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Rearing techniques and nutritional quality of two mysids from Gran Canaria (Spain)

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Abstract

This paper presents the preliminary results of different trials carried out with two species of mysids from Gran Canaria: Leptomysis lingvura (G.O. Sars, 1866) and *Paramysis nouvel*. Experiments lasting 21 days showed significantly higher fecundity and survival in *L. lingvura* than in *P. nouveli* ($P < 0.05$). We also report the biochemical profile of both species fed 48-h-*Artemia* nauplii enriched with Easy-DHA-Selco® for 7 days. A comparison of our results with those of for *Artemia* and rotifers, organisms frequently used as live food in aquaculture, showed that mysids have a high percentage of protein per dry mass (73.38% in *P. nouveli*, and 74.19% in *L. lingvura*). Furthermore, the percentage of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) in total fatty acids was higher in both species than that reported by Roo and colleagues for rotifers and *Artemia*. In addition to the content of these fatty acids, their ratios between them are also important for normal growth and larval development. We found that the ratio, DHA:EPA, was 0.85 0.02 and 0.89 0.01; the ratio, DHA: AA, 6.25 0.26 and 4.74 0.14; and the ratio, EPA:AA, 7.32 0.26 and 5.32 0.2, respectively, for *P. nouveli* and *L. lingvura* in cultures and these ratios do not significantly differ ($P > 0.05$) from organisms in the wild. Here, we argue that as mysids are prey for many commercially important fish, cephalopods and rays, it is likely that the biochemical composition of mysids in their natural environment is "optimal" for these predators. Therefore, we studied the lipid profile of both species as they naturally occur in their environment. The results indicate that these mysids could be used to develop high quality live fish food.

Keywords: mysids, *Leptomysis lingvura*, *Paramysis nouveli*, live prey, nutritional quality, production

Introduction

The order Mysidacea comprises 780 species in about 120 genera, all included in the superorder Peracarida (Mauchline 1980; Bowman & Abele 1982). Mysids are omnivorous. The stomachs of mysids collected near the coast contain detritus, bodies and appendages of small crustaceans, and small amounts of diatom shells (Murano 1999).

Studies on the relationships between fish and mysids indicate that mysids are a keystone food for fish, especially in coastal environments, where they are abundant (Mauchline 1980; Murano 1999).

The stomach contents of chub mackerel (*Scomber japonicus*) indicate that mysids have a trophic importance even greater than euphausiids in the waters around the island of Gran Canaria (Castro 1995). This mackerel represents 52% of mid-sized pelagic fish in the region. It daily consumes 8% of its body mass in crustaceans and 2.5% in fish (anchovy). Accordingly, Castro (1995) estimated that annually, this mackerel consumes about 242 000 tonnes of mysids and 29 000 tonnes of euphausiids. These data give us an idea of the trophic importance of mysids as food in the region.

In aquaculture, mysids have proven to be a high-quality food for the juvenile stages of cuttlefish *Sepia officinalis* (Domingues, Sykes & Andrade 2001) and adult seahorse *Hippocampus abdominalis* (Woods & Valentino 2003) and *Hippocampus hippocampus*

(Otero-ferrer, Molina, Socorro, Herrera, Fernández-palacios & Izquierdo 2009).

In culturing fish larvae, *Artemia* and rotifers are normally used as food, but this sparce diet, according to Izquierdo (1996) leads to malnutrition and should be improved.

Three fatty acids are essential for normal development of marine fish: docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA). They fill a fundamental role in developing both the structure and the function of integral cell membranes. Furthermore, these fatty acids and the EPA:AA ratio serve as precursors or are otherwise important for the development of a group of highly active hormones known as eicosanoids (Izquierdo 1996; Sargent, Bell, McEvoy, Tocher & Estevez 1999; Roo, Hernández-cruz, Socorro, Fernández-palacios, Montero and Izquierdo 2009). However, not only is the content of these fatty acids important, but their inter-relationships: DHA:EPA:AA are also important. Knowing the optimal ratios is difficult in practice because it is likely to differ in each species (Sargent *et al.* 1999). Consequently, we suggest analysing the prey of each species in its natural environment, as predator and prey are well adapted to the same environment conditions.

This paper is a pilot study of the survival and production of *Leptomysis lingvura* and *Paramysis nouveli* in captivity. Here, we analyse the nutritional quality (lipid and protein profiles) of both species to determine their suitability as live prey in aquaculture.

We present the protein and fatty acid profiles of both species in their natural environment in order to determine whether the diet used during cultivation changes their natural biochemical composition. Other investigators have cultivated mysids, mainly from the genus of *Mysidopsis*, and used them for laboratory experimentation and for water toxicity testing (Reitsema 1980; Ward 1984; Lussier, Kuhn, Chammas & Sewall 1988; Domingues, Turk, Andrade & Lee 1999; Verslycke, Fockedey, Mckenney Jr, Roast, Jones, Mees & Janssen 2004). We intend to use our results to facilitate the development of fish food for cultivating ornamental fish as well as commercially important fish.

Material and methods

Survival and production experiments

On the east coast of Gran Canaria, in Risco Verde bay (27°51′N and 15°23′W), samples were taken weekly from August to October 2008. Sampling took place at depths between 5 and 15 m in areas near the rocks using SCUBA equipment and a hand net of 500 μm mesh. Species identification was performed using a binocular microscope (Wild M8, Heerbrugg, Switzerland), following the work of Tattersall and Tattersall (1951), Labat (1953), Wittmann (1986) and Barberá Cebrián, Ribeiro Da Cunha, Sánchez Jerez and Ramos Esplá (2001).

To study the survival and production, samples of *L. lingvura* and *P. nouveli*, two of the most abundant species in our samples, were taken in October 2008. After acclimatization for 2 days, 10 males and 10 females of each species were then placed in small 1 L farrowing containers, which in turn were placed in larger 14 L open flow tanks of filtered seawater with a salinity of 37 g L^{-1} (PSU). The seawater, common to the farrowing containers and the 14 L tanks, was maintained at 18.2 ± 0.4 °C, renewed every 12 h and monitored for pH, oxygen, ammonium, nitrate and nitrite. The pH was maintained at 8.2 ± 0.1, the O_2 at 7.1 ± 0.1 mg L^{-1} and the NH_4^+, NO_3^- and NO_2^- at concentrations below 0.2, 1 and 0.02 mg L^{-1} respectively. The photoperiod was 14 h:10 h light and dark. Mysids were fed twice daily using 100 *Artemia* nauplii per mysid. The *Artemia* (EG type) were enriched with Easy-DHA Selco®; INVE aquaculture, Dendermonde, Belgium).

Mysids were counted daily. Survival of adults was expressed as a percentage of the original number. Relative production was estimated by dividing the number of hatchlings per day by the number of females alive. Production rates were expressed as young per female. The experiments were carried out in three replicates.

To measure the standard length (from the rostrum in between the eye stalks to the end of the last abdominal segment) of young, we used a binocular microscope with a reflex digital camera of 10 megapixels (Canon EOS 1000D, Tokyo, Japan) and the software IMAGE J 1.40 g (National Institutes of Health, USA) to estimate the length from the megapixels in the photograph.

Nutritional quality experiments

Samples for lipid and protein analysis were also collected in Risco Verde between March and April 2009. Samples of *P. nouveli* and *L. lingvura* were separated immediately after capture using a binocular microscope and kept frozen at − 80 °C for further analysis. For culture experiments, the mysids were separated by

species and after an acclimatization period of 2 days, were maintained for 7 days, fed twice daily using 100 *Artemia* nauplii per mysid (as above). The culture conditions were identical to those used in the survival and production experiments. At day 7, the organisms were placed on filters, washed with distilled water and stored at − 80 °C until analysis was performed.

Moisture was determined in the samples by drying them to a constant weight in an oven at 110 °C (AOAC 1995). The ash content was determined by incinerating the samples to a constant weight in a muffle furnace at 600 °C (AOAC 1995).

Protein was calculated from total nitrogen in the samples as determined using the Kjeldhal technique (AOAC 1995). Crude lipids (% wet mass) were extracted following the method of Folch, Lees and Sloane-stanley (1957). Fatty acid methyl esters from total lipids were prepared by transmethylation as described by Christie (1982), separated and quantified by Gas–Liquid chromatography as described by Izquierdo, Watanabe, Takeuchi, Arakawa and Kitajima (1989). Proteins, lipids, ash and moisture were expressed as % dry mass. Fatty acids are expressed as % of total.

Statistical analysis

The Mann–Whitney non-parametric test with significance $P < 0.05$ was used to determine statistical differences in the survival and production of each species and Kruskal–Wallis one-way ANOVA with significance $P < 0.05$ was performed for the three replicates.

All the biochemical data were expressed as means ± SD. To evaluate the homogeneity of variances between wild and cultured mysids, we applied Levene's test, and to study the differences between them, we applied the Student *t*-test with significance level $P < 0.05$. These statistical analyses were performed using SPSS Statistical Software version 14.0 (1999, SPSS Chicago, IL, USA).

Results

Survival and production experiment

At the end of the experiment, the average survival for *L. lingvura* was 65 ± 8.7% (mean ± SD) and 16 ± 5.8% for *P. nouveli* (Fig. 1). The cultures of the two mysids showed no significant differences in survival until day 9, since then the values were higher ($P < 0.05$) in *L. lingvura*. The total hatchling production was 166 ± 2 and 45 ± 7 for *L. lingvura* and *P. nouveli* (Fig. 2) and the hatchling average standard length was 2.03 ± 0.23 and 1.86 ± 0.17 mm, respectively, showing significant differences between species ($P < 0.05$). The relative production (young per female) was significantly higher ($P < 0.05$) in *L. lingvura* (18.2 ± 2) than that in *P. nouveli* (4.6 ± 0.8) at day 21. No hatchlings of *P. nouveli* were found from day 12 of the experiment.

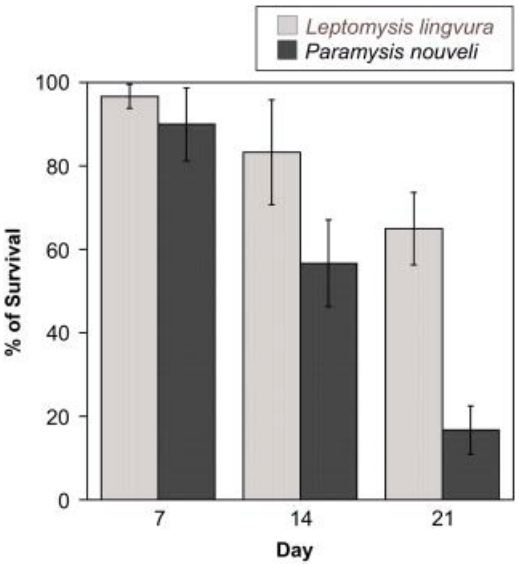

Figure 1 Survival in percentage of *Leptomysis lingvura* and *Paramysis nouveli* at days 7, 14 and 21 of the experiment.

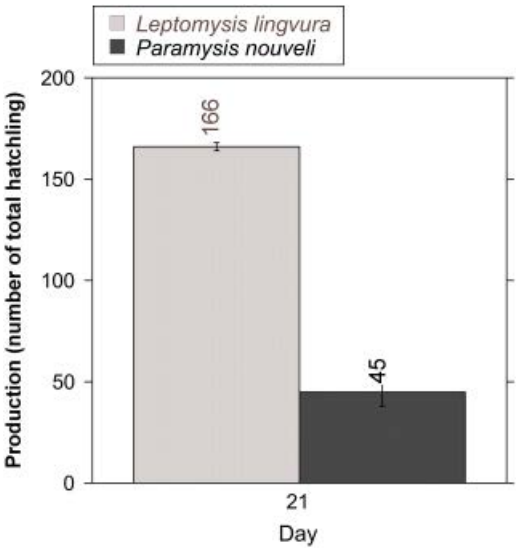

Figure 2 Total hatchling production of *Leptomysis lingvura* and *Paramysis nouveli* at day 21 of the experiment.

Nutritional quality experiments

Lipid and protein analysis was the first step in determining the nutritional quality of the cultured mysids. The proteins and lipids as a % of dry mass were 73.38 ± 1.77% and 15.01 ± 1.12% for *P. nouveli* and 74.19 ± 5.22% and 14.79 ± 2.66% for *L. lingvura* (Table 1). The most abundant fatty acids in both species were oleic acid 18:1 n-9, palmitic acid 16:0, EPA 20:5 n-3, DHA 22:6 n-3, α-linoleic acid (ALA) 18:3 n-3 and linolenic acid 18:2 n-6 (Fig. 3). The omega-3 (n-3) and the omega-6 (n-6) polyunsaturated fatty acids (PUFA) in *P. nouveli* and *L. lingvura* accounted for 39.45 ± 0.73% and 8.43 ± 0.22%, and 42.4 ± 0.36% and 8.34 ± 0.06% of the total lipids respectively (Table 1). The ratio DHA:EPA was 0.85 ± 0.02 and 0.89 ± 0.01, DHA:AA 6.25 ± 0.26 and 4.74 ± 0.14 and EPA:AA 7.32 ± 0.26 and 5.32 ± 0.2% respectively (Table 1).

In mysids collected in the wild, lipids, protein and ash as a % of dry mass for *P. nouveli* were 17.83 ± 0.12%; 74.24 ± 1.28% and 2.69 ± 0.2%, respectively, and 16.25 ± 4.96%; 77.34 ± 1.24% and 3.72 ± 0.31%, respectively, for *L. lingvura*.

Fatty acids as a per cent of total are presented in Table 1 and represented with the percentages obtained for mysids fed *Artemia* in culture in Fig. 3 for *L. lingvura* and Fig. 4 for *P. nouveli*.

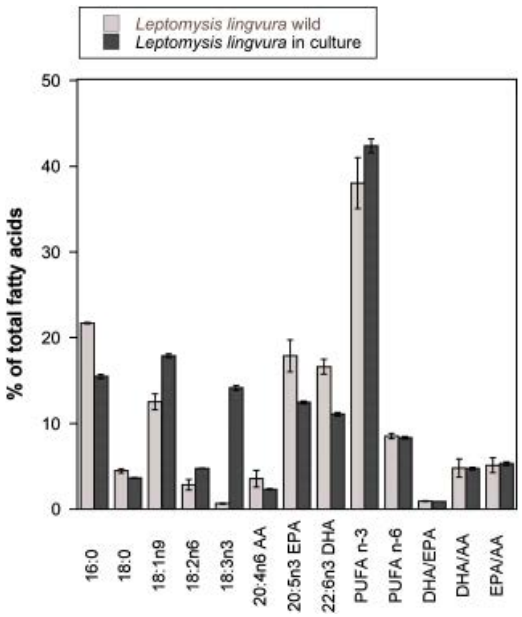

Figure 3 The most abundant fatty acids as a percentage of total for wild *Leptomysis lingvura* and for the same species cultured for 7 days on *Artemia* nauplii enriched for 48 h with Easy–DHA, INVE, Belgium. The fatty acids are identifiable by the following key: 16:0 (Palmitic acid), 18:0 (Stearic acid), 18:1 n-9 (Oleic acid), 18:2 n-6 (Linolenic acid), 18:3 n-3 (α-linoleic acid), 20:5 n-3 (EPA), 22:6 n-3 (DHA) and 20:4 n-6 (AA).

Table 1 Lipids, proteins and ash composition (% dry mass) and fatty acids (% total fatty acids) of wild and cultured *Paramysis nouveli* and *Leptomysis lingvura* and two live prey used frequently in aquaculture (rotifers and *Artemia*) reported by Roo *et al.* (2009)

		Wild *P. nouveli*	Cultured *P. nouveli**	Wild *L. lingvura*	Cultured *L. lingvura* *	Enriched rotifers	Enriched *Artemia*
% Lipids (dm)		17.83 ± 0.12	15.01 ± 1.12	16.25 ± 4.96	14.79 ± 2.66	22.05 ± 3.84	26.04 ± 0.41
% Proteins (dm)		74.24 ± 1.28	73.38 ± 1.77	77.34 ± 1.24	74.19 ± 5.22	54.28 ± 4.57	56.39 ± 4.84
% Ash (dm)		2.69 ± 0.2	2.99 ± 0.07	3.72 ± 0.31	3.63 ± 0.21	1.48 ± 0.5	0.75 ± 0.02
16:0	Palmitic acid	22.88 ± 0.34†	16.94 ± 0.62†	21.71 ± 0.07†	15.48 ± 0.23†	13.0 ± 2.48	15.22 ± 3.8
18:0	Stearic acid	4.28 ± 0.14	4.01 ± 0.1	4.48 ± 0.25	3.64 ± 0.05	4.73 ± 1.21	4.42 ± 0.37
18:1 n-9	Oleic acid	12.85 ± 0.46†	19.11 ± 0.38†	12.53 ± 0.93	17.9 ± 0.24	20.1 ± 1.72	20.36 ± 7.38
18:2 n-6	Linolenic acid	3.15 ± 1.07	4.79 ± 0.24	2.86 ± 0.63	4.76 ± 0.02	8.14 ± 1.31	3.78 ± 2.61
18:3 n-3	α-Linoleic acid	0.78 ± 0.12†	8.22 ± 0.19†	0.67 ± 0.08†	14.18 ± 0.26†	1.62 ± 0.11	10.81 ± 4.23
20:5 n-3 EPA	Eicosapentaenoic acid	19.39 ± 0.68	14.77 ± 0.2	17.89 ± 1.85	12.45 ± 0.15	6.51 ± 0.62	11.10 ± 4.27
22:6 n-3 DHA	Docosahexaenoic acid	16.98 ± 1.22	12.63 ± 0.37	16.62 ± 0.86	11.10 ± 0.2	9.68 ± 0.93	4.47 ± 1.43
20:4 n-6 AA	Arachidonic acid	2.92 ± 0.01†	2.02 ± 0.06†	3.57 ± 0.96	2.34 ± 0.09	1.46 ± 0.73	1.49 ± 0.37
∑ PUFA n-3		40.31 ± 0.38	39.45 ± 0.73	38.04 ± 2.95	42.4 ± 0.36	21.12 ± 0.48	31.14 ± 11.43
∑ PUFA n-6		7.99 ± 1.18	8.43 ± 0.22	8.53 ± 0.31	8.34 ± 0.06	10.77 ± 2.11	7.03 ± 3.73
DHA/EPA		0.88 ± 0.09	0.85 ± 0.02	0.93 ± 0.05	0.89 ± 0.01	1.49 ± 0.01	0.4 ± 0.34
DHA/AA		5.81 ± 0.44	6.25 ± 0.26	4.8 ± 1.06	4.74 ± 0.14	8.1 ± 4.45	2.99 ± 3.87
EPA/AA		6.64 ± 0.21	7.32 ± 0.26	5.13 ± 0.86	5.32 ± 0.2	5.45 ± 2.99	7.43 ± 11.53

Values (mean ± SD).
*Fed *Artemia* nauplii enriched with Easy-DHA Selco®.
†Significant differences between wild and cultivated.

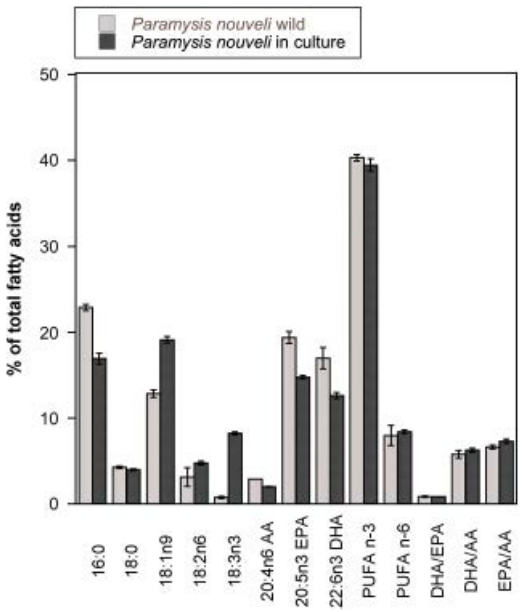

Figure 4 The most abundant fatty acids as a percentage of total for wild *Paramysis nouveli* and for cultured *P. nouveli* fed for 7 days on *Artemia* nauplii enriched for 48 h with Easy–DHA, INVE, Belgium. The fatty acids are identifiable by the following key: 16:0 (Palmitic acid), 18:0 (Stearic acid), 18:1 n-9 (Oleic acid), 18:2 n-6 (Linolenic acid), 18:3 n-3 (∝-linoleic acid), 20:5 n-3 (EPA), 22:6 n-3 (DHA) and 20:4 n-6 (AA).

Discussion

From the results obtained in the preliminary experiments with survival and production, we determined that *L. lingvura* is the more suitable of the two species for culture in our facilities. These results could vary if we changed the culture conditions and feeding treatment because the mysids are omnivorous and feed on copepods, rotifers, diatoms and organic detritus in the natural environment (Mauchline 1980; Murano 1999; Domingues *et al.* 1999; Domingues, Fores, Turk, Lee & Andrade 2000), and may not be receiving adequate food in cultures.

As reported previously by Domingues *et al.* (2000), the complete replacement of *Artemia* nauplii by rotifers caused decreased production and survival of juvenile and adult *Leptomysis* sp.; however, the partial replacement of *Artemia* by rotifers (1/3 *Artemia*+2/3 rotifers) showed no significant differences in the production and survival of offspring and adults as compared with being fed 100% *Artemia* nauplii. In general, our results with *L. lingvura*, especially around day 20, were similar to those of Domingues *et al.* (2000).

To optimize the culture conditions, further experiments with different types of prey, for example, different algae, rotifers as well as *Artemia* must be carried out. In addition, one should experiment with environmental conditions by modifying temperature, density and salinity, as they directly affect survival and growth production (Mauchline 1980; Domingues *et al.* 1999; Murano 1999; Fockedey, Mees, Vangheluwe, Verslycke, Janssen & Vincx 2005).

The study of lipid and protein composition revealed that both species have a high potential as live food in aquaculture. The levels of proteins and lipids and fatty acids in *P. nouveli* and *L. lingvura* meet the nutritional requirements for fish according to FAO (Tacon 1989).

Both mysids species in culture showed higher levels of PUFA n-3, *P. nouveli* (39.45%) and *L. lingvura* (42.4%) in comparison with *Artemia* (31.14%) and rotifers (21.12%) according to Roo *et al.* (2009) (Table 1).

Polyunsaturated fatty acids, DHA, EPA and AA are required, by themselves and in specific dietary ratios, for the normal growth and development of fish. Both mysids have a composition of DHA, EPA and AA higher than that reported by Roo *et al.* (2009) for rotifers and *Artemia* enriched with DHA Protein Selco® (INVE) and Selco® (INVE) respectively (Table 1).

Otero-ferrer, Molina, Socorro, Herrera, Fernández-palacios and Quierdo (2010) reported results of DHA (6.6%), EPA (5.5%) and AA (1.3%) close to those obtained by Roo *et al.* (2009) (4.47%; 11.5% and 1.46% respectively) for the same type of *Artemia* sp. enrichment under similar conditions; the values for rotifers (2.2%; 1.8% and 0.6% respectively) are lower than those obtained by Roo *et al.* (2009) (9.68%; 6.5% and 1.49% respectively). The results of DHA, EPA and AA obtained for *L. lingvura* (11.10%; 12.45%; 2.34% respectively) and *P. nouveli* (12.63%; 14.77%; and 2.02% respectively) are higher than those obtained by both authors for rotifers and *Artemia* (Table 1). We suspect that mysid fatty acid composition would be more optimum for organisms that normally prey on mysids in the wild. This is in keeping with the ideas expressed by Sargent *et al.* (2009).

Domingues *et al.* (2001) conducted experiments on survival and growth in cuttlefish (*S. officinalis*) fed at an early stage of growth with two different treatments: *Artemia* and mysids (*P. nouveli*). In both experiments, the hatchlings, fed mysids, reached larger sizes and survival was higher. These results support our hypothesis that mysids are a higher quality food for the cultivation of the commercially important species that prey on mysids in nature.

However, the preliminary results do not show a high production, which argues against using the mysids for cultivation on a commercial level. It is clear that mysid cultivation is more expensive and less productive than that of *Artemia* and rotifers. Nevertheless, they may serve as food for ornamental fish or as supplementary food for cultures suffering high mortality at certain stages of development. This is the case in cultured paralarvae of *Octopus vulgaris*, where high mortality and low growth have been observed (Iglesias, Sánchez, Bersano, Carrasco, Dhont, Fuentes, Linares, Muñoz, Okumura, Roo, Van Der Meeren, Vidal & Villanueva 2007). In this situation, the mysids could complement other cheaper food as the mysid hatchlings have a size appropriate for the *O. vulgaris* paralarvae (1.8–2 mm). Furthermore, the data presented for *P. nouveli* and *L. lingvura* can be useful in determining the composition of "optimal" food for natural predators such as mackerel, *S. officinalis, O. vulgaris and Hippocampus* sp.

The study of lipids in wild mysids and in their natural food shows differences between the wild and cultured mysids. In the wild, palmitic acid (16:0) in both *P. nouveli* and *L. lingvura* was present at higher percentages ($P < 0.05$) of total lipids than it was in cultures; however, in both mysids, ALA (18:3 n-3) was significantly higher ($P < 0.05$) in culture than in the wild (Figs 3 and 4; Table 1). *Paramysis nouveli* also showed significant differences in the percentages of oleic acid (18:1 n-9) and AA (20:4 n-6) ($P < 0.05$). These differences are likely due to the wide variety of foods the mysids consume in the wild.

However, the ratios DHA:EPA, DHA:AA and EPA:AA do not show significant differences ($P > 0.05$) between wild and cultured organisms.

Research in mysid cultures growing on different prey suggests ways in which the diet could be modified to attain optimum lipid ratios in the mysids themselves.

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