Survival, growth and composition of *Poecilia latipinna* fry fed enriched *Artemia* nauplii

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***Artemia* nauplii (II instar) were enriched with soybean oil emulsion (SOE), sardine oil emulsion (SAOE) or shrimp head oil emulsion (SHOE) at selected concentrations (0.25, 0.5 and 1.0 ml/l sea water) and durations (9, 12 and 18 h). The nauplii enriched with 0.5 ml/l oil emulsion (individually) for 12 h fed to freshwater ornamental fish, *Poecilia latipinna* yielded appreciable results. The experimental fish fry were assessed for their survival, growth and biochemical composition and compared with the control. Survival of the fry fed SAOE-enriched *Artemia* nauplii was higher (98%) than the control (78%). Maximum specific growth rate of 22% was displayed by the fish fry fed SAOE-enriched *Artemia* nauplii and the minimum of 18% by the control. Total polyunsaturated fatty acid content in the fry fed on SAOE-enriched *Artemia* nauplii was the highest (36%).**

Keywords: Composition, enriched PUFA, growth, shrimp head oil emulsion, survival.

Introduction

SUCCESS in hatchery production system largely depends on the availability of suitable live feed organisms for feeding the sensitive fry and fingerling stages of fish[1]. Live feed is an essential food source for the fry of cultured species, especially those without a fully developed digestive system. In the freshwater ornamental fish culture, *Artemia* nauplii are used as the live feed[2]. Two major concerns of aquaculturists are: (i) providing organisms appropriate to the size of the feed to the first feeding stage and (ii) supplying adequate number of feed organisms to ensure higher survival and faster growth[3]. Hence the present investigation was aimed at nutritional evaluation of the indigenous *Artemia* nauplii and enriching them with selected exogenous source of oil emulsions. The enriched nauplii were tested on *Poecilia latipinna* fry for its survival, growth and composition.

It is known that invertebrates like *Artemia* and vertebrates like fishes do not synthesize the essential fatty acids such as eicosapentaenoic acid (20: $5n-3$), docosahexaenoic acid (22: $6n-3$) and arachidonic acid (20: $4n-6$)[4]. Hence there is a need for the live feed organisms like *Artemia* nauplii to be enriched with a substance rich in polyunsaturated fatty acid (PUFA). In view of the importance of this fact, this article throws light on how these fatty acids present in the selected oils enrich the *Artemia* nauplii and ensure higher survival and faster growth of fish.

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Experimental design

The orange molly *P. latipinna* were obtained from a commercial ornamental fish farm (Southern India Aquarists Pvt Ltd) near Chennai, reared in the laboratory, and their fry were collected and maintained in the culture tank. After measuring the length and weight of the fry, they were stocked at a density of 10 fry/l in a circular container (capacity 5 l) with 2 l of mildly aerated freshwater. Three replicates were maintained for every experimental trial.

Selection of nutrient sources

To enrich the locally available *Artemia* nauplii, soybean, sardine and shrimp head oils were used. Soybean oil was purchased commercially, while sardine and shrimp head oils were prepared in the laboratory following the procedure of Immanuel *et al.*[5]. The oil emulsions of soybean (SOE), sardine (SAOE) and shrimp head (SHOE) were prepared adopting the method of Tamaru *et al.*[6]. The freshly prepared emulsions were stored at –4°C until use.

Methods and standardization of enrichment

Artemia cyst collection and hatching procedures were accomplished following the standard procedure of Sorgeloos and Kulasekarapandian[7]. After 24 h of incubation, the first instar nauplii appeared; they did not feed as their mouth and anus were still closed. After 12 h the larva molts into the second stage the nauplius (instar II) started feeding on small particles and this stage of the nauplius was enriched with oil emulsions[8]. The second-instar *Artemia* nauplii were separated from the hatching container using 120 μm sieve and transferred into a glass enrichment container (capacity 2 l) at a density of 120 nauplii/ml of 1 l filtered sea water, at temperature of 25 ± 1.5°C.

They were enriched for 9, 12 or 18 h separately with selected oil emulsions at three selected concentrations (0.25, 0.5 and 1 ml/l of sea water). Strong aeration was provided to keep the O_2 level at 5 ppm. Each enrichment was performed in triplicate. The *Artemia* nauplii were collected from the enriched media. They were washed thoroughly with tap water and stored at –20°C for fatty acids analyses. As the nauplii were transparent, the presence of the emulsion could readily be assessed by the yellowish gut. The time required for filling the gut with emulsions in the nauplii was observed periodically under light microscope (Figure 1).

Feeding schedule

The experimental fry were fed with 12 h enriched *Artemia* nauplii (0.5 ml oil emulsion/l), and the control fry with unenriched *Artemia* nauplii. Both the experimental and control fry were fed at a density of 4 nauplii per fry each, three times a day (at 8, 13 and 20 h). The unfed dead nauplii were removed from the culture tank. The experimental duration was restricted to 20 days, as the fry reach marketable size by that time. At the end of the experiment, both control and experimental fry were randomly selected, and the total length and body weight were measured. The remaining fry available at the end of experiment were counted and per cent survival was calculated. Experimental samples of fry were taken at a specified time interval and stored for further analyses.

Proximate composition

Total proteins[9], carbohydrates[10], ash[11]and moisture[12] were estimated to know the effective utilization of *Artemia* nauplii by *P. latipinna* fry. Total lipids were extracted according to Folch *et al*[13]. One gram of sample was homogenized in 5 ml of chloroform/methanol (2 : 1 v/v) and shaken for 20 min in an Erlenmeyer flask with a magnetic stirrer. After filtering, the liquid phases were mixed and washed with 0.5 ml 0.9% sodium chloride solution. The lower phase was collected after phase separation, and the solvent was evaporated. The residue was transferred to a 10 ml glass vial and maintained at –20°C until analysis was performed. The total lipid content was determined according to Barnes and Blackstock[14]. Fatty acid methyl esters were separated using a Hewlett Packard 5890 gas chromatograph equipped with a fused silica capillary column (12 m long × 0.22 mm inner diameter) coated with BPX70. The oven temperature was 180°C and helium was used as the carrier gas at 2 ml/min.

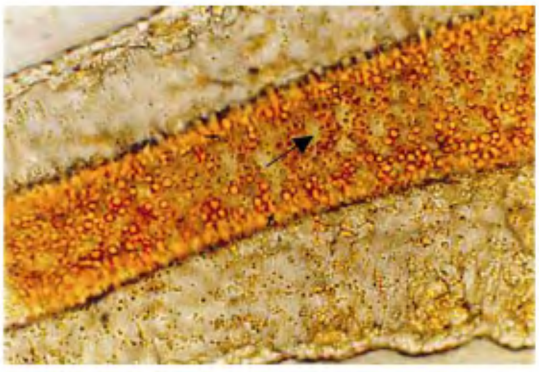

Figure 1. Photomicrograph showing the gut region of enriched *Artemia* nauplius (note massive intake of oil emulsion after 12 h enrichment).

Statistical analyses

Data obtained in a selected experiment on survival, growth and composition of the *Artemia* nauplii and fish were subjected to one-way analysis of variance using standard statistical methods of Sokal and Rohlf[15]. Specific growth rate (SGR; %/d) was calculated as SGR = 100 × (final weight/initial weight)/duration of feeding trial in days.

Results

The proximate composition of the freshly hatched *Artemia* nauplii and selected oils indicate that: (i) the nauplii have high water content (88%), as against 2–4% moisture present in the selected oils, (ii) the oils have 43–54% fat, as against 15% fat present in the nauplii and (iii) the protein (52%), carbohydrate (17%) and ash (8%) contents of the larvae were low in comparison to those in the oils (Table 1).

Fatty acid profiles of the nauplii and oils

The PUFA content of the freshly hatched *Artemia* nauplii and selected oils is summarized in Table 2. The PUFA content of sardine oil (49%), soyabean oil (46%) and shrimp head oil (44%) was high compared to freshly hatched *Artemia* nauplii (12%). Notably, the essential fatty acids like eicosapentaenoic (20: 5n – 3), docosahexaenoic (22: 6n – 3), arachidonic (20: 4n – 6), docospentaenoic (22: 5n – 3), linoleic (18: 2n – 6) and linolenic acids (18: 3n – 3) were also high in the selected oils.

Fatty acid profiles of the nauplii enriched with the oil emulsions

Figure 2 shows the trends observed for incorporation of PUFA into the nauplii at selected concentrations against the selected durations of exposure. It is clear that an exposure duration of 12 h at 0.5% concentration of SOE, SAOE and SHOE was optimum. The fish fry fed with nauplii enriched for duration of 12 h at 0.5% concentration of SOE, SAOE and SHOE ensured higher survival and faster growth of fish fry.

Table 1. Proximate composition of freshly hatched *Artemia* nauplii (% live weight) and selected oils (% liquid weight) (Mean ± SD represents three estimates)

Substance	Nauplii	Soybean oil	Sardine oil	Shrimp head oil
Moisture	88.1 ± 1.05	4.4 ± 0.47	2.3 ± 0.79	2.1 ± 0.41
Protein	52.0 ± 1.02	20.2 ± 0.44	18.4 ± 0.21	19.3 ± 0.54
Lipid	15.3 ± 0.45	43.3 ± 0.56	54.3 ± 0.38	47.4 ± 0.38
Carbohydrate	17.8 ± 1.38	18.6 ± 0.99	17.1 ± 1.30	18.2 ± 0.45
Ash	8.7 ± 1.09	13.2 ± 1.04	8.2 ± 0.55	15.3 ± 0.56

Table 2. Composition (%) of selected fatty acids present in the nauplii and the selected oils (Mean ± SD represents three estimates)

Fatty acids	Freshly hatched nauplii	Soybean oil	Sardine oil	Shrimp head oil
18: 2*n* – 6	3.5 ± 0.12	21.9 ± 0.35	18.2 ± 0.11	10.7 ± 0.07
18: 3*n* – 3	6.1 ± 0.08	6.7 ± 0.07	7.2 ± 0.11	6.9 ± 0.07
20: 4*n* – 6	1.7 ± 0.04	5.7 ± 0.07	6.9 ± 0.10	6.5 ± 0.07
20: 5*n* – 3	0.3 ± 0.02	4.6 ± 0.06	6.0 ± 0.07	8.7 ± 0.08
22: 5*n* – 6	1.2 ± 0.04	1.8 ± 0.06	1.6 ± 0.07	1.7 ± 0.09
22: 6*n* – 3	0.0 ± 0.00	6.0 ± 0.09	8.9 ± 0.09	9.8 ± 0.08
ΣPUFA	12.9 ± 0.22	46.7 ± 0.16	49.0 ± 0.31	44.5 ± 0.15

Table 3. Survival and growth of the fry *Poecilia latipinna* fed unenriched and enriched *Artemia* nauplii with selected oil emulsions for 20 days. (Each value represents the mean performance of ten fry)

Parameter	Control	SOE	SAOE	SHOE
Survival (%)	78.3 ± 1.68	92.7 ± 1.93	97.7 ± 0.70	96.6 ± 2.89
Total length (mm)	17.1 ± 0.25	26.0 ± 1.02	29.1 ± 1.17	25.8 ± 1.00
Live weight (mg)	42.8 ± 0.08	80.6 ± 0.59	85.0 ± 0.08	80.4 ± 0.24
Specific growth rate (SGR) (%/d)	18.3 ± 0.23	21.5 ± 0.48	21.8 ± 0.29	21.5 ± 0.28

Growth of fish fry

The fish fry fed on the enriched nauplii showed an overall increase in total length, weight, survival and SGR, compared to those of the control. Highest survival, maximum length, weight and SGR were recorded in the fry fed SAOE-enriched nauplii, viz. 97.7%, 29.2 mm, 85.0 mg and 21.8%, respectively (Table 3). The fry fed with unenriched *Artemia* nauplii showed slower growth and SGR, and lower survival. Difference in the growth, survival and SGR of *P. latipinna* fish fry fed with enriched nauplii was significant, compared to fry fed unenriched nauplii ($P < 0.001$).

Fatty acid profile of fish fry

The enriched *Artemia* nauplii had considerable influence on the PUFA content of fish fry (Table 4). Essential fatty acids of fry fed with enriched *Artemia* nauplii contain high levels of eicosapentaenoic (20: 5*n* – 3) and docosahexaenoic acids (22: 6*n* – 3) compared to fry fed with unenriched nauplii. The overall PUFA content of fish fry fed unenriched nauplii amounted to 29%. On the other hand, fish fry fed enriched nauplii with selected oil emulsions showed comparatively higher PUFA content of 34% (SOE), 36% (SAOE) and 34% (SHOE). The total PUFA content of fish fry fed enriched *Artemia* nauplii with selected oil emulsions showed significant differences when compared to control ($P < 0.001$).

Discussion

The nutritional quality of *Artemia* nauplii is often unpredictable, thus making quality strain costly. Hence, several enrichment diets such as micro-algae, highly unsaturated fatty acid (HUFA)-modified yeast, compound diets coated micro particles, oil-based emulsions and microencapsulated preparation have been successfully used[16]. In the present study fatty acid profiles of oil emulsions such as soybean, sardine and shrimp head showed higher levels of PUFA than the freshly hatched nauplii. Emulsion-type preparation has a major advantage over dried enrichment diet, as it has high lipid and HUFA content[17–19]. Consequently, marine oils in which the DHA and EPA levels are high due to their origin in specific fish tissues (cod liver oil and tuna orbital oil), are prepared through special

extraction procedures (silage and cold acetone), and have been recommended for both broodstock diet and larval rearing enrichment preparations[20].

Our results confirm the earlier report of Walford and Lam[21], who used protein-walled microcapsules AR 121 (Frippak Feeds, UK) to enrich nauplii for 8 h and obtained 1.4% increase in EPA and 3.6% increase in DHA. Stottrup and Attramadal[19] gained 3.3% increase in EPA and 0.8% increase in DHA by feeding nauplii with 'Protein Selco' (*Artemia* Systems, Belgium), twice over a period of 12 h. However, they showed 10.2% increase in EPA levels and 7.5% increase in DHA levels, when 'Super Selco' (*Artemia* System, Belgium) was used as the enrichment diet. Studies on (n – 3) HUFA have demonstrated that the EPA and DHA play a key role in sustaining normal growth, survival, pigmentation, resistance to stress and disease in many species[22–24].

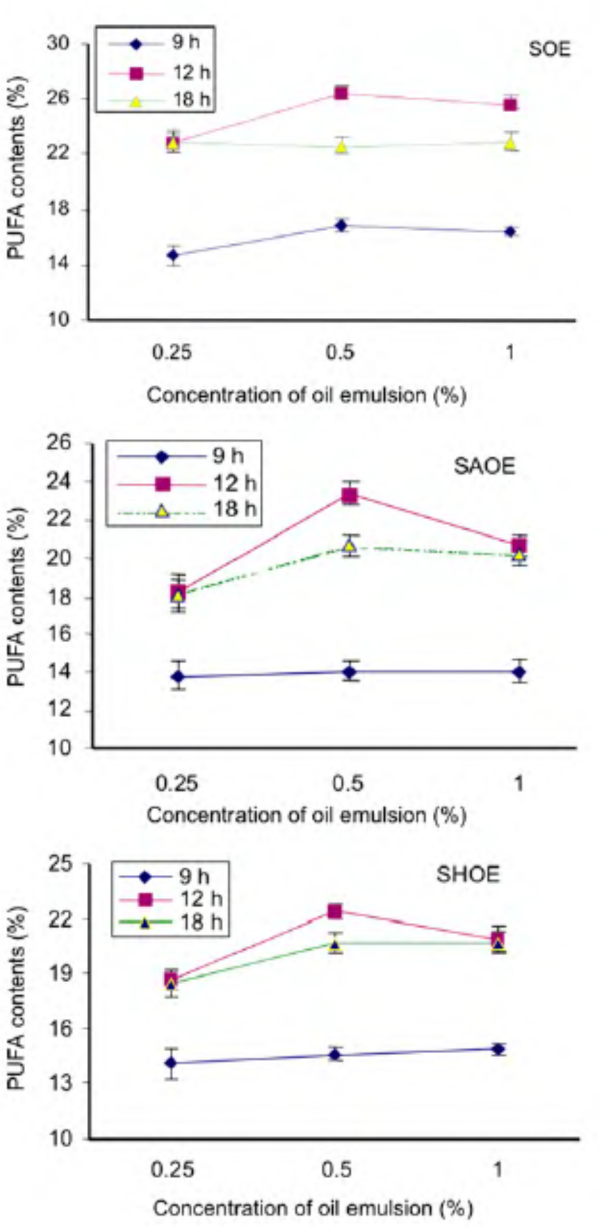

Figure 2. Polyunsaturated fatty acid content (%) of *Artemia* nauplii enriched with selected concentrations of SOE, SAOE and SHOE for different durations.

Interestingly, *P. latipinna* fry fed with enriched *Artemia* nauplii with selected oil emulsions showed an overall increase in growth, survival and SGR when compared to the control. Maximum total length and body weight were recorded in fish fry fed with enriched *Artemia* nauplii with SAOE. Similar observation was also made by Jones *et al.*[24] with *Artemia* fed on gelatin–acacia microcapsules containing either cod liver oil or pollack oil, which significantly supported faster growth rate and higher survival in post-larval gobies than feeding with unenriched *Artemia*. Compared to *Moina*, the use of *Artemia* nauplii for feeding would result in significant improvement in the growth performance of guppy adults and fry, and better survival rate in the adult fish[25]. The freshwater cladoceran, *Moina* is frequently used as a food source in freshwater ornamental fish culture because of its size (0.6–0.9 mm) and its ability for mass production. But in the present investigation, *Artemia* nauplii showed better influence on the growth and survival of ornamental fish than *Moina*.

Moreover, recently, Ozkizilcik and Chu[26] have reported that *Artemia* nauplii enriched with gelatin–acacia microcapsules containing menhaden oil supported significantly faster growth of striped bass (*Morone saxatilis*) larvae than unenriched nauplii with lower EPA content. The 100% cod liver oil-enriched *Artemia* nauplii were found superior as food for walleye larvae and juveniles over any other tested enrichment containing (n – 3) HUFA concentrate as a lipid source[27]. Lemm and Lemarie[28] showed significant improvement in the survival of striped bass larvae at 24 days post-hatch when fed with *Artemia* containing 8.2% EPA and 3.1% DHA in their lipids. Haddock larvae (*Malanogrammus aeglefinus*) similarly fed with *Artemia* nauplii enriched with Algamac 2000 (Aquafauna – BioMarine, California, USA) resulted in higher survival and body weight gain[29].

Fatty acid profiles of fish fry fed with enriched *Artemia* with selected oil emulsions had also enhanced PUFA content, particularly in fish fry fed enriched *Artemia* nauplii with SAOE. The occurrence of elongation and desaturation among fatty acid families has already been registered for several freshwater fish species[30,31]. The high body tissue DHA : EPA ratios of 1.21 (in fish fed HUFA-enriched live food) and 1.02 (in fish fed HUFA + vitamin C-enriched live food) compared to a low 0.29 (in fish fed unenriched diet) seems to suggest higher biological value of DHA over EPA in fish, notably marine species[32,33].

Therefore, the nutritional quality of *Artemia* nauplii is of prime importance in aquaculture, especially at the hatchery level. The presence of PUFA determines the quality of feed for shellfish and finfish culture. Hence the present study provides a comprehensive account on assessment of fatty acid composition of *Artemia* nauplii

Table 4. PUFA content (%) of the fry *P. latipinna* fed unenriched and enriched *Artemia* nauplii with selected oil emulsion for 20 days. (Mean ± SD represents three estimates)

Fatty acids	Fry fed unenriched *Artemia* nauplii	Fry fed enriched *Artemia* nauplii		
		SOE	SAOE	SHOE
18: 2*n* – 6	5.64 ± 0.08	6.47 ± 0.07	7.51 ± 0.08	6.46 ± 0.07
18: 3*n* – 3	11.92 ± 0.10	12.14 ± 0.05	12.24 ± 0.07	12.18 ± 0.09
20: 4*n* – 6	4.71 ± 0.09	5.31 ± 0.07	5.92 ± 0.05	5.36 ± 0.08
20: 5*n* – 3	5.73 ± 0.08	7.13 ± 0.06	7.20 ± 0.09	7.18 ± 0.07
22: 5*n* – 6	0.61 ± 0.05	0.74 ± 0.05	0.63 ± 0.06	0.78 ± 0.06
22: 6*n* – 3	1.03 ± 0.06	2.90 ± 0.09	3.04 ± 0.06	2.89 ± 0.11
ΣPUFA	29.64 ± 0.11	34.69 ± 0.16	36.54 ± 0.14	34.85 ± 0.16

before and after enrichment, prior to feeding the fry. All the three oil emulsions used in this study emerged as the most promising enrichers, because of the required PUFA content, ease of application and their reasonable costs. Thus the indigenous *Artemia* strain could be an ideal, cost-effective live feed with required nutrients for ornamental fish culture, especially in the early stages.

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