasus edwardsii* phyllosoma profiles (target predator species) are included for comparison (Smith 1999). Data are presented as mean ± *s.d.* (n = 3, except for starved 6 h, where n = 6). Enrichment composition: A1 DHA Selco (INVE Group, Belgium).

	Jasus edwardsii phyllosoma	A1 DHA Selco enrichment	Ongrown Day 1 *Artemia*	24h enrichment (Day 2 *Artemia*)	6h starved (Day 2 *Artemia*)	Ongrown Day 4 *Artemia*	24h enrichment (Day 5 *Artemia*)	6 h starved (Day 5 *Artemia)*
14:0	0.9	3.3 ± 0.0	0.8 ± 0.0 [c]	1.5 ± 0.0 [a]	1.2 ± 0.1 [b]	1.8 ± 0.0	1.6 ± 0.1	1.5 ± 0.1
16:1(n-7)c	2.3	4.2 ± 0.2	3.2 ± 0.2 [b]	3.6 ± 0.1 [b]	4.0 ± 0.1 [a]	2.8 ± 0.0 [c]	4.1 ± 0.1 [b]	4.4 ± 0.2 [a]
16:0	13.1	21.4 ± 0.6	11.6 ± 0.1 [c]	12.7 ± 0.4 [a]	10.7 ± 0.4 [b]	12.3 ± 0.3 [a]	11.0 ± 0.2 [b]	10.6 ± 0.3 [b]
18:4(n-3)	0.0	0.0 ± 0.0	3.7 ± 0.1 [a]	1.3 ± 0.0 [c]	1.4 ± 0.0 [b]	3.5 ± 0.2 [a]	1.6 ± 0.1 [b]	1.6 ± 0.1 [b]
18:2(n-6)	1.8	5.4 ± 0.2	10.3 ± 0.3 [ab]	8.9 ± 0.3 [b]	10.2 ± 0.6 [a]	22.8 ± 0.1 [a]	13.2 ± 0.1 [b]	14.2 ± 0.6 [b]
18:1(n-9)c/18:3(n-3)[1]	13.2	18.0 ± 3.0	42.9 ± 0.6 [a]	27.9 ± 0.1 [c]	30.6 ± 1.0 [b]	29.5 ± 0.4 [a]	24.0 ± 0.1 [b]	24.9 ± 0.8 [b]
18:1(n-7)c	4.8	3.0 ± 0.2	8.4 ± 0.2 [a]	5.6 ± 0.1 [c]	6.2 ± 0.3 [b]	5.9 ± 0.1 [a]	4.7 ± 0.2 [c]	5.0 ± 0.1 [b]
18:0	7.2	6.0 ± 0.2	5.8 ± 0.1 [a]	4.7 ± 0.2 [a]	4.3 ± 0.1 [b]	6.9 ± 0.0 [a]	4.2 ± 0.1 [b]	4.1 ± 0.2 [b]
20:4(n-6), AA	8.2	1.7 ± 0.1	0.6 ± 0.0	1.9 ± 0.0	2.1 ± 0.2	1.2 ± 0.0 [b]	2.7 ± 0.7 [a]	2.4 ± 0.2 [a]
20:5(n-3), EPA	20.3	5.3 ± 0.3	3.1 ± 0.0 [b]	7.1 ± 0.1 [a]	7.7 ± 0.2 [a]	2.8 ± 0.0 [b]	8.7 ± 0.3 [a]	8.2 ± 0.3 [a]
22:6(n-3), DHA	11.3	20.7 ± 0.6	0.0 ± 0.0 [c]	15.8 ± 0.3 [a]	12.4 ± 0.4 [b]	1.2 ± 0.0 [c]	15.4 ± 1.1 [a]	13.6 ± 0.7 [b]
Other[2]	16.9	11.0	9.6	9.0	9.2	9.3	8.8	9.5
Sum (n-3)	33.1	28.1 ± 1.1	7.5 ± 0.1 [b]	25.6 ± 0.4 [a]	22.8 ± 0.7 [a]	8.0 ± 0.3 [c]	27.0 ± 0.8 [a]	24.6 ± 0.8 [b]
Sum (n-6)	12.2	9.2 ± 0.4	11.5 ± 0.2 [a]	12.4 ± 0.3 [b]	13.9 ± 0.4 [b]	25.2 ± 0.1 [a]	17.8 ± 0.7 [b]	18.4 ± 0.8 [b]
Sum (n-3)/(n-6)	2.7	3.1	0.7 [a]	2.1 [b]	1.6 [b]	0.3 [c]	1.5 [a]	1.3 [b]
Ratio EPA/AA	2.5	3.2	5.0 [a]	3.7 [b]	3.6 [b]	2.3 [b]	3.2 [a]	3.4 [a]
Ratio DHA/EPA	0.6	3.9	0.0 [a]	2.2 [b]	1.6 [b]	0.4 [b]	1.8 [a]	1.6 [a]
Total fatty acids (mg/g)			144.3 ± 10.6 [a]	247.0 ± 31.0 [b]	245.1 ± 18.9 [b]	138.4 ± 7.4 [a]	261.0 ± 17.7 [b]	236.2 ± 54.1 [b]

See Table 3 for additional information.

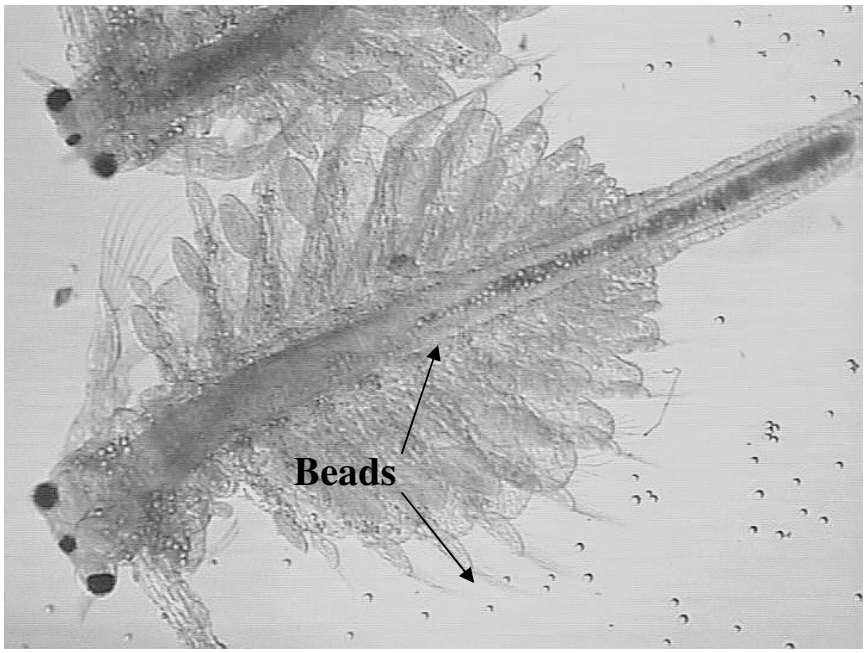

Figure 1. Within a few minutes of suspending 20-30 um plastic beads in the water column, they are visible within the gut cavity of *Artemia* due to continuous mechanism of filtration and ingestion.

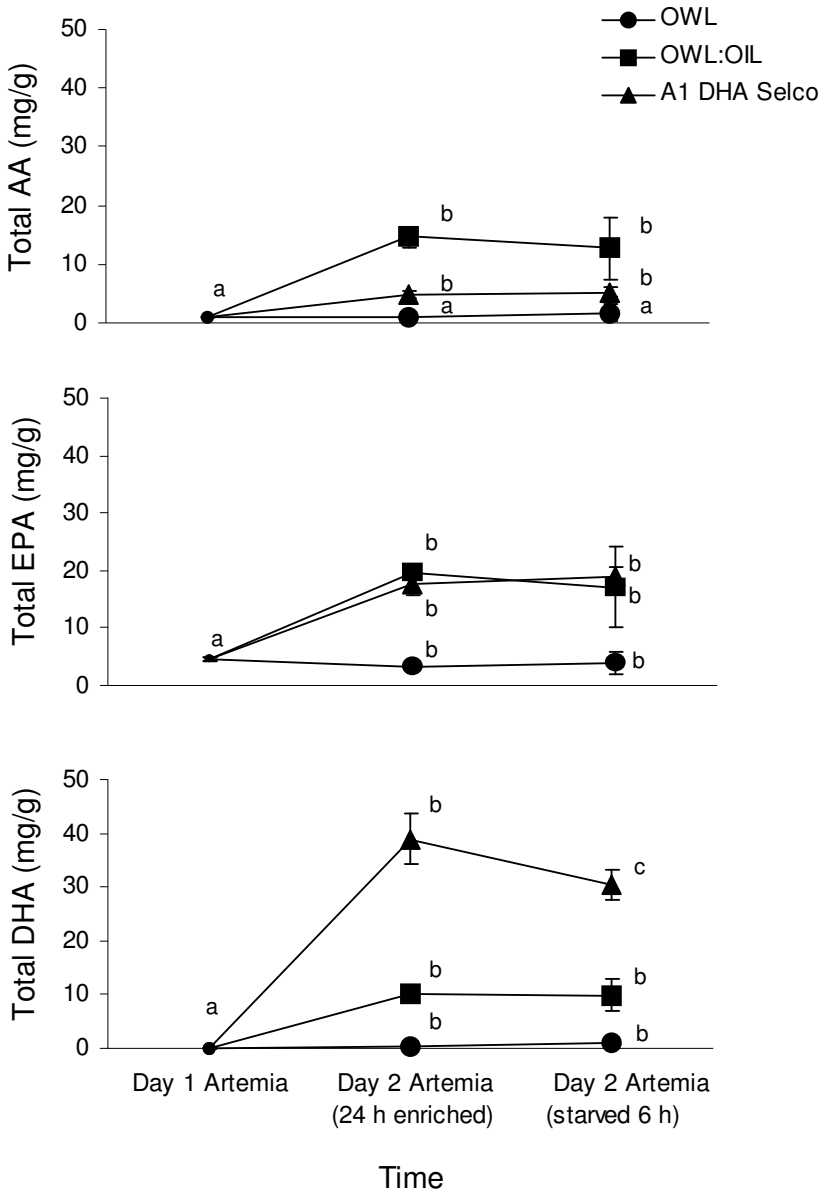

Figure 2. Changes in the quantitative contribution of arachidonic (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to the total lipid in Day 1 *Artemia*, after 24 h enrichment (Day 2) and a further 6 h starvation. Different superscripts denote significant differences within a dietary treatment over time.

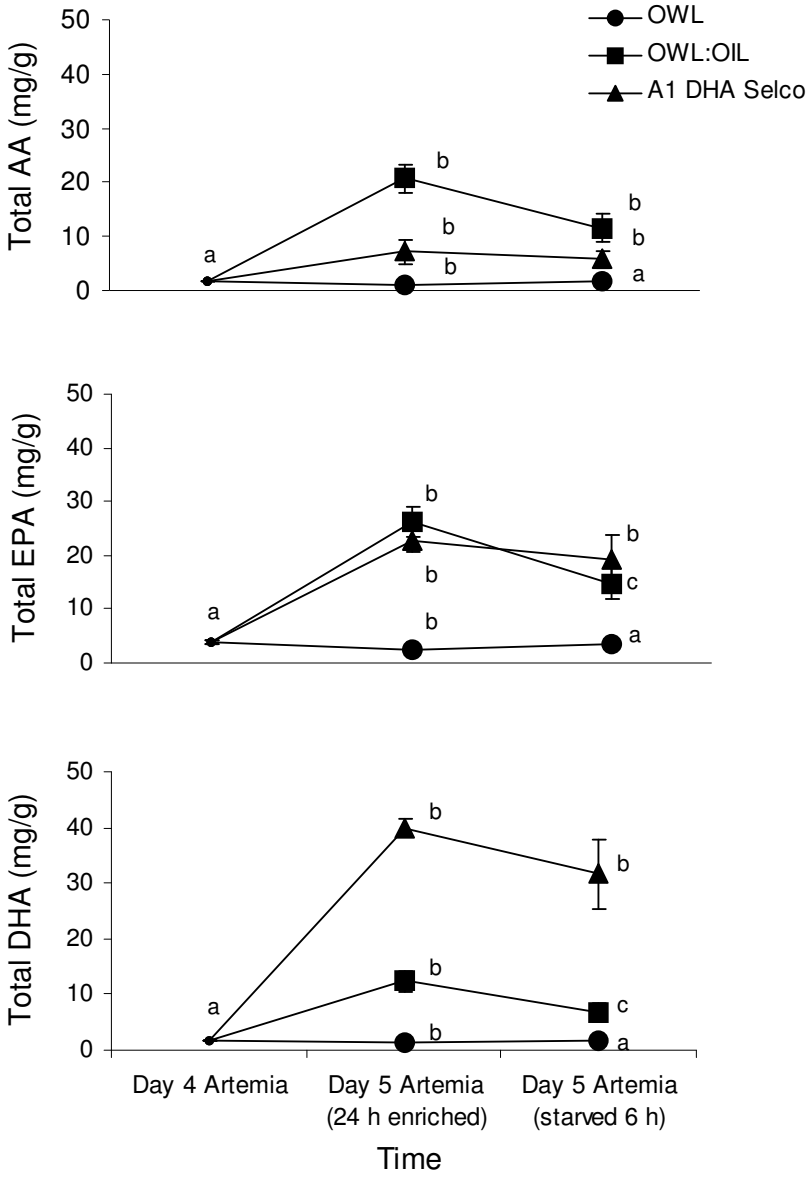

Figure 3. Changes in the quantitative contribution of arachidonic (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to the total lipid in Day 4 *Artemia*, after 24 h enrichment (Day 5) and a further 6 h starvation. Different superscripts denote significant differences within a dietary treatment over time.