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

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Abstract

This paper presents preliminary results of different trials carried out with two species of mysids from Gran Canaria: *Leptomysis lingvura* (G.O. Sars, 1866) and *Paramysis nouveli* (Labat, 1953). 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-hour-*Artemia* nauplii enriched with Easy-DHA-Selco® (INVE, Belgium) for 7 days. A comparison of our results with those of Roo *et al.* (2009) 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 DHA (docosahexaenoic acid), EPA (eicosapentaenoic acid), and AA (arachidonic acid) in total fatty acids was higher in both species than reported by Roo *et al.* (2009) for rotifers and *Artemia*. In addition to the content of these fatty acids, the ratios between them is 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 (Bowman & Abele 1982; Mauchline 1980). 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 (Murano 1999; Mauchline 1980).

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 *et al*. 2001) and adult seahorse, *Hippocampus abdominalis* (Woods & Valentino 2003) and *H. hippocampus* (Otero-Ferrer *et al*. 2009). In culturing fish larvae, only *Artemia* and rotifers are used traditionally as food and this poverty of choice can lead to nutritional imbalances (Izquierdo 1996), and other foods are needed to improve this situation.

Three fatty acids are essential for normal development of marine fish: DHA, EPA, AA. They fill a fundamental role in developing both the structure and function of integral cell-membranes. Furthermore, they 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 *et al.* 1999, Roo *et al.* 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 analyzing 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 *L. lingvura* and *P. nouveli* in captivity. Here, we analyze 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 if 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 *et al*. 1988, Domingues *et al*. 1999, Verslycke *et al*. 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 (27º51'N and 15°23'W), samples were taken weekly from August to October 2008. Sampling took place at depths between 5 and 15 meters in areas near the rocks using SCUBA equipment and a hand net of 500-µm mesh. Species identification was performed with a binocular microscope (Wild M8, Heerbrugg, Switzerland), following the work of Tattersall and Tattersall (1951), Labat (1953), Wittmann (1986) and Barberá-Cebrián *et al.* (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 1L farrowing containers that in turn, were placed in larger 14L 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 ± 0.4ºC, renewed every 12 hours,

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 14h:10h 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 1.40g (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 -80ºC 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 made.

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

Protein was calculated from total nitrogen in the samples as determined by the Kjeldhal technique (AOAC 1995). Crude lipids (% wet mass) were extracted following the method of Folch *et al.* (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 *et al* (1989). Proteins, lipids, ash and moisture were expressed as % dry mass. Fatty acids are expressed as % of total.

Statistical analysis

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 differences between them we applied the Student t-test with significance level, $P<0.05$. These statistical analyses were done using SPSS Statistical Software version 14.0 (SPSS Chicago, Illinois, 1999).

Results

Survival and production experiment

At the end of the experiment the average survival for *L. lingvura* was 65 ± 8.7% (mean ± S.D.) and for *P. nouveli* 16 ± 5.8% (Fig. 1). The cultures of the two mysids showed no significant differences in survival until day 9, since then 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 mm and 1.86 ± 0.17 mm, respectively, showing significant differences between species ($P<0.05$). The relative production (young.female^{-1}) was significantly higher ($P<0.05$) in *L. lingvura* (18.2±2) that *P. nouveli* (4.6±0.8), at day 21. No hatchlings of *P. nouveli* were found from day 12 of experiment.

(Insert Figure 1 here)

(Insert Figure 2 here)

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, for *P. nouveli* were 73.38 ± 1.77% and 15.01 ± 1.12% and for *L. lingvura*, 74.19 ± 5.22% and 14.79 ± 2.66% (Table 1). The most abundant fatty acids in both species were oleic acid 18:1 n-9, palmitic acid

16:0, eicosapentaenoic acid (EPA) 20:5 n-3, docosahexaenoic acid (DHA) 22:6 n-3, α-linoleic acid (ALA) 18:3 n-3 and linolenic acid (LA) 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: arachidonic acid (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 were for *P. nouveli*: 17.83 ± 0.12%; 74.24 ± 1.28% and 2.69 ± 0.2% respectively; and for *L. lingvura*: 16.25 ± 4.96%; 77.34 ± 1.24% and 3.72 ± 0.31%, respectively.

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

(Insert Table 1 here)

(Insert Figure 3 here)

(Insert Figure 4 here)

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 in the natural environment feed on copepods, rotifers, diatoms and organic detritus (Mauchline 1980, Murano 1999, Domingues *et al.* 1999, 2000), and in cultures may not be receiving adequate food.

As previously reported 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 production and survival of offspring and adults as compared to 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

204 salinity, as they directly affect survival and growth production (Mauchline 1980, Murano
205 1999, Domingues *et al.* 1999, Fockedey *et al.* 2005).
206 The study of lipid and protein composition revealed that both species have a high potential
207 as live food in aquaculture. The levels of proteins and lipids and fatty acids in *P. nouveli*
208 and *L. lingvura* meet nutritional requirements for fish according to FAO (Tacon 1989).
209 Both mysids species in culture showed higher levels of PUFA (polyunsaturated fatty
210 acids) n-3; *P. nouveli* (39.45%) and *L. lingvura* (42.4%) in comparison with *Artemia*
211 (31.14%) and rotifers (21.12%) according to Roo *et al*. (2009). (Table 1).
212 PUFA, DHA, EPA and AA are required, by themselves and in specific dietary ratios, for
213 normal growth and development of fish. Both mysids have a composition of DHA, EPA
214 and AA, higher than that reported by Roo *et al.* (2009) for rotifers and *Artemia* enriched
215 with, DHA Protein Selco® (INVE, Belgium) and Selco® (INVE, Belgium) respectively
216 (Table 1).
217 Otero-Ferrer *et al.* (2010) reported results of DHA (6.6%), EPA (5.5%) and AA (1.3%)
218 close to Roo *et al.* (2009) (4.47%; 11.5% and 1.46 respectively) for the same type of
219 *Artemia sp.* enrichment under similar conditions; the results for rotifers (2.2%; 1.8% and
220 0.6% respectively) are lower than those obtained by Roo *et al*. (2009) (9.68%; 6.5% and
221 1.49% respectively). The results of DHA, EPA and AA obtained for *L. lingvura* (11.10%;
222 12.45%; 2.34% respectively) and *P. nouveli* (12.63%; 14.77%; and 2.02% respectively)
223 are higher than those obtained by both authors for rotifers and *Artemia* (Table 1). We
224 suspect that these differences in fatty acid composition could make mysid food more
225 likely, than rotifer or *Artemia* food, to satisfy the nutritional requirements of aquaculture,
226 especially the aquaculture of those species that in the wild prey naturally of mysids.
227 Domingues *et al*. (2001) made experiments with survival and growth in cuttlefish (*Sepia*
228 *officinalis*), fed at an early stage of growth with two different treatments: *Artemia* and
229 mysids (*P. nouveli*). In both experiments, the hatchlings, fed mysids, reached larger sizes
230 and survival were higher. These results support our hypothesis that mysids are a higher
231 quality food for the cultivation of the commercially important species that prey on mysids
232 in nature.
233 However, the preliminary results do not show a high production, which argues against
234 using the mysids for cultivation on a commercial level. It is clear that mysid cultivation
235 is more expensive and less productive than that of *Artemia* and rotifers. Nevertheless,
236 they may serve as food for ornamental fish or as supplementary food for cultures suffering
237 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 *et al.* 2007). In this situation, the mysids could complement other cheaper food since 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, *Sepia officinalis, Octopus vulgaris, Hippocampus* sp.

The study of lipids in wild mysids and in their natural food show 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 α-linoleic acid (18:3 n-3) was significantly higher ($P<0.05$) in culture than in the wild (Fig. 3 and 4; Table 1). *P. nouveli* also showed significant differences in the percentages of oleic acid (18:1n9) and arachidonic acid (20:4n6) ($P<0.05$). 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 suggest ways in which the diet could be modified to attain optimum lipid ratios in the mysids, themselves.

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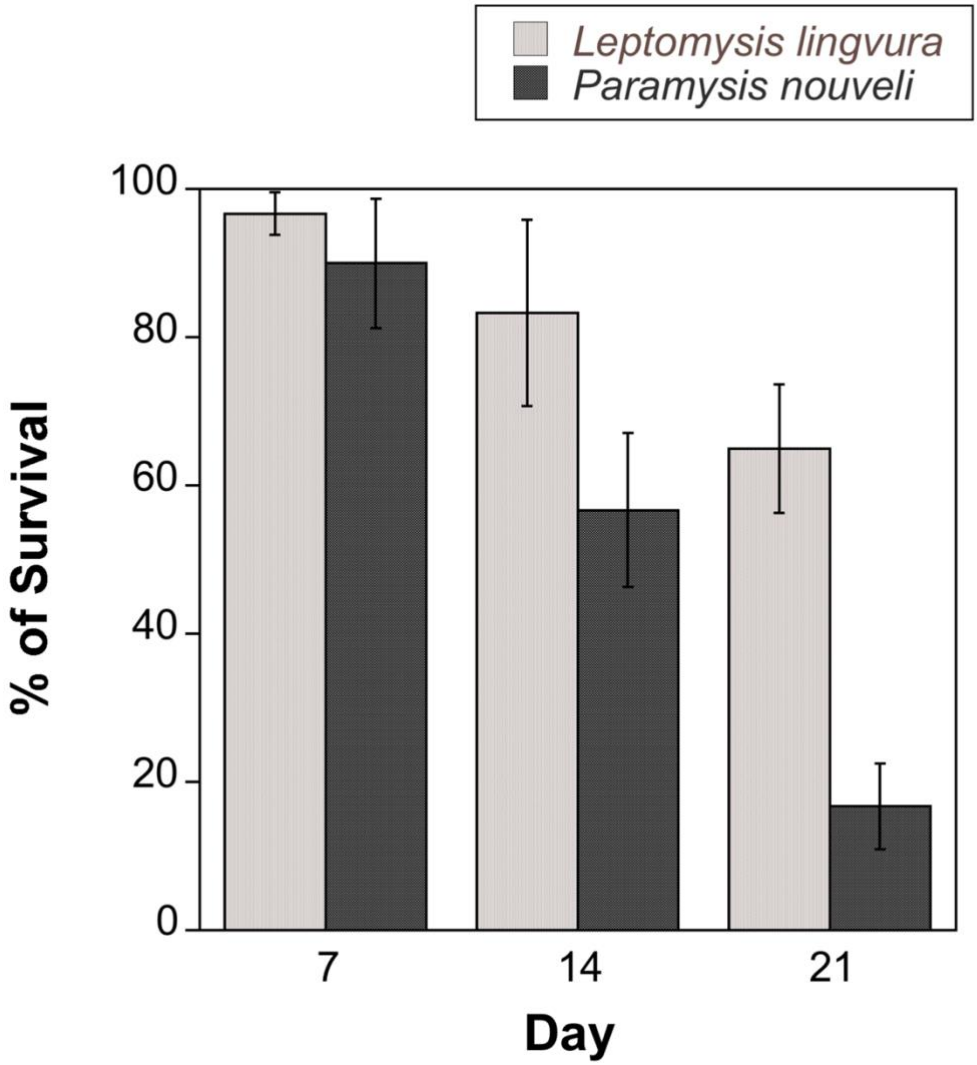
Figures legends

Figure 1 Survival in percentage of *L. lingvura* and *P. nouveli* at day 7, 14 and 21 of the experiment .

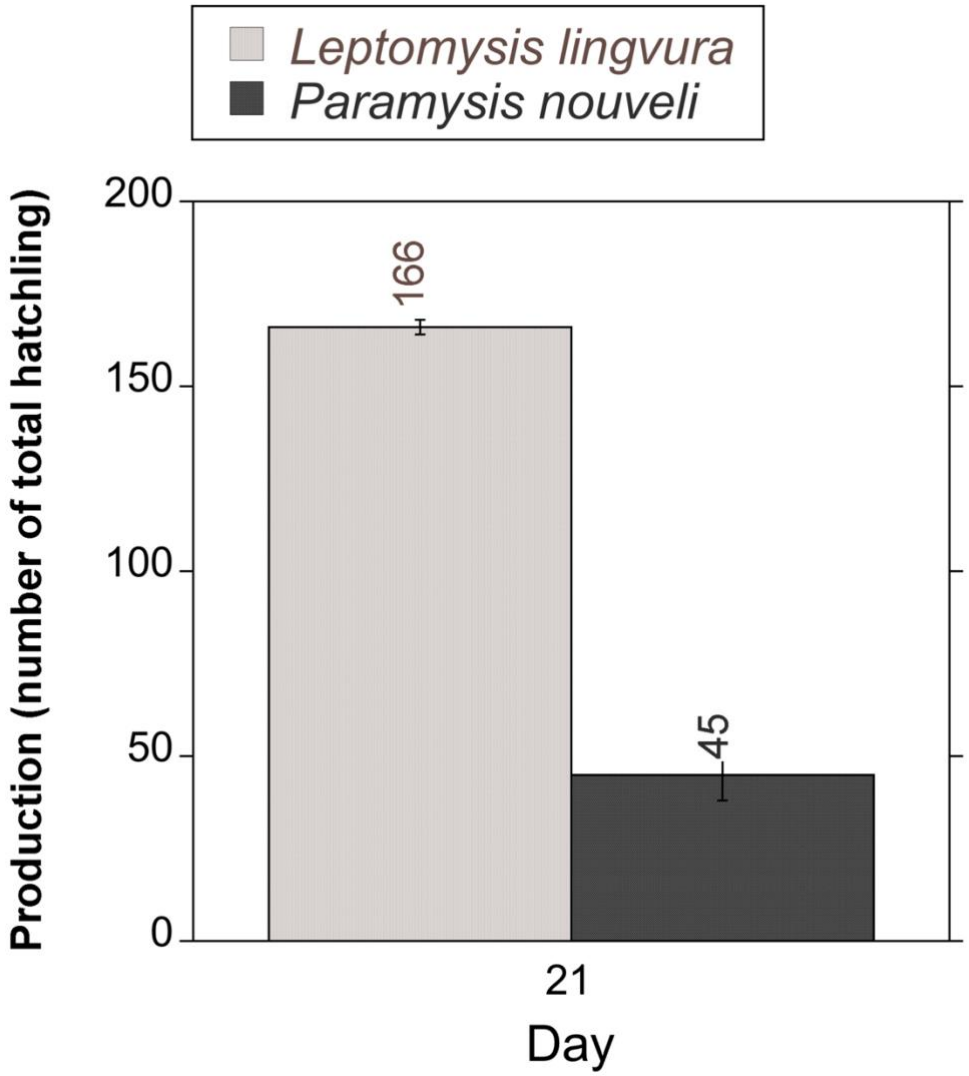
Figure 2 Total hatchling production of *L. lingvura* and *P. nouveli* at day 21 of the experiment .

Figure 3 The most abundant fatty acids as a percentage of total for wild *L. 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 20:4n-6 (AA).

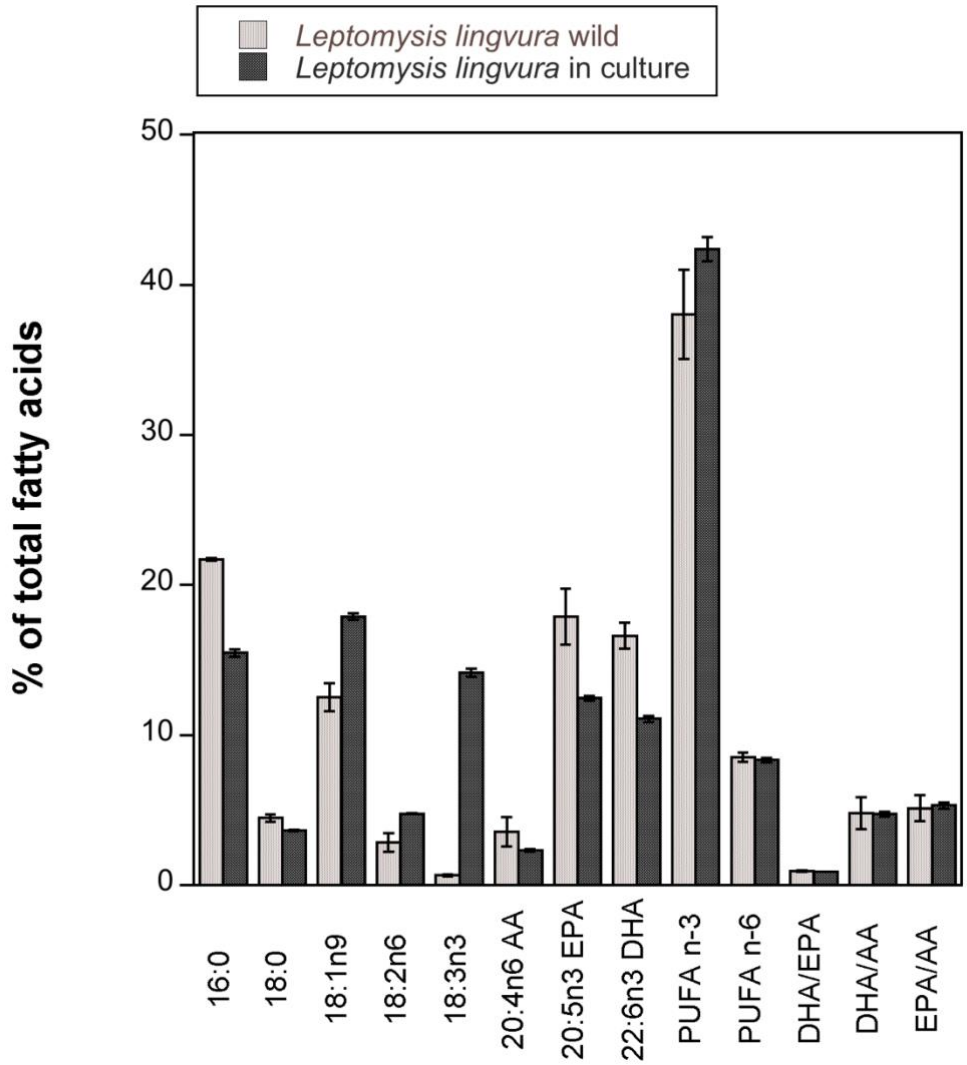
Figure 4 The most abundant fatty acids as a percentage of total for wild *P. nouveli* and for cultured P. nouveli fed for 7 days on *Artemia* nauplii enriched for 48 h with Easy–DHA, INVE, Belgium. The fatty acids are identifiable by the following key, 16:0 (Palmitic acid), 18:0 (Stearic acid), 18:1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


Leptomysis lingvura
Paramysis nouveli
100
80
60
40
20
0
% of Survival
7
14
21
Day

358

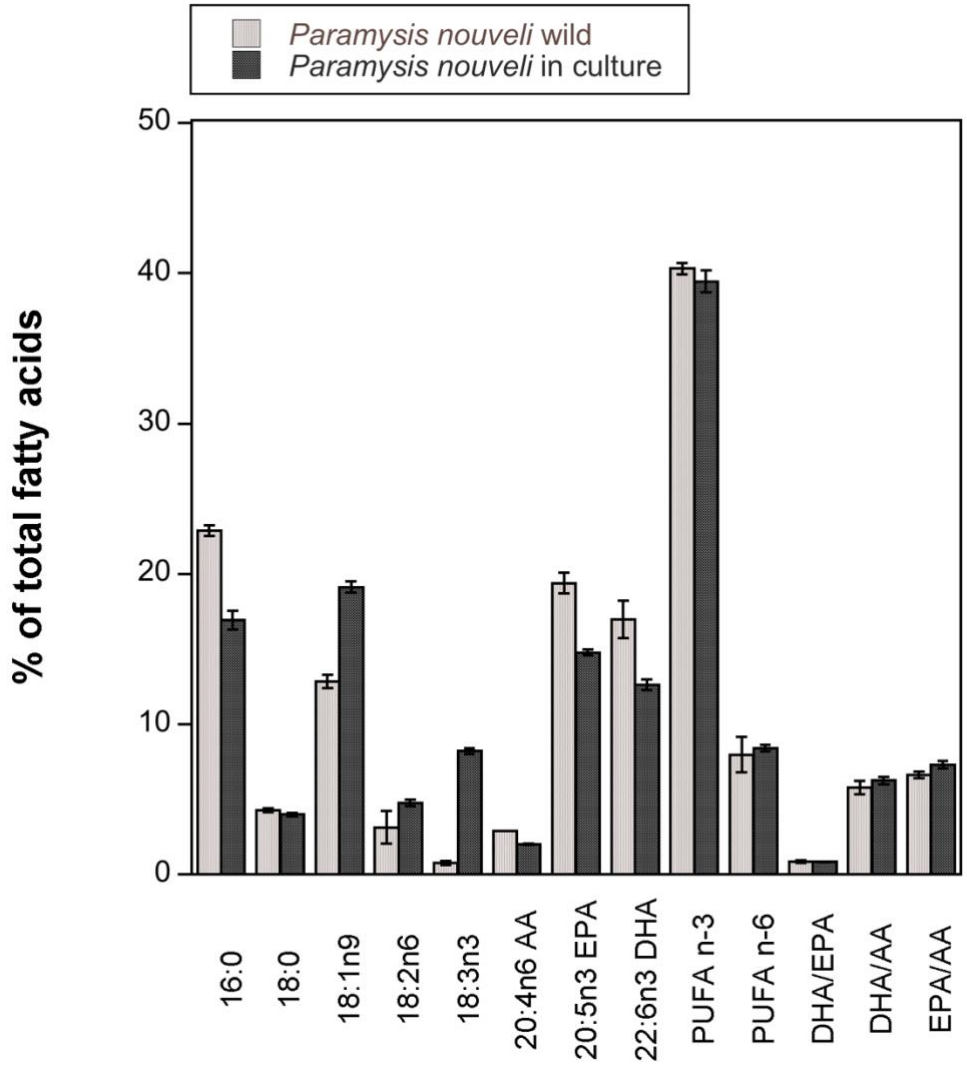

Leptomysis lingvura
Paramysis nouveli
Production (number of total hatchling)
200
150
100
50
0
166
45
21
Day

359


Leptomysis lingvura wild
Leptomysis lingvura in culture
% of total fatty acids
50
40
30
20
10
0
16:0
18:0
18:1n9
18:2n6
18:3n3
20:4n6 AA
20:5n3 EPA
22:6n3 DHA
PUFA n-3
PUFA n-6
DHA/EPA
DHA/AA
EPA/AA

360

361


Paramysis nouveli wild
Paramysis nouveli in culture
% of total fatty acids
50
40
30
20
10
0
16:0
18:0
18:1n9
18:2n6
18:3n3
20:4n6 AA
20:5n3 EPA
22:6n3 DHA
PUFA n-3
PUFA n-6
DHA/EPA
DHA/AA
EPA/AA

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† Fed *Artemia* nauplii enriched with Easy-DHA Selco®.

*Significant differences between wild and cultivated.

		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
18:1n9	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
18:2n6	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
18:3n3	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
20:5n3 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
22:6n3 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
20:4n6 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

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). Values (mean±SD).