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Abstract

The common octopus (*O. vulgaris*) is an important fishery species with potential for aquaculture industry. However, the lack of a standardized rearing methodology for the paralarvae stage prevents the commercial culture of this species.

The aim of this study was to evaluate the effects of tank volumes (100L and 500L) and alternative prey (*Grapsus adscensionis* zoeae, *Artemia* spp. *nauplii* and juveniles enriched with *Nannochloropsis* spp.) on the growth, survival and lipid composition of octopus paralarvae. The rearing was performed in a flow-through seawater system, at a density of 3 paralarvae/L, under a light regime of 200 lux and photoperiod 12L:12D. After 15 days of rearing, the diets and tank volumes used had a clear influence on growth and survival of paralarvae. In 100 L tanks, the best growth performance were obtained with *G. adscensionis* (Instantaneous Growth Rate, IGR, 5.97% BW/d), which promoted a survival of 28.67%, where individuals gained more than twice of its initial weight and were larger than paralarvae fed *Artemia* spp. ($P<0.05$). In 500 L tanks and with *G. adscensionis* as first prey, even better growth results were obtained. The size of paralarvae at the 16th day of culture was significantly larger (1.90 ± 0.09 mm Ventral Mantle Length, VML) than that obtained in 100 L tanks (1.76 ± 0.20 mm VML). The survival rates were 2.5% at day 77th and 0.1% after 160 rearing days. At the end of this experiment, (223 days) one benthic-stage octopus with a VML of 13.00 mm and wet weight of 2.17 g was obtained.

Determination of total lipid content (TL), lipid classes (LC) and fatty acid (FA) profiles were only possible on hatchlings and 15-day paralarvae reared in the 100 L tanks. In general terms, TL content increased after rearing. Newly hatched paralarvae showed dominance in total polar lipid content (62.05 ± 1.21%DW), while all 15-day paralarvae shifted its LC profile towards a dominant neutral lipid content (increased content in triacylglycerides and sterol esters).

Regarding the FA profile, hatchlings were rich in essential fatty acids (EFA), mainly 20:5 n-3 (EPA) and 22:6 n-3 (DHA), with DHA/EPA ratios of 1.43 ± 0.01, and relatively low contents in 20:4 n-6 (ARA). After 15 days of rearing, paralarvae fed *Artemia* spp. displayed no changes, while significant changes were observed in octopus fed *G. adscensionis*. The latter displayed a rise on levels of DHA and more specifically in ARA, which lead to a significant increase in total n-3 and n-6 HUFA. Increments in ARA made the EPA/ARA and DHA/ARA ratios to sharply decrease.

The present results showed that with increased tank volume (500 L) and *G. adscensionis* as first prey, the best growth results and significantly improved survival rates of paralarvae were obtained compared those for 100 L tank volumes.

Keywords: Tank volume; *Grapsus adscensionis*; Alternative prey; Nutritional composition; *Octopus vulgaris*; Paralarvae stage; Aquaculture.

Resumo

O polvo comum (*O. vulgaris*) é um recurso pesqueiro importante e com potencial para a aquicultura. No entanto, a falta de uma metodologia de cultivo para a fase paralarvar impede a cultura comercial desta espécie.

O objetivo deste estudo foi avaliar os efeitos de volumes de tanques (100L e 500L) e regime alimentar (*Grapsus adscensionis* zoea, *Artemia* spp. sob a forma de náuplios e juvenis enriquecidos com *Nannochloropsis* spp.) no crescimento, sobrevivência e composição lipídica de paralarvas de polvo. O cultivo foi realizado em sistema abeto de água do mar, a uma densidade de 3 paralarvas / L, sob um regime de luz de 200 lux e fotoperíodo 12L: 12D. Após 15 dias de cultivo, as dietas e os volumes de tanques usados tiveram uma clara influência sobre o crescimento e a sobrevivência das paralarvas. Em tanques de 100 L, os melhores resultados de desempenho de crescimento foram obtidos com *G. adscensionis* (taxa de crescimento instantâneo, IGR, 5,97% BW / d), que também promoveu uma sobrevivência de 28,67%, onde os indivíduos ganharam mais que o dobro de seu peso inicial e foram maiores do que as paralarvas alimentadas com *Artemia* spp. ($P < 0,05$). Em tanques de 500 L e com *G. adscensionis* como primeira presa, os resultados do crescimento obtidos foram ainda melhores. O tamanho da paralarva no 16º dia de cultura foi significativamente maior (1,90 ± 0,09 mm de comprimento do manto ventral, VML) do que o obtida em tanques de 100 L (1,76 ± 0,20 mm VML). As taxas de sobrevivência foram de 2,5% no dia 77 e 0,1% após 160 dias de criação. No final desta experiência, obteu-se um polvo na fase bêntica, com um VML de 13,00 mm e um peso húmido de 2,17 g.

A determinação do teor de lipído total (TL), classes lípidicas (LC) e perfis de ácidos gordos (FA) só foram possíveis em recém-nascidos e paralarvas de 15 dias cultivados em tanques de 100 L. Em termos gerais, o conteúdo de TL aumentou após o cultivo. As paralarvas recém-eclodidas mostraram uma predominância no teor de lipídos polares totais (62,05 ± 1,21% DW), enquanto que todas as paralarvas com 15 dias mudaram o seu perfil de LC para uma predominância no teor de lipídos neutros (aumento em triacilglicerídeos e ésteres de esteróis).

Em relação ao perfil de FA, os animais recém-eclodidos eram ricos em FA essenciais, principalmente 20:5 n-3 (EPA) e 22:6 n-3 (DHA), com racio DHA / EPA de 1,43 ± 0,01, e relativamente baixo teor de 20 : 4 n-6 (ARA). Após 15 dias de cultivo, as paralarvas alimentadas com *Artemia* spp. não apresentaram mudanças, enquanto que alterações significativas foram observadas nos polvos alimentados com *G. adscensionis*. Estas últimas exibiram um incremento nos níveis de DHA e mais especificamente em ARA, que por seu lado levaram a um aumento significativo no conteúdo total de n-6 e n-3 HUFA. Por outro lado, os rácios de EPA / ARA e DHA / ARA foram dramaticamente reduzidos.

Os resultados obtidos demonstram que um incremento do volume do tanque (500 L) e o uso de *G. adscensionis* como primeira presa promoveram o melhor crescimento e sobrevivência das paralarvas em contraste com volumes de 100 L.

Palavras-chave: Volume do tanque; *Grapsus adscensionis*; Vítima alternativa; Composição nutricional; *Octopus vulgaris*; Fase paralarvae; Aquicultura.

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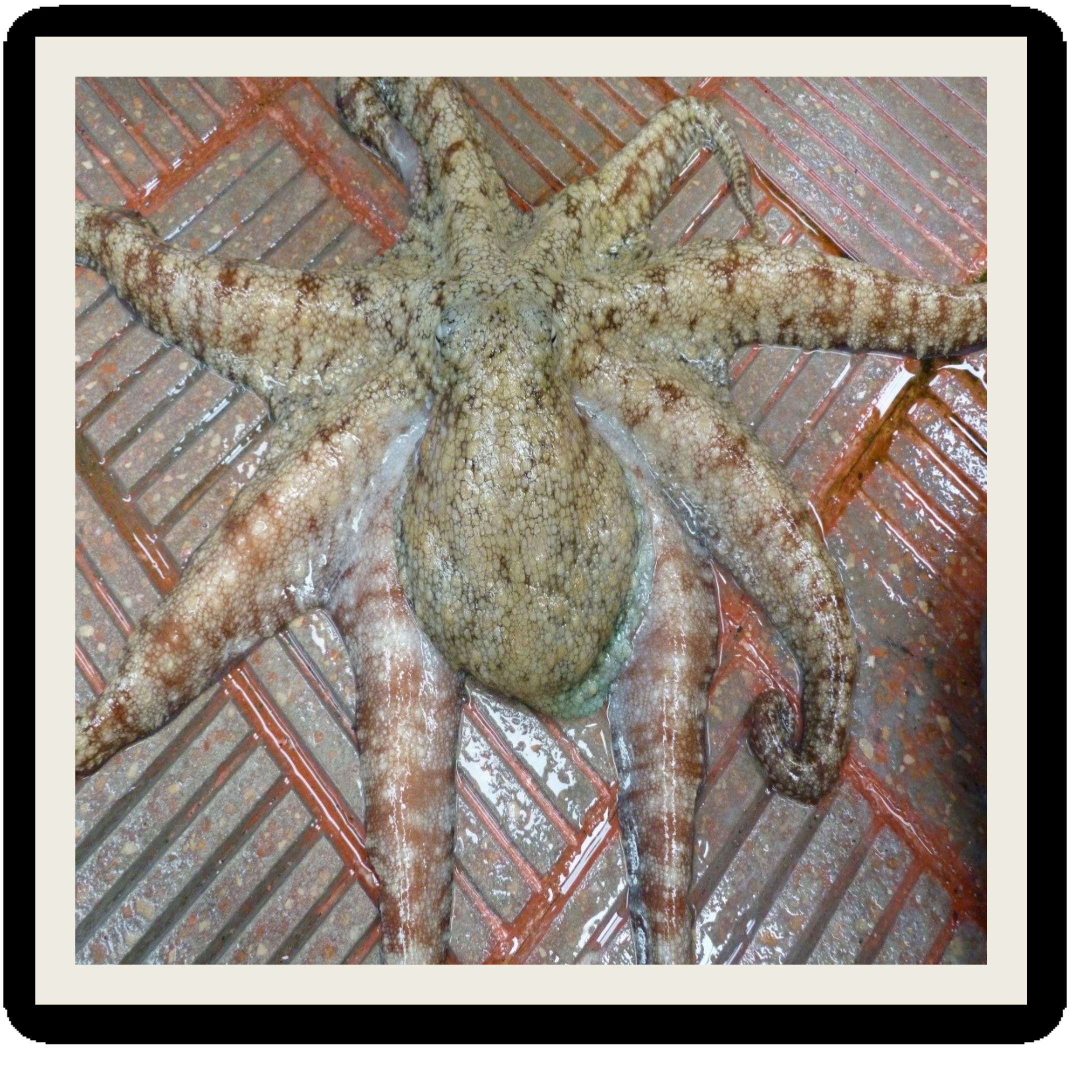
I. INTRODUCTION



1.1. State of the world cephalopod fisheries

With the increasing exploitation of finfish resources, many coastal areas are becoming fished close to or even beyond the level of maximum sustainable yield. In the search for resources that can support the demand of constantly growing human population, attention was paid to cephalopods because of their abundance and their undeniable nutritional qualities.

Historically, the consumption of cephalopod products has been highest in the countries of South - East Asia. Countries such as Japan, Korea, Thailand, Taiwan and China have been most prominent in the sale and trade of fished cephalopods and the development of specialized fishing methods, notably the use of jigging machines with high-intensity lights (Boyle & Rodhouse, 2005). Large-scale cephalopod fisheries of the world have developed since 1960, when Japan expanded its fishing effort worldwide and 83% of the world cephalopod catches were made by Japanese boats (Caddy, 1983). Their exploitation has steadily increased in significance since then (Fig. 1).

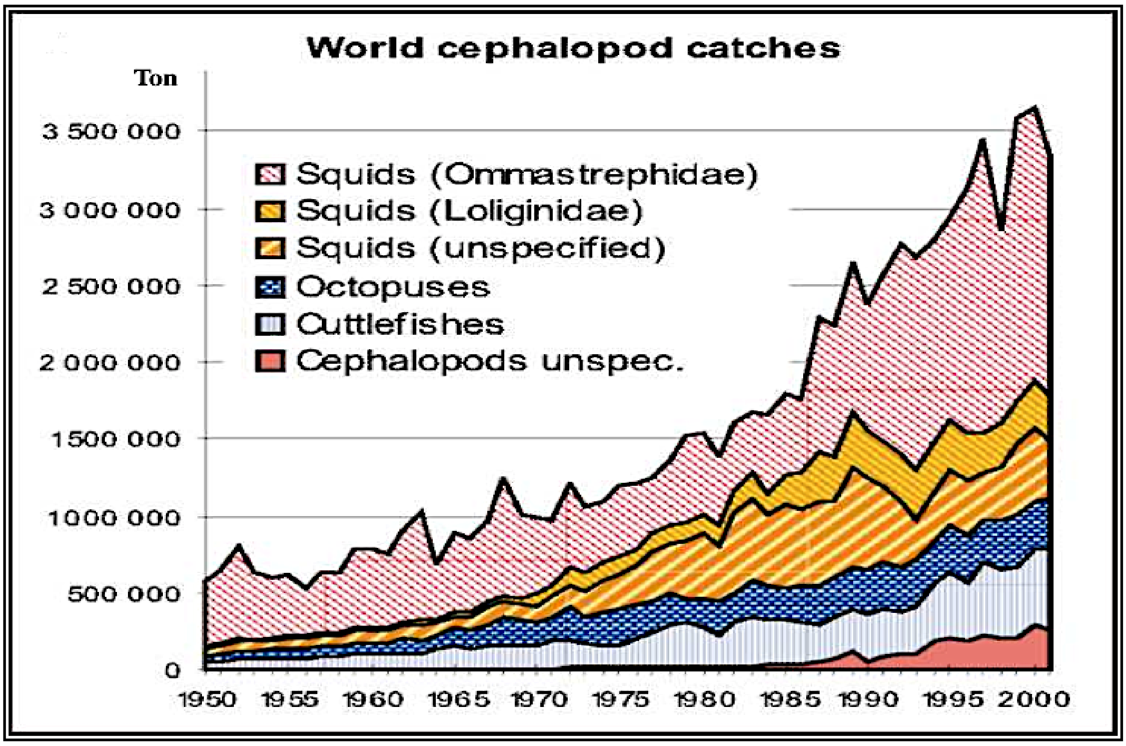


Figure 1 World cephalopod catches (Jereb & Roper, 2005).

Since the early 60's, the world cephalopods catch has increased from around 0.5 million tons to more than 1.3 million in 1980 (Jereb & Roper, 2005). Between 1990 and

1999, the total world annual catch of cephalopods increased from 2.4 to 3.4 million tons per year (Boyle & Rodhouse, 2005), and to almost 4 million tons in 2004 (FAO, 2010). Cephalopod catches reached a peak in 2006 at 4.3 million tones (FAO, 2008) and, by 2008, the total annual catches of cephalopod remained relatively stable at about 3.6 – 3.8 million tons (FAO, 2010).

In European waters, cephalopods have always been considered as a minor fishery resource. Once located mostly in southern Europe, cephalopod fisheries have grown since the 1980's (Pierce et al., 2010). At the present, the main importers of cephalopod products are Spain, Italy, Greece, Portugal and France; which account for 49.6% of the world total. Spain has been the leading European importer (by tonnage) in the world since 1997. The highest import values however, remain those spent by Japan, mainly for frozen *Octopus* spp., and reached US $ 812.8 million in 2001 (Jereb & Roper, 2005).

Among all cephalopods, substantial increases have been observed in landings of common octopus (*Octopus vulgaris*; Figure 2). Catches have increased steadily since 1950 and subsequently climbed to over 100 000 tons in the 1980s, and declined by 2000. Landings of just below 40 000 tons were recorded in 2007 (FAO, 2010).

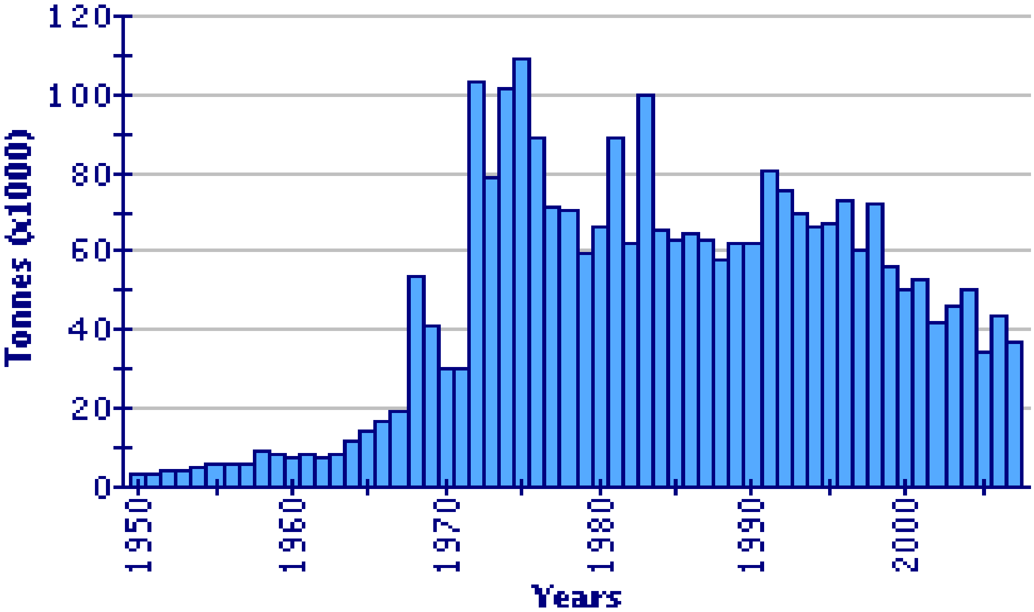


Figure 2 Global capture production for *O. vulgaris* (FAO, Fishery Statistic, 2010).

1.2. Biology and aquaculture state of *O. vulgaris*

It is clear that fishing cannot supply the growing demand for marine products as the natural stocks are overexploited, thus aquaculture as a method of producing food take place. Cephalopods were introduced recently to aquaculture and, among cultivated species, the common octopus (*Octopus vulgaris*) received more attention as one of the emerging new species (Vaz-Pires et al., 2004). It meets many of the criteria for intensive aquaculture, such as: short life cycle and fast growth (Sanchez et al., 1998), readily adaptation to captivity conditions and the species is able to sustain at high densities with minimum disease problems (Boyle & Rodhouse, 2005), good acceptability of frozen food, high feed efficiency and reproductive rate (Mangold, 1983). Some criteria such as, high market price and high nutritional value make this species an excellent candidate for aquaculture but, despite the various attempts to rear the early planktonic life stage of *O. vulgaris*, the culture of this cephalopod species is limited to ongrowing sub-adults individuals captured from the wild (Prato et al., 2010).

O. vulgaris belongs to Class Cephalopoda, Order Octopoda, Family Octopodidae and Genus Octopus. This species has worldwide distribution in temperate and tropical waters of coastal and shelf zones (Fig. 3).

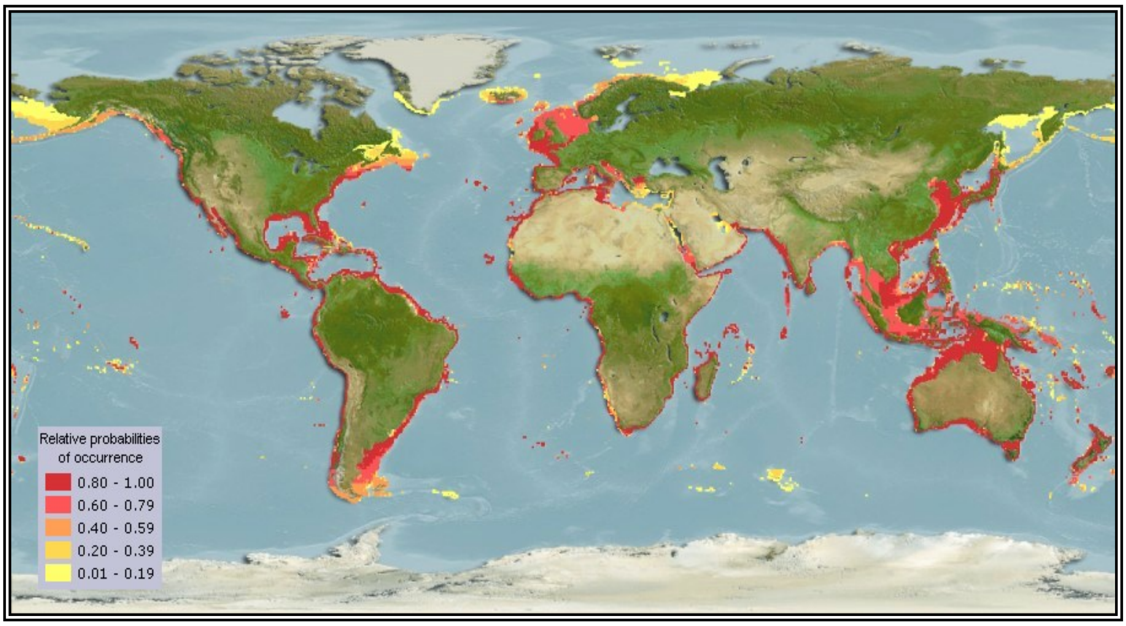


Figure 3 Map of *O. vulgaris* distribution (Source: AquaMaps)

It is a benthic, versatile opportunistic predator with preference for crustaceans. *O. vulgaris* females brood the eggs, keeping them clean and ventilating them during embryonic development to hatching, and dies shortly after the eggs have been brooded. Hatching larvae, named "paralarvae" by Young & Harman (1988), with wet weigh about 1.4 mg and have a temperature dependable planktonic phase lasting 33–54 days (27–21°C) or even up to 3 months at lower temperatures. When juveniles have reached about 200 mg, they become benthic and settlement to seabed occurs (Boyle & Rodhouse, 2005).

Big attention was recently paid to rear the paralarvae of *O. vulgaris*. Nonetheless, a standardized system for paralarval rearing is not developed so far. According to Iglesias et al. (2007), different technologies with respect to tank color, size and shape; larval and prey densities; and environmental factors (light, water flow, temperature etc.,) were investigated by different research groups, but no methodology was established until now, although, all of them could influence survival. Apart from that, the high mortality rates and the poor paralarval growth resulting from nutritional aspects are seen as the main constraint in this species aquaculture development (Navarro & Villanueva, 2000). Therefore, the question of resolving this problem has been set as of high priority.

Decapod crustacean larvae are probably one of the main natural preys of planktonic *O. vulgaris* paralarvae and most of the successful long-term laboratory rearing was obtained by using it as the primary prey (Villanueva & Norman, 2008). Alvaro et al. (2012) used a PCR-based method with group-specific primers to identify prey consumed by *O. vulgaris* paralarvae. The detected prey consisted mainly of crustaceans - 97.4% of the clones detected, from which prawns (37.1%), crabs (37.1%) and krill (19.8%). The remaining 2.6% corresponded to fishes. The first attempt to culture *O. vulgaris* from paralarvae was carried out in Japan, where Itami et al. (1963) succeeded in rearing hatched octopus paralarvae up to benthic juveniles, feeding them on larvae of Palaemon serrifer. These authors obtained 8% survival after 40 days of rearing. In Europe and more recently, Villanueva et al. (1994, 1995) were able to obtain benthic juveniles of this species, with a survival of 9% at 47 days, using *Liocarcinus depurator* and *Pagurus prideaux* decapod crab zoeae as a prey.

For the first time, the closing of a completed life cycle of *O. vulgaris* under culture conditions was achieved by Iglesias et al. (2002) and, subsequently, by Carrasco et al. (2003, 2005). *Artemia* spp. and larval stages of crustaceans Palaemon serrifer, *Maja brachydactyla*, and *Pagurus prideaux* were used as prey for both experiments; although, survival rates were very low in general.

Together with different diets being tested, the rearing methodology also differs considerably. Different volumes of rearing tanks from 25 L to 6000 L (Vidal et al., 2002; Villanueva et al., 2004; Okumura et al., 2005; De Wolf et al., 2011) were tested with paralarval densities from one up to 25 paralarvae per L (Villanueva et al., 2004; Carrasco et al., 2003, 2005; De Wolf et al., 2011). The higher survival rates were attained in tank volumes with more than 100 L and lower paralarval densities, and the best results in this sense were obtained from 1 to 3 paralarvae per L (Iglesias et al., 2002; De Wolf et al., 2011). According to De Wolf et al. (2011) these results could be explained by the fact that the physical variations, such as fluctuations in water temperature, salinity, pH etc., are less expressed in larger volumes and lower paralarvae stocking densities reduce competition for space and prey captures.

1.3. Prey

O. vulgaris paralarvae start to feed during the first 24 h after hatching (Villanueva et al., 2002; Morote et al., 2005; Iglesias et al., 2006) and as they have external digestion they can capture prey of their own size using their well-developed arms and suckers (Villanueva & Norman, 2008). Therefore, prey density, its size and distribution within the tanks could influence the rearing and nutritional aspects of octopus paralarvae (Villanueva & Norman, 2008). Young cephalopods are very selective of the prey they will take, with relative size being an important criterion. Villanueva, (1994) defined that prey size for octopus paralarvae should represent 50-100% of mantle length. On the other hand, the effect of different prey densities also needs to be studied in more detail. The most successful studies used decapod crustacean zoeae ranged from 0.01 to 1 zoeae per mL (Iglesias et al., 2002; Carrasco et al., 2003, 2005).

Together with prey size and quantity, there is another question associated with the prey quality. *O. vulgaris*, as all cephalopods, have a predominant amino acid metabolism (Lee, 1994; Villanueva, 2004) but, recently, more attention has been paid to their lipid and fatty acids profile (Navarro & Villanueva, 2000; Miliou et al., 2006, 2007; Prato et al., 2010; Reis et al., 2011). Based on this studies, it was defined that octopus paralarvae require a diet with high content in phospholipids, cholesterol and, especially, highly unsaturated fatty acids (HUFA). HUFA such as eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (ARA, 20:4n-6) are known as the precursors of eicosanoids, which have a wide range of physiological actions, such as, assisting in blood clotting, the immune response, the inflammatory response, cardiovascular tone, renal function, neural function, and reproduction (Sargent et al., 1995). The most expressive HUFAs found in cephalopods are docosahexaenoic acid (DHA, 22:6n-3) and EPA, and its amount in paralarvae are strongly dependent on the fatty acids composition of their prey (Navarro and Villanueva, 2000, 2003; Okumura et al., 2005). In order to find a suitable diet for paralarvae, enriched *Artemia* spp. and different crustacean zoeae are by far the main food items used by far in laboratory experiments.

The use of *Artemia* spp. has undoubtfully benefits due to its availability and large-scale production, and the possibility of manipulating its nutritional content. However, the poor growth and mortality of paralarvae fed with *Artemia* seem to be primarily related to its nutritional quality. Having a low HUFA content, where the amount of EPA is low and DHA basically absent (Berger, 2010) make *Artemia* an unsuitable diet for octopus paralarvae. Only Hamazaki et al., (1991) achieved 28.9% of survival at day 25 of *O. vulgaris* juveniles using *Artemia* spp. enriched with *Nannochloropsis* spp. as sole prey. In order to enrich the HUFA content of *Artemia*, Seixas et al. (2008) suggested improvement with enrichment of selected microalgae. However, due to the low DHA content results, these enrichments revealed to be far from adequate to achieve the correct FA composition that cephalopod diets should require.

Decapod crustacean zoeae from *Liocarcinus depurator*, Palaemon serrifer, *Pagurus prideaux* and *Maja brachydactyla* were used successfully as alternative prey in most of the long-term laboratory rearing (Itami et al., 1963; Villanueva, 1994, 1995; Shiraki, 1997; Carrasco et al., 2003, 2005; Iglesias et al., 2004). The biochemical composition of these crustaceans is characterized by a high phospholipid content, lack of triglycerides and high percentage of HUFA, specifically DHA and ARA (Navarro & Villanueva, 2000). Arachidonic acid is the primary precursor of eicosanoids in fish and mammals and an essential dietary fatty acid for marine fish (Tocher, 2003). Although, the role of ARA in cephalopods has not been studied sufficiently, Almansa et al., (2006) and Miliou et al., (2006) suggested that ARA may play a similar important role in the maintenance of cell membrane and function, and increased level of this fatty acid is associated with an improvement of growth in cephalopods as in fish.

These characteristics seem to explain the lipid requirements of octopus, and suggest shrimp and crab zoeae to be very valuable during the first period of paralarval feeding on live feed (Iglesias et al., 2007). In fact, laboratory experiments using these prey obtained the best growth and survival rates during the first half of *O. vulgaris* planktonic life (Itami et al., 1963; Villanueva, 1995; Carrasco et al., 2003, 2005; Iglesias et al., 2004). In the work of Reis (2011) the higher survival rate was obtained with decapod crab zoeae *Grapsus adscensionis* as a first prey and, to continue progress on those results, the present experiments were performed.

1. 4. Objectives.

The main purpose of the present study was to evaluate the effect of tank volume on growth and survival of paralarvae fed with different alternative prey (*Grapsus adscensionis* zoeae, *Artemia nauplii* and *Artemia* juveniles enriched with *Nannochloropsis* spp.) and on the resultant paralarval lipid composition.

II. MATERIAL AND METHODS

Two experiments were set up in order to evaluate the effects of different diets and tank volume on growth performance, survival and lipid composition of octopus paralarvae. Different volumes of 100 L and 500 L culture tanks were tested at the IEO (Instituto Espanol de Oceanografía – Tenerife, Canary Islands, Spain). For each experiment paralarvae from different broodstocks were used.

2.1. Effects of alternative prey and 100 L tank volume on *O. vulgaris* paralarvae

In this experiment, three different diets were tested in 100L tanks: *Artemia* spp. juveniles, *Artemia* spp. *nauplii* (EG Type, INVE AQUACULTURE, Belgium) and *G. adscensionis* zoeae. The experiment lasted for 15 days, from 1st to 15th of June 2011.

For this experiment, paralarvae hatched in the same day from one egg batch were used. They were siphoned from the broodstock tank and transferred with a jar to the 18 trial tanks (6 replicates per each diet) at a density of 3 paralarvae/L (300 paralarvae per tank).

2.1.1. *O. vulgaris* broodstock

The capture of wild *O. vulgaris* broodstock individuals was performed by professional artisanal fishermen on the Tenerife island coast (Canary Islands, Spain). After being caught, the 18 broodstock individuals were kept in six 1000 L circular fiberglass tanks, where individuals of similar weight were placed with a sex ratio of 2 females per male (2:1). Sex determination was performed by verifying the existence of the hectocotilized arm in males. The tanks were maintained in natural photoperiod (from 10L:14D to 11L:13D hours of light and dark), with a mean water temperature of 20.69 ± 1.04ºC and salinity of 36.8 ± 0.14 ppm. 50% of each tank surface was covered with a shady net. The tanks were part of a flow through seawater system with a flow of 6 L/minute which entered the tank by the top of the water column and exited through a filter mesh (1 cm) located on the bottom. A mix of frozen squid (Loligo opalescens), mussels (Mytilus edulis) and prawns (Parapenaeus longirostris) were fed to the ongrowing adults ad libitum. PVC pipes and clay pots were placed inside the tanks to provide dens.

The presence of eggs was verified once a week to avoid disturbing the breeders. When an egg mass was observed, the remaining individuals were removed and placed in a different tank, leaving the ovate female alone with the egg mass. When paralarvae were detected, the tank filter was changed to a 363 μm mesh and 0 days paralarvae (hatchlings) were removed to the experimental tanks.

2.1.2. Experimental design

100 L fiberglass cylinder-conical tanks (Fig. 4), with black walls and white bottom, were tested for paralarvae rearing in a flow-through seawater system.

Figure 4 100 L fiberglass tanks used for *O. vulgaris* paralarvae rearing.

Water quality of the culture system was promoted through the use of a filtration system, consisting of three inline mesh filters (with a porosity of 20, 5 and 1 μm) and of an UV filter, prior it entering each tank. Water was supplied through the top of the tanks, with a flow of 70 mL/min, ensuring at least 100% water renovation per day. Dissolved oxygen was provided through the use of moderate aeration (by one porous plastic aeration stone - 3 cm in length), placed in the lateral side of tanks. The tanks were under a light regime of 200 lux of intensity and a photoperiod of 12L:12D. The green water technique was used by adding 2 L of 200000 cells/ml of Chlorella spp. per day. In order to reduce the stress of paralarvae, cleaning of the tanks was not performed.

Water temperature and dissolved oxygen were daily measured with a DO METER PRO ODO device. Ammonia (NH3), nitrites (NO2) and pH were measured every 6 days with a TETRA test aquarium kits for NH3 and NO2 and with a Hanna-HI-98107 pH Metter (Table 1).

Table 1. Physicochemical parameters of the rearing water used during the experiment.

Parameters/diets	**G. adscensionis (zoeae)**	**Artemia spp. nauplii**	***Artemia* spp. juveniles**
Temperature (°C)	21.76 ± 0.39	21.78 ± 0.37	21.83 ± 0.37
Oxygen (%)	100.90 ± 1.08^{b}	101.39 ± 0.90^{a}	101.71 ± 0.89^{a}
Salinity (‰)	36.8 ± 0.14	36.8 ± 0.14	36.8 ± 0.14
pH (ppm)	7.93 ± 0.06	7.93 ± 0.06	7.93 ± 0.06
NH_3 (ppm)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
NO_2 (ppm)	< 0.30 ± 0.00	< 0.30 ± 0.00	< 0.30 ± 0.00

Different superscript letters within the same row indicate statistical differences ($P<0.05$).

2.1.3. Diets

Three different crustacean diets were used to feed the Octopus paralarvae in 6 replicate tanks per diet:

- Paralarvae fed crab *G. adscensionis* zoeae, further defined as P-GR;
- Paralarvae fed *Artemia* spp. *nauplii*, defined as P-AN;
- Paralarvae fed enriched *Artemia* spp. juveniles, defined as P-AJ.

2.1.3.1. *Artemia* spp. production protocol

Artemia* spp. *nauplii

Artemia spp. cysts (EG Type, INVE AQUACULTURE, Belgium) were placed in a hatching tank and maintained at 28°C in seawater (salinity of 36 ‰), under strong aeration and with a light intensity of 2000 lux, according to the protocol described by Sorgeloos (1986). After 24 hours, newly hatched *nauplii* were siphoned and placed in an enrichment tank, with a density of 350 000 *nauplii* per tank. The enrichment 100 L cylinder-conical fiberglass tanks (Fig. 5) were filled with 50 L of seawater and 2 g of lyophilized powder of *Nannochloropsis* spp. After 24 hours of enrichment, *nauplii* were filtered from the tank to a jar, counted and divided between paralarvae tanks to be fed *Artemia nauplii*.

Figure 5. 100 L cylinder-conical fiberglass tanks used for *Artemia* spp. culture.

***Artemia* spp. juveniles**

Newly hatched *nauplii* were also placed in similar 100 L cylinder-conical fiberglass tanks for growth, at a density of 10 *nauplii*/mL (105 *nauplii* per tank), water temperature of 22°C, salinity of 36 ‰, under strong aeration and a photoperiod of 12L:12D. Every day, 10% of the tank volume was flushed and refilled with clean seawater together with 2 g of lyophilized powder of Tetraselmis chuii. After 7 days, *Artemia* was enriched for 24 hours with *Nannochloropsis* spp., filtered from the tank, counted and divided between paralarvae tanks to be fed *Artemia* juveniles.

2.1.3.2. *G. adscensionis* zoeae production

The *G. adscensionis* adult broodstock was captured in Tajao (28º06´N/16º28´W SE Tenerife, Canary Islands, Spain) and in Tacoronte coast (28º30´N/16º25´W N Tenerife) at night, with new moon conditions and during low tide. Forty-eight crabs were caught on the 11th March 2011 and another 74 crabs on the 19th and 25th May 2011. Crabs were transported, in 30 L containers and without water, to the culture facilities of the Spanish Institute of Oceanography (IEO - Centro Oceanográfico de Canarias).

All *G. adscensionis* individuals were reared in 3000 L fibre glass cylinder tanks, under a natural photoperiod 12L:12D and 21.8 ± 1.2°C. Water column level was low (~20 cm) and water flow was 6 L/min. Some stones, net boxes and PVC tubes were placed in the tanks to act as shelters and diminish territorial competition, allowing crabs to stay out of the water. Animals were daily fed ad libitum on a diet of frozen mackerel and squid.

In order to determine if the broodstock offspring production was enough to cover the prey requirements of the experiments, individual fecundity and periodicity egg batch production of female crabs were studied. Twelve females bearing eggs, with a mean size of 54 ± 6.1 mm carapace width (CW) and mean weight of 69.8 ± 23.59 g were used to gather the required data. Egg sampling was accomplished by, firstly, anaesthetizing females in cold seawater (-1°C) for one minute. Secondly, eggs were carefully removed by scrapping the pleopods with tweezers and a scalpel, but taking care not to cause any injury to the animals (Fig. 6). Reproductive investment (RI) was calculated as eggs wet weight/female wet weight x 100.

Individual fecundity (eggs/female) was determined by counting the amount of eggs present in 10 mg of egg mass and extrapolating this data for the whole egg mass of each female. The mean fecundity weight (eggs/female weight) was estimated according to weight of female and expressed as the amount of eggs per g of female mass.

Figure 6. Anesthetized *G. adscensionis* ovate female just before egg removal.

The number of zoeae supplied to paralarvae varied according to broodstock offspring production. Zoeae were filtered through 365 µm mesh from the crab broodstock tank every day. To count the zoeae amount they were concentrated in a 4 L jar supplied with strong aeration for equal distribution, then 100 ml were taken 5 times and zoeae counted and volumetrically estimated. This procedure was repeated 5 times, being the total amount on volume of 4 L jar was in this way estimated. Excess zoeae were placed into a cylinder 1000 L fiberglass tank and maintained with rotifers (6 rot/mL/day). This tank had water temperature of 22°C, salinity of 36 ‰, dissolved oxygen above 100% saturation, 100% water renewal per day, and a photoperiod of 12L:12D. When needed, these zoeae were used to complete the next day paralarval feeding.

2.1.3.3. Paralarvae feeding tables

Paralarvae were fed once a day, an initial quantity of *Artemia* spp. *nauplii* adjusted as 0.15 *nauplii*/ml or 0.06 juveniles/ml. The initial number of zoeae was 0.2 zoeae/ml. After that, the amount of prey was daily corrected by determining the remaining prey density, according to the following scale:

0 – no prey remained in the tank – density was increased;

1 – some prey remaining in the tank – provided the same density as before;

2 – a lot of prey remaining in the tank – no prey was added.

The determination of prey remains was made prior to each new feeding by visual observation. The amount of added zoeae depended on amount of zoeae produced by females in a given day. Tables 2 provide the information on the average number of prey used throughout the whole experiment for *G. adscensionis* zoeae, Table 3 for *Artemia nauplii* and Table 4 for *Artemia* juveniles.

Table 2. Number of *G. adscensionis* zoeae provided to *O. vulgaris* paralarvae each day of rearing.

G. adscensionis						
Days of rearing	Tanks №					
	2	7	11	15	20	24
1	15000	15000	15000	15000	15000	15000
2	8300	8300	8300	8300	8300	8300
3	2250	2250	2250	2250	2250	2250
4	8200	8200	8200	8200	8200	8200
5	9500	9500	9500	9500	9500	9500
6	12000	12000	12000	12000	12000	12000
7	2200	2200	2200	2200	2200	2200
8	2500	2500	2500	2500	2500	2500
9	5000	5000	5000	5000	5000	5000
10	7000	—	7000	7000	7000	7000
11	10000	10000	10000	10000	10000	10000
12	3500	3500	3500	3500	3500	3500
13	6300	—	—	—	6300	6300
14	5600	5600	5600	5600	5600	5600
Average	6978 ± 3815					

Table 3. Number of *Artemia* spp. *nauplii* provided to *O. vulgaris* paralarvae each day of rearing.

Artemia* spp. *nauplii						
Days of rearing	Tanks №					
	3	5	9	13	17	19
1	13300	13300	13300	13300	13300	13300
2	—	—	—	—	—	—
3	5000	5000	5000	5000	5000	5000
4	—	—	—	—	—	—
5	—	—	—	—	—	—
6	—	—	—	—	5000	—
7	5000	5000	5000	5000	—	5000
8	2500	8000	8000	—	5000	—
9	3000	8000	8000	5000	8000	5000
10	—	8000	—	—	8000	—
11	5000	8000	5000	5000	5000	5000
12	—	—	5000	—	—	—
13	—	—	—	—	—	—
14	5000	8000	5000	—	8000	5000
Average	6793 ± 3039					

Table 4. Number of *Artemia* spp. juveniles provided to *O. vulgaris* paralarvae each day of rearing.

***Artemia* juveniles**						
Days of culture	Tanks №					
	1	4	10	18	21	23
1	5800	5800	5800	5800	5800	5800
2	6000	6000	8000	6000	6000	6000
3	—	—	—	—	5000	8000
4	5000	5000	5000	5000	5000	5000
5	5000	5000	5000	5000	5000	5000
6	—	—	8000	—	5000	—
7	5000	8000	8000	5000	5000	5000
8	8000	8000	8000	5000	5000	5000
9	—	—	10000	—	8000	—
10	—	8000	—	—	—	—
11	5000	—	—	5000	5000	5000
12	—	5000	5000	—	5000	—
13	—	8000	8000	5000	8000	5000
14	—	—	—	—	—	—
Average	5978 ± 1349					

2.1.4. Growth and survival

Thirty newly hatched paralarvae were taken from the broodstock tank at the first day of the experiment for determination of ventral mantle length (VML) and another 60 paralarvae for the dry weight.

At the end of the experiment (day 15), 10 paralarvae from each tank were measured and used for dry weight estimation. In those tanks where survival was less than 10 paralarvae, all of them were used to this purpose.

Paralarvae were anaesthetized with MgCl2, which was prepared by dissolving 8.1 mg of MgCl2 in 100 ml of filtered seawater. Before using, 50% of this solution was mixed with 50% of seawater. One by one, paralarvae were placed in a jar with the solution and removed when they stopped moving. The size of mantle length (ML) was measured under a magnifying glass (Nikon SMZ-10A – 4x magnification). For recovery, paralarvae were placed back in a plastic flask containing seawater.

These paralarvae were then used to calculate dry weight. Individuals were sacrificed in ice-cold seawater at - 2°C. After, they were kept in distilled water for 20 min to remove salt from the tissues. Samples were then placed on Whatman GF/C glass fiber filters, washed 3 times with distilled water and left for 24 hours under 110°C in an oven (SELECTA, DIGITHEAT), until constant weight was obtained. Collected data was used to calculate: (1) mean dry weight (MDW – mg); (2) mean instantaneous growth rate (IGR - %BW day^{-1}) = (LnW2 – LnW1)/t*100 where, W1 and W2 are the initial and final mean dry weight of each diet treatment, respectively, Ln is the natural logarithm and t the number of days of the experiment (Sykes et al., 2010); (3) mean absolute growth rate (AGR – mg/day^{-1}) = (W2 – W1)/t where, W1 and W2 are the initial and final mean dry weight of each diet treatment, and t is the number of days of the experiment (Miliou et al., 2006).

Survival was accounted at the end of the experiment. Alive paralarvae were counted in each tank, and the mean survival rate (S-%) was estimated as S% = (Nf/Ni)*100, where Ni and Nf are the initial and final number of paralarvae of each diet treatment.

2.1.5. Lipid analysis

Octopus paralarvae of 0 and 15 days after hatching (DAH) and its prey (*Artemia* spp. *nauplii*, *Artemia* spp. juveniles and *G. adscensionis* zoeae) were sampled for the following lipid analysis: total lipid content (TL), lipid classes (LC) and fatty acids (FA) composition. Moisture (M) was also measured in order to express lipid and fatty acid contents as dry weight basis (DW).

Sample collection was carried out by filtering animals from the tanks to Eppendorff tubes placed over dry ice and then to a cryo-freezer at -80°C, until utilization.

M and TL content determinations were performed in quadruplicate (n=4) in 0 DAH paralarvae and preys. 15 DAH paralarvae replicates were based on the availability of samples (data shown in Table 5).

Table 5. Number of replicates for Moisture and Lipid extraction.

Group	**Moisture**	**Lipid extraction**
G. *adscensionis* zoeae	4	4
Artemia spp. juveniles	4	4
Artemia spp. *nauplii*	4	4
Paralarvae 0 DAH	4	4
P-GR	5	5
P-AJ	4	3
P-AN	1	3

Samples were set at 1100C for 24 hours, until constant weight was obtained, in agreement with the Official Method of Analysis of the Association of Official Analytical Chemistry (A.O.A.C., 2006), which is an adaptation of Horwirtz method, (1980). Collected data were used to calculate mean percentage of moisture content (MC-%) = [100*(CSi – CSf)]/(CSi – C), where CSi is initial fresh weight of the sample with the container and CSf is the final weight of the same dry sample, and C is the weight of the empty container respectively.

The lipid extraction was performed according to Folch et al. (1957) method. Lipids were extracted by homogenizing the tissue with 2:1 chloroform – methanol (v/v). The filtered homogenate was mixed with a salt solution of KCl 0.88% and centrifuged for 5 minutes at 1500 r.p.m., in order to obtain two phases and where the upper phase contains all of the non-lipid substances and must be removed. The lower organic phase, which contains all the lipids, was evaporated under a nitrogen flux and left for 24 hour in vacuum. Total lipid was then determined gravimetrically.

All samples were redissolved in chloroform : methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, and to a final concentration of 10 mg/ml. Lipid classes were separated by high performance thin layer chromatography (HPTLC) using the method of Olsen & Henderson (1989). Separation of neutral lipids (NL) and polar lipids (PL) was accomplished using silica gel plates (10x10). Plates were loaded with a standard of cod roe and experimental samples, and developed with the polar solvent using Isopropanol : Chloroform : Methyl acetate : Methanol : KCl 0.25% (5 : 5 : 5 : 2 : 1.8 ml) for approximately 15 minutes. After drying in a desiccator for 30 minutes, plates were developed in neutral solvent containing n-Hexane : Diethyl ether : Acetic acid (22.5 : 2.5 : 0.25 ml). Dry plates were sprayed with 3% copper acetate, 8% phosphoric acid reagent and placed into the oven at 160°C for 10-15 minutes. Plates were then analyzed by densitometry using a SHIMADZU CS – 9001 PC DUAL – WAVELENGHT FLYING SPOT SCANNER.

Regarding the fatty acids analysis, lipids were transmethylated according to the Christie (1982) method and using nonadecanoic acid (C 19:0) as an internal standard. Methyl esters were extracted with hexane : diethyl ether (1 : 1 v/v), and purified by thin layer chromatography (TLC), using as solvent hexane : diethyl ether : acetic acid (90 : 10 : 1). The resultant FAMEs (fatty acids methyl esters) were analyzed by Gas Chromatography using a THERMO SCIENTIFIC TRACE GC ULTRA equipped with a flame ionization detector. The identification of each fatty acid was carried out using a well-identified multistandard containing the most abundant fatty acids in marine organisms (50 fatty acids). Data were then transformed into absolute amounts (µg.g DW-1) by means of a known amount of an internal fatty acid standard (19:0).

2.2. Effects of 500 L tank volume on *O. vulgaris* paralarvae growth and survival

The second experiment was conducted using 1500 newly hatched paralarvae from another female of the same broodstock group.

2.2.1. *O. vulgaris* broodstock

For this experiment, the individuals were maintained under the same conditions as described in the previous experiment (Section 2.1.1.).

On 30.04.11, when water temperature was 19.8°C, the beginning of spawning was detected from a female of 1.350 g weight. The first hatchlings were observed 40 days later, with water temperature of 20.7 ± 0.51 °C and removed to the experimental tank.

2.2.2. Experimental design

1500 paralarvae were placed in one 500 L fiberglass tank, with black walls and bottom, in a flow-through seawater system. Replication was not possible in this experiment due to the limited production of paralarvae by a single female.

Water quality was assured through the same filtration system (Section 2.1.2.). Water flow was 5 mL/sec, with 86% of renovation per day. One porous plastic aeration stone of 3 cm length was placed in the middle of the tank to provide moderate aeration. Light intensity of 150 lux (by an incandescent bulb) and a photoperiod of 12L:12D was provided. No green water technique was performed. Once a week water temperature, salinity and dissolved oxygen were measured with a DO METER PRO ODO device. Ammonia (NH3), nitrites (NO2) and pH were measured with a TETRA test aquarium kits for NH3 and NO2 and with a Hanna-HI-98107 pH Metter (Table 6). All measurements were taken weekly.

Table 6. Parameters of the rearing water during the second experiment.

Parameters	
Temperature °C	24.12 ± 1.30
Oxygen (%)	92.57 ± 5.29
Salinity (‰)	36.8 ± 0.14
pH (ppm)	7.93 ± 0.06
NH_3 (ppm)	0.00 ± 0.00
NO_2 (ppm)	< 0.30 ± 0.00

2.2.3. Diets

During the first 50 DAH, paralarvae were fed life prey once a day in a co-feeding regime. Since thereafter, paralarvae were still alive, frozen and bigger preys were needed.

2.2.3.1. Paralarvae feeding tables

Figure 7 shows timetables the experimental feeding regimes related to paralarval age in days. Diet.

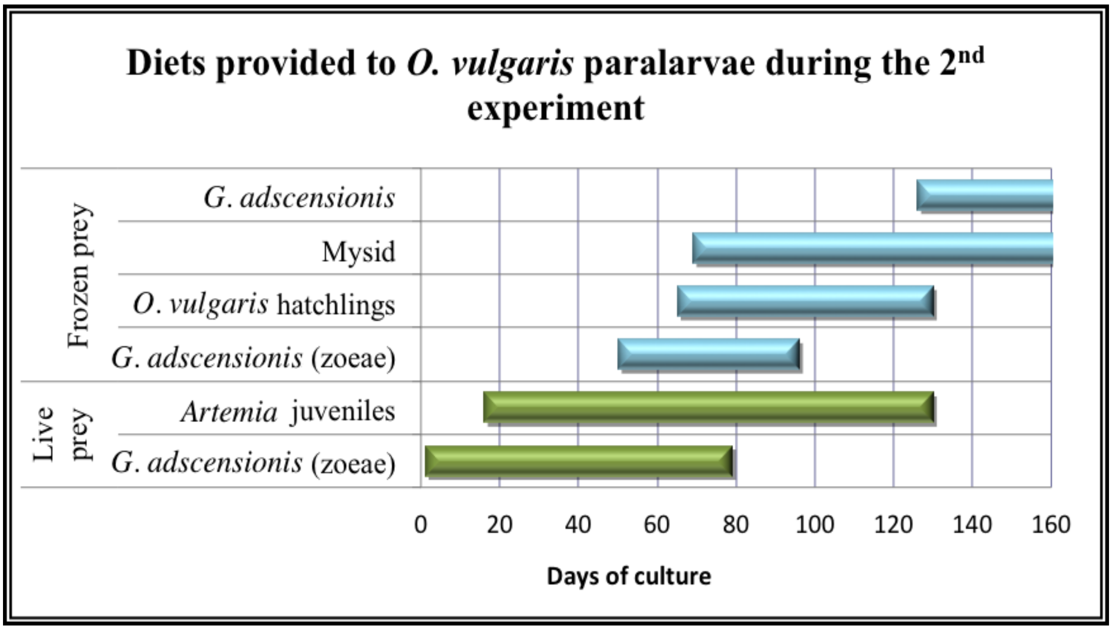


Figure 7. Diets of *O. vulgaris* paralarvae.

Life prey

G. adscensionis zoeae were obtained from the same crab broodstock of the previous experiment and using the same technique already described (Section 2.1.3.2.). The amount of zoeae given to paralarvae were dramatically dependent on production of zoeae by crab broodstock, therefore a big standard deviation during the period of feeding was observed (Table 7).

The Artemia (EG Type, INVE AQUACULTURE, Belgium) production followed the process of decapsulation and incubation, described in Section 2.1.3.1. (Sorgeloos 1986), and was added according to paralarvae demand (Table 7).

Table 7. Average amount of live prey (*G. adscensionis* zoeae and *Artemia* spp. *juveniles*) supplied to *O. vulgaris* paralarvae for each 10 days of culture, per 500 L.

Diet/days	Life prey	
	G. adscensionis (zoeae)	*Artemia* juveniles
10	39700 ± 36630	-
20	49710 ± 37544	49000 ± 42485
30	26300 ± 40111	52400 ± 30383
40	39540 ± 54472	69500 ± 17393
50	52900 ± 72631	107875 ± 33043
60	51000 ± 33813	90000 ± 31623
70	49680 ± 35965	120000 ± 49721
80	4111 ± 6168	139700 ± 26323
90	-	170500 ± 11655
100	-	159300 ± 34999
110	-	193500 ± 13754
120	-	197000 ± 6749
130	-	197143 ± 12536

Frozen prey

G. adscensionis zoeae and *O. vulgaris* hatchlings were also used as frozen food from days 60-70 (Table 8). Samples were stored in Eppendorff tubes in a cryo-freezer at -80°C and defrosted prior feeding. Finally, commercial mysids (Ocean Nutrition) or mysid shrimps of Mysidae family were used as frozen food as well.

Table 8. Average amount (grams per paralarvae) of frozen prey supplied to *O. vulgaris* paralarvae for each 10 days of culture.

Diet/days	Frozen prey in g per paralarvae		
	G. adscensionis (zoeae)	*O. vulgaris* hatchlings	Mysid
10	-	-	-
20	-	-	-
30	-	-	-
40	-	-	-
50	-	-	-
60	0.73 ± 1.01	-	-
70	0.80 ± 0.92	0.14 ± 0.38	-
80	0.50 ± 0.53	0.30 ± 0.48	1.25 ± 0.87
90	0.90 ± 0.32	0.40 ± 0.52	2.00 ± 0.00
100	0.60 ± 0.52	0.50 ± 0.53	2.00 ± 0.00
110	-	1.00 ± 0.00	2.00 ± 0.00
120	-	1.00 ± 0.00	1.80 ± 0.42
130	-	1.00 ± 0.00	1.14 ± 0.38

2.2.4. Growth and survival

Mantle length of paralarvae were measured at 0 days after hatchling (0 DAH) and every 15 days until day 60. After that, measurements were carried out monthly.

To perform the required measurements, 30 paralarvae were anaesthetized one by one in a solution of 50% MgCl2 and 50% of filtered seawater of 36 ‰. Mantle length was measured under a magnifying glass (Nikon SMZ-10A – 4 x magnification). After, paralarvae were placed in a plastic flask with seawater for recovery and released back to the rearing tank.

Survival was determined from 77 day-old onwards, by visual observations with use of a white disc, counting the live paralarvae into the tank from 77 day-old onwards. This procedure was not performed earlier, due to the big amount of paralarvae in the tank and to avoid paralarval disturbance. The mean survival rate (S-%) was estimated as S% = (Nf/Ni)*100, where Ni and Nf are the initial and final number of paralarvae of each diet treatment.

2.3. Statistics

Statistical analyses were performed with the program SPSS Statistics 17.0. Data is presented as means ± standard deviation (SD) from replicate tanks of given treatments. All data was tested for both normal distribution and homogeneity of variances using the one-sample Kolmogorov-Smirnov test and the Levene's test, respectively (Zar, 1999). To all data expressed as percentage, arcsin square root transformation was applied (Fowler, Cohen & Jarvis, 1998). Statistical difference was considered for $P<0.05$. When differences were found, a Tukey post hoc test was used to compare the groups (Zar, 1999).

One-way ANOVA analysis was performed to determine differences in water temperature, salinity, dissolved oxygen, lipid classes and fatty acids between treatments.

At the end of the experiment, nested ANOVA test was performed to determine differences in size, dry weight and survival between treatment groups (Zar, 1999) and in order to compare them with newly hatched animals.

For a better understanding of the LC and FA results, a principal components analysis (PCA) was performed to determine the differences between prey and octopus paralarvae composition. All the most expressive LC and FA were considered, sums were not included.

III. RESULTS

3.1. Effects of alternative prey and 100 L tank volume on *O. vulgaris* paralarvae

3.1.1. Growth and survival

By the end of rearing period, the diets had significant impact on growth and survival performances of *O. vulgaris* paralarvae. Obtained results are shown in Table 9 and correspond to growth (Size, DW, IGR, AGR) and survival of paralarvae after 15 days of culture. The maximum weight increase was found in the dietary groups based on crab zoeae (P-GR; 0.58 ± 0.11) although, no statistical difference was found among the treatment groups. A percentage of body weight gain per day (IGR) of 5.97% and an absolute growth rate of 0.023 mg per day in P-GR group were also the biggest.

Table 9. Growth and survival data of *O. vulgaris* paralarvae after 15 days of culture.

Parameters/diet	P - 0 DAH	15 DAH		
		P - GR	P - AN	P - AJ
VML (mm)	1.57 ± 0.09^{b}	1.76 ± 0.20^{a}	1.62 ± 0.16^{b}	1.59 ± 0.19^{b}
DW (mg)	0.24 ± 0.02^{b}	0.58 ± 0.11^{a}	0.48 ± 0.18^{a}	0.45 ± 0.17^{ab}
IGR (%)	—	5.97	4.69	4.30
AGR (mg)	—	0.023	0.016	0.014
SR (%)	—	28.67^{a}	5.61^{b}	9.61^{b}

VML – ventral mantle length; **DW** – dry weight per octopus paralarvae; **IGR** – instantaneous growth rate (percentage of body weight gain per day); **AGR** – absolute growth rate (weight gain in mg per day); **SR** – survival rate. Different superscript letters within the same row indicate statistical differences (P<0.05).

The increase of weight in relation to size showed a similar pattern, where paralarvae fed with crab zoeae (Fig. 8) had the biggest mean value of ML 1.76 ± 0.20 mm. Paralarvae fed with diet of *Artemia* spp. juveniles and *nauplii* were smaller in size and did not differ between themselves (P>0.05).

Survival of paralarvae at the end of 15 days ranged between 5.61 and 28.67%. The best results were also obtained in paralarvae fed with crab zoeae. Survival rate of paralarvae fed with both *Artemia* spp. juveniles and *nauplii* were very low, and the differences were not statistically significant due to the high standard deviation in these groups (P>0.05).

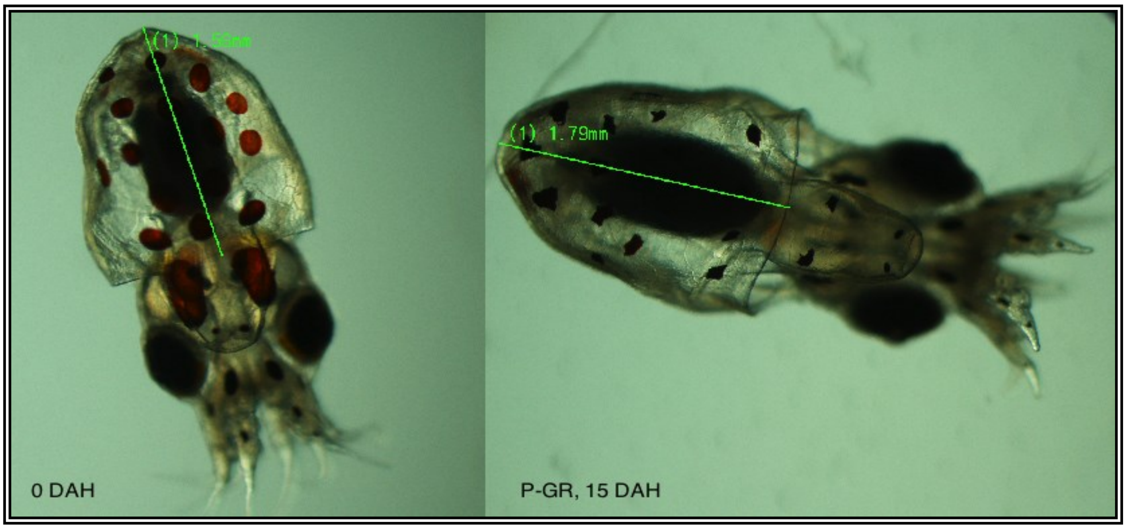


Figure 8. Mantle length of 0 and 15th DAH paralarvae from the experimental P-GR group.

3.1.2. Lipid composition

3.1.2.1. Prey

Table 10 shows moisture and total lipid content (% of DW) of the three live preys used. Significant differences were found between all types of prey ($P<0.05$). *Artemia* spp. juveniles had higher moisture ($P<0.05$) while *Artemia* spp. *nauplii* displayed higher total lipid. On the other hand, *G. adscensionis* zoeae displayed the lowest lipid content ($P<0.05$).

Table 10. Moisture (%) and total lipid content (% of DW) of life preys (n=4) provided to *O. vulgaris* paralarvae.

	G. adscensionis	***Artemia* spp. *nauplii***	***Artemia* spp. juveniles**
Moisture	84.06 ± 0.24^{c}	90.17 ± 0.82^{b}	93.20 ± 0.07^{a}
Total Lipid	5.29 ± 0.47^{c}	11.21 ± 0.64^{a}	7.10 ± 0.66^{b}

All data are expressed as mean percentages ± SD; Different superscript letters within the same row indicate statistical differences ($P<0.05$).

Lipid class composition of preys is shown in Table 11. Generally, all three diets presented significant differences in their profile ($P<0.05$).

Artemia juveniles showed the highest content of total polar lipids (61.27 ± 1.89%), displaying the highest values of PC and PE ($P<0.05$). Despite the lowest value in total neutral lipids, the amount of cholesterol in *Artemia* juvenile was the biggest one ($P<0.05$).

Neutral lipids were the major lipid class in *G. adscensionis*, where the amount of TAG was similar to that of *Artemia nauplii* ($P>0.05$), and SE displayed the biggest concentration of all preys ($P<0.05$). This prey also presented the highest content in pigments ($P<0.05$).

Table 11. Lipid class composition of experimental diets.

Lipid Class/Prey	*G. adscensionis*	*Artemia nauplii*	*Artemia* juvenile
SM	0.57 ± 0.28	0.58 ± 0.34	0.86 ± 0.41
PC	15.01 ± 0.67^{c}	16.86 ± 0.28^{b}	20.36 ± 1.23^{a}
PS+PI	5.87 ± 0.40^{b}	7.06 ± 0.51^{b}	10.54 ± 1.68^{a}
PG	3.13 ± 0.14^{c}	5.25 ± 0.23^{b}	7.29 ± 0.49^{a}
PE	11.53 ± 0.58^{c}	13.26 ± 0.60^{b}	15.18 ± 0.36^{a}
MAG	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	4.70 ± 0.59^{a}
DAG	1.33 ± 0.38^{b}	0.89 ± 0.25^{b}	3.46 ± 0.54^{a}
CHO	16.59 ± 1.21^{c}	21.01 ± 0.19^{b}	27.86 ± 1.42^{a}
FFA	1.95 ± 0.38	1.71 ± 0.19	2.27 ± 0.68
TAG	29.93 ± 1.58^{a}	28.72 ± 0.49^{a}	2.54 ± 0.50^{b}
SE	6.44 ± 0.56^{a}	2.72 ± 0.08^{b}	2.60 ± 0.48^{b}
Pigments	7.31 ± 0.56^{a}	0.58 ± 0.14^{b}	0.00 ± 0.00^{c}
UK	0.34 ± 0.07^{c}	1.36 ± 0.11^{b}	2.35 ± 0.28^{a}
TPL	36.45 ± 1.12^{c}	44.37 ± 0.79^{b}	61.27 ± 1.89^{a}
TNL	63.55 ± 1.12^{a}	55.05 ± 0.79^{b}	38.73 ± 1.89^{c}

Data presented in percentage of total lipid content ± SD; **SM** – sphingomyelin; **PC** – phosphatidylcholine; **PS+PI** - phosphatidylserine+phosphatidylinositol; **PG** – phosphatidylglycerol; **PE** – phosphatidylethanolamine; **CB** – cerebrosides; **MAG** – monoacylglycerol; **DAG** – diacylglycerol; **CHO** – cholesterol; **FFA** – free fatty acids; **TAG** – triacylglycerol, **SE** – sterol ester; **TPL** – total polar lipids; **TNL** – total neutral lipids; UK – unknown. Different superscript letters within the same row indicate statistical differences ($P<0.05$).

Main fatty acid composition of preys expressed in absolute terms (µg.g DW^{-1}), is shown in Annex I. FA profiles are significantly different between different prey ($P<0.05$).

A high content of palmitic (16:0) and oleic (18:1n-9) acids were detected in all three types of prey, with the highest concentration in *Artemia* spp. *nauplii* ($P<0.05$). No differences ($P>0.05$) were found between *G. adscensionis* zoeae and *Artemia* spp. juveniles in 16:0, but the content of 18:1n-9 in *G. adscensionis* zoeae was the lowest.

Among physiologically essential FA, crab zoeae had the biggest concentration in µg.g DW^{-1} of arachidonic acid (ARA; 20:4 n-6), the only prey containing docosahexaenoic acid (DHA; 22:6 n-3) and also were rich in eicosapentaenoic acid (EPA; 20:5 n-3), although *Artemia* spp. juveniles displayed the highest EPA content ($P<0.05$). The amount of ARA in *Artemia* spp. juveniles was statistically similar to that of *Artemia* spp. *nauplii* and DHA was not found in either *Artemia* prey.

The FA profile of *Artemia* spp. *nauplii* exhibited a significant dominance of SFA, MUFA, PUFA, n-3 and n-9 FA's ($P<0.05$). From SFA, palmitic acid (16:0) displayed the highest values. MUFA showed the highest value due to the high content in 18:1n-9 and 18:1n-7. Among PUFA's, alpha-linolenic acid (18:3 n-3) had the highest value ($P<0.05$) that led to the greatest amount of n-3 FA among preys ($P<0.05$).

A significantly higher amount of ARA, was found in *Grapsus* zoeae and therefore, the ratio of n-3/n-6 and EPA/ARA was the lowest one ($P<0.05$) whereas the high concentration of DHA and EPA gave the best ratio of 0.58 ± 0.11 μg.g DW^{-1} among tested diets.

It is well established that FA profiles expressed as percentages, gives a general and interesting view of the existing different proportions among FAs, highlighting those FA which might be particularly relevant in a given sample. Annex II shows prey relative percentages of FAs and comparing this data with those given in absolute terms (μg.g DW^{-1}), some significant aspects can be pointed out. For instance, 20:4n-6 and 22:6n-3 seem to be quite relevant FAs in G adscensionis whereas 18:3n-3 is comparatively the most expressive FA in *Artemia nauplii* or EPA in case of *Artemia* juveniles. It is also noticeable that percentages of palmitic acid (16:0) was found to be the highest FA in *G. adscensionis* zoeae ($P<0.05$) with no differences between *Artemia* spp. *nauplii* and *Artemia* spp. juveniles ($P>0.05$) while in μg.g DW^{-1}, when taking into account the samples different TL contents, this FA showed the opposite trend, with a similar situation applying for oleic acid.

Regarding the essential FA, only minor differences were found among the two ways of data expression. The amount of ARA in *Artemia* spp. juveniles was statistically bigger than in *Artemia* spp. *nauplii*, although no difference was detected in its absolute value.

The EPA % was statistically different among all the groups and remained the highest in *Artemia* spp. juveniles ($P<0.05$) while for *Artemia* spp. *nauplii* became the lowest ($P<0.05$).

3.1.2.2. Paralarvae

In Table 12, moisture and total lipid content of paralarvae at 0 and 15 days after culture is presented. Paralarvae at 0 DAH had the biggest moisture content ($P<0.05$), while cultured octopus did not show any difference according to the dietary regime ($P>0.05$). In general terms, lipid content of paralarvae increased after 15 days of culture. The lowest lipid content was observed for 0 DAH paralarvae whereas the group fed *Artemia* spp. *nauplii* displayed the highest content of lipid 12.65 ± 3.7 ($P<0.05$).

When comparing the fed paralarvae moisture and total lipid content with the corresponding prey, statistical similarities were found only in the P-AN group and for total lipid ($P>0.05$).

Table 12. Moisture (%) and Total Lipid content (% DW) of 0 and 15 days-old *O. vulgaris* paralarvae fed with different diets.

	P-0 DAH	P-GR	P-AN	P-AJ
Moisture	83.45 ± 2.57^{a} G	77.67 ± 0.52^{b}	77.61 ± 0.00^{b}	77.85 ± 1.17^{b}
Total Lipid	8.14 ± 0.69^{b} J	11.52 ± 0.97^{ab} N	12.65 ± 3.87^{a} N	10.25 ± 1.10^{ab} N

All data are expressed as mean percentage ± SD; Different superscript letters within the same row indicate statistical differences ($P<0.05$). Capital letters in the right column of data represent: J – statistical similarity between paralarvae and *Artemia* juvenile ($P>0.05$); N - statistical similarity between paralarvae and *Artemia nauplii* ($P>0.05$); G - statistical similarity between paralarvae and *G. adscensionis* zoeae ($P>0.05$).

The lipid class composition of octopus hatchlings and paralarvae fed with different diets is shown in Annex III.

All paralarvae were rich in polar lipids, mainly PC and PE. Newly hatched paralarvae showed a dominance in TPL ($P<0.05$). Significantly lower amount of PC, PS+PI and PE was observed in paralarvae after 15 days of rearing ($P<0.05$). Regarding neutral lipids, hatchlings were mainly rich in CHO. Nonetheless, the content of this LC decreased significantly after 15 of culture with any prey ($P<0.05$). This change was generally characterized by an increase in TAG and SE of the TNL content.

Similarities between P-GR and P-AN groups and its prey were not so evident. Only the P-AJ group was similar in cholesterol and free fatty acids to *Artemia* juvenile; although, the total amount of polar and neutral lipids were different ($P<0.05$).

The FA profile (μg.g DW^{-1}) of 0 DAH and 15 days paralarvae is presented in Annex IV. The 3 groups of cultured paralarvae were similarly rich in palmitic (16:0) and stearic (18:0) saturated fatty acids ($P>0.05$). P-AN and P-AJ groups were rich in MUFA, mainly due to oleic 18:1 n-9 and 18:1 n-7 acid, while the P-GR group showed the lowest value in these two fatty acids ($P<0.05$).

Although the amount of PUFA and n-3 HUFA in all 3 groups was statistically similar ($P>0.05$), differences in essential fatty acids among cultured paralarvae were found. Individuals from P-AJ and P-AN groups had statistically similar high content of EPA ($P<0.05$) and both groups were similarly low in DHA and ARA while its highest contents in μg.g DW^{-1} were found in the P-GR group ($P<0.05$).

Comparing the absolute FA values of reared groups with newly hatched animals, as changes as and similarities could be observed. 0 DAH paralarvae contained 4.19 ± 0.65 μg.g DW^{-1} of EPA, 5.99 ± 0.94 μg.g DW^{-1} of DHA and 1.21 ± 0.20 μg.g DW^{-1} of ARA and were statistically similar to reared P-AN and P-AJ groups. Significant changes occurred in P-GR group, the amount of DHA and ARA raised considerably compared to 0 DAH and P-AN, P-AJ.

Regarding the sums of FA's, the increase in all the groups occurred after the rearing period with *G. adscensionis* zoeae which displayed the highest values in SFA, PUFA, n-3 HUFA and n-6 FA's. Despite that, the DHA/EPA ratio in P-GR group was similar to that found in octopus hatchlings ($P>0.05$) and in P-AN and P-AJ groups were significantly lower. The lowest EPA/ARA and DHA/ARA ratios in P-GR group was related to a high ARA content in this group.

Similarities in the absolute value of FA content of cultured paralarvae and its prey were found in all the treatment groups.

The P-GR group was statistically similar to its diet in MUFA and n-9 fatty acids, mainly 16:1n-7, 18-1n-9 and 18:1n-7 ($P>0.05$). Similarities to crab zoeae in n-3/n-6 and EPA/ARA ratios were found as well ($P>0.05$).

In the P-AN group, main similarities with *Artemia* spp. *nauplii* were observed in SUM of all fatty acids and total amount of PUFA, n-3 and n-6 FA ($P>0.05$) were found. Therefore the n-3/n-6 and EPA/ARA ratio was also similar ($P>0.05$).

P-AJ reflected the FA content of *Artemia* juveniles in 14:0, 18:2n-6, 18:3n-3 and 18:4n-3 and in the total amount of MUFA and n-9 fatty acids.

Annex V shows paralarvae percentages of FAs in and comparing these data with absolute values (µg.g DW^{-1}) some minor differences were found.

P-0 DAH showed significantly higher amount of 16:0 and 20:1n-9 ($P<0.05$) compared to the reared groups, while in absolute value it had the lowest amount ($P<0.05$).

Among physiologically essential FA differences were observed between all the groups in percentage of ARA ($P<0.05$) with the highest % in P-GR group (as well as in µg.g DW^{-1}), while in absolute value ARA content was similar in P-0 DAH, P-AN and P-AJ. The percentage of DHA in P-0 DAH group was significantly bigger compared to others ($P<0.05$) but in absolute terms was similar among P-0 DAH, P-AN and P-AJ ($P>0.05$) and had the highest value ($P<0.05$) in P-GR.

Statistical difference between P-GR, P-AN and P-AJ, for saturated fatty acids, was only found in percentage, being higher in the P-GR group. Finally, the percentage of PUFA, n-3 FA and therefore n-3 HUFA, was the highest in P-0 DAH group ($P<0.05$) although in total amount its content was very low compared to other groups. The principal component analysis (PCA) regarding the lipid classes and fatty acids profile was performed. A bi-dimential representation of the first 2 extracted factors for LC is shown in Figure 9.

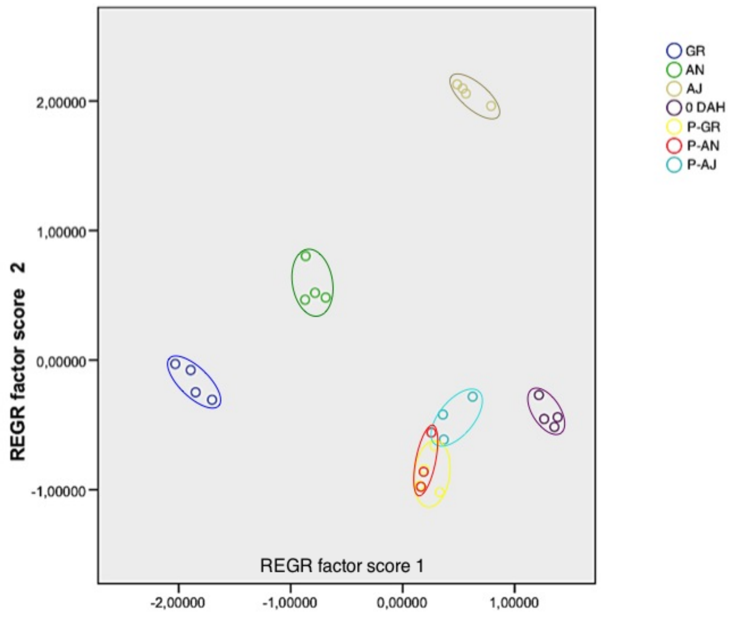


Figure 9. Score plot of LC profile (%) of prey and paralarvae in relation to the first two PCA factors.

Factors were differentiated by type of prey, hatchlings and feeding groups. Prey groups were the most distant by both factors from each other. Between the experimental paralarvae there was similarity in its content by Factor 1, where the group P-AJ was similar to its prey *Artemia* juveniles. Regarding the Factor 2, clear similarity was observed in P-GR and P-AN, and also between crab zoeae, 0 DAH and P-AJ.

Comparing hatchlings with reared paralarvae, it was possible to observe slight changes of LC towards its preys.

Regarding the FA profile of prey by bi-dimential principal component analysis, crab zoeae were the most distant by two Factors from all the prey, while *Artemia nauplii* and *Artemia* juveniles were close to each other in its FA content but not similar statistically (Fig. 10).

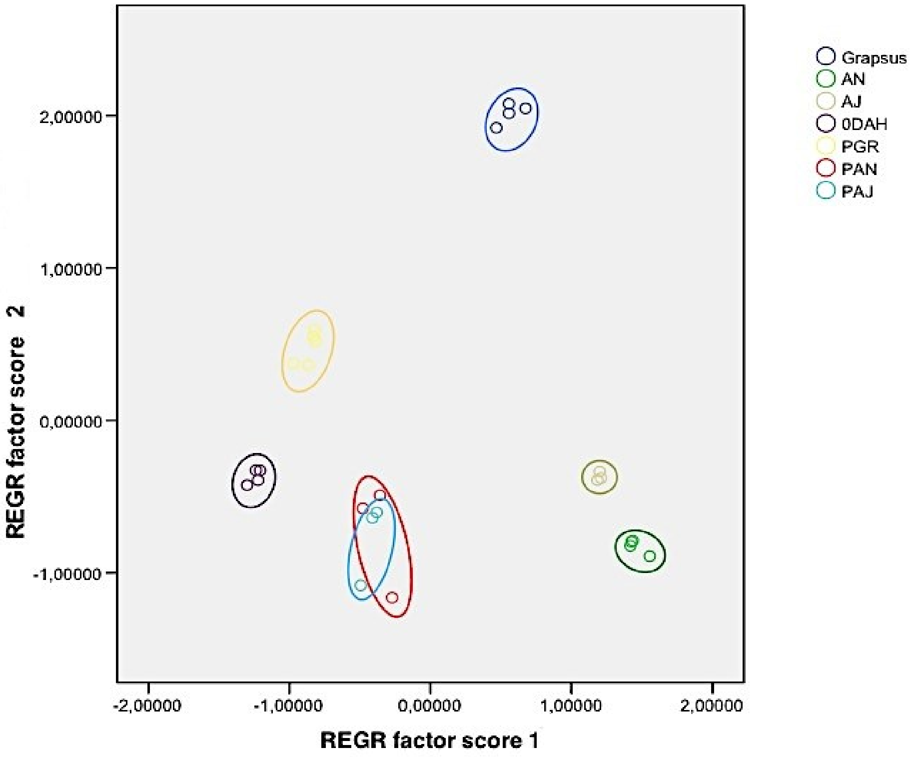


Figure 10. Score plot of FA profile (%) of prey and paralarvae in relation to the first two PCA factors.

Octopus hatchlings displayed statistical similarity with *Artemia* juveniles (AJ) and paralarvae fed on *Artemia nauplii* (P-AN) by Factor 2. Also, P-AN were close to its prey and P-AJ. These paralarvae were similar by Factor 1 as well.

Thus, it was observed a tendency of 0 DAH paralarvae in changing the FA profile during the rearing period towards to the prey they were fed.

3.2. Effect of 500 L tank volume on *O. vulgaris* paralarvae growth and survival

The results showed that compared with 100 L tank experiment a higher growth and survival rate were obtained in a larger tank volume with the same paralarvae density.

Paralarvae used for this experiment were statistically bigger at 0 DAH (1.68 ± 0.09 mm), compared with those for 100 L (1.57 ± 0.09 mm) ($P < 0.05$). At the 16th DAH, statistical analysis was performed to compare the size of VML with the groups P-GR (1.76 ± 0.20 mm), P-AN (1.62 ± 0.16 mm), P-AJ (1.59 ± 0.19 mm) from previous experiment at day 15th (Fig.11). Paralarvae of the 500 L trial were significantly bigger among all the tested treatments (1.90 ± 0.09, $P < 0.05$). On the other hand, when we compared the IGR (%) of the VML for all treatments, we found similar values in paralarvae fed with crab zoeae, both in 100 and 500L (0.77 and 0.76 respectively) and lower values for P – AN (0.21) and P – AJ (0.08).

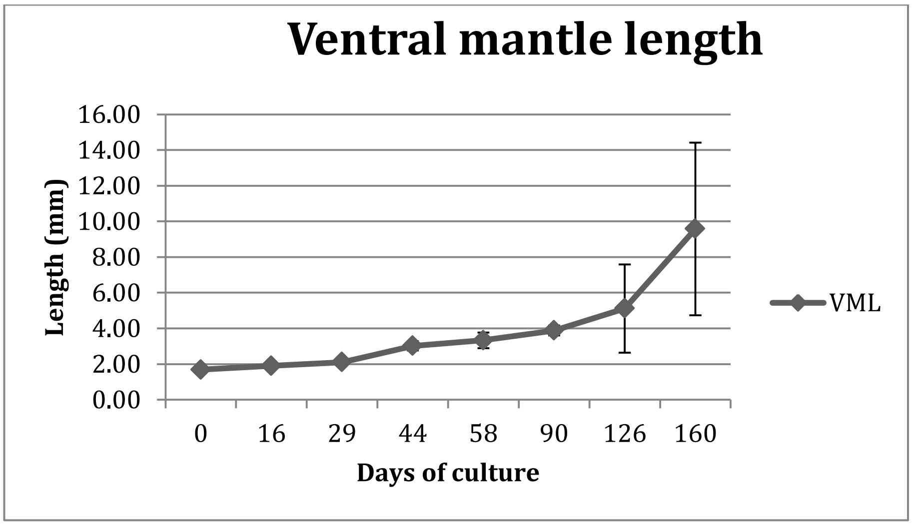


Figure 11. Ventral mantle length (VML; mm) of paralarvae reared in 500 L tank.

The survival rates of the octopus paralarvae in the 500 L tank were 2.5% at 77 day of culture and 0.1% at 160th day (Fig. 12).

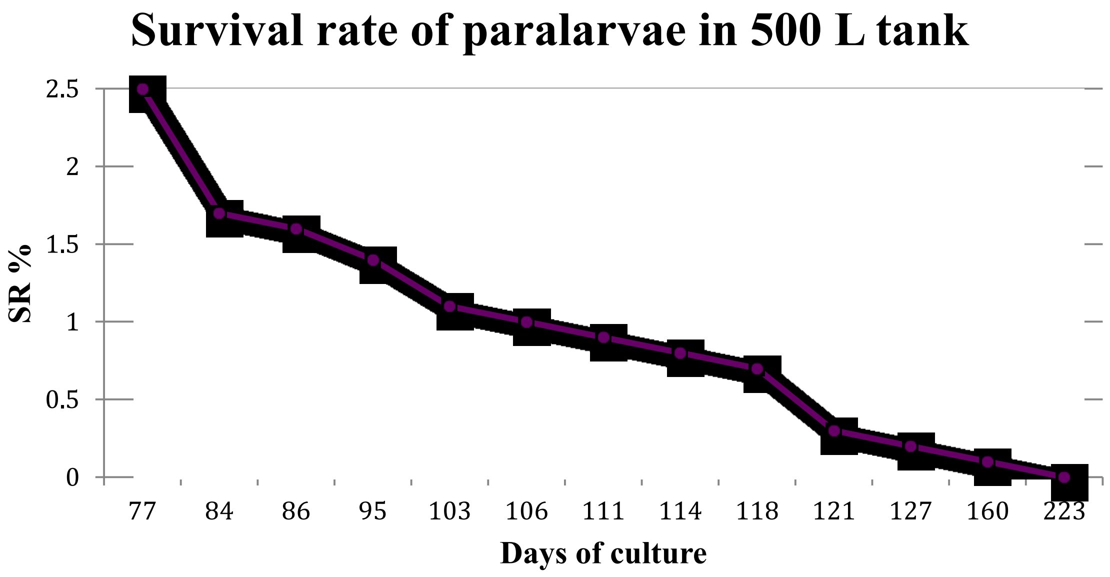


Figure 12. Survival rates of paralarvae reared in a 500 L tank.

At the end of this experiment (223 days), one benthic stage of octopus with the size 13.00 mm of VML and wet weight of 2.17 g was obtained (Fig.13).

Figure 13. 223-day old octopus - Pablito.

IV. DISCUSSION

The size range (mm of mantle length) and dry weight of newly hatched *O. vulgaris* paralarvae varies according different studies. Iglesias et al. (2007), reported 1.0 – 1.5 mm of ML, which is similar to this study, while Villanueva & Norman, (2008) reported bigger values of 2.1 – 2.3 mm and 0.5 mg (DW). The same results 0.24 ± 0.02 of hatchlings dry weights as in this study were obtained by Vidal et al. (2002). Differences in size and weight could be due to the broodstock rearing conditions. Influence of environmental parameters such as salinity, showed a significant effect on broodstock reproduction, embryotic development and hatching rate of cephalopods (Palmegiano & D'Apote, 1983; Bouchad & Galois, 1990) while, as concluded by Sakaguchi et al. (2002), female body weight and water temperature are positively correlated with the egg size and therefore hatchlings size. On the other hand, according to Quintana et al. (2009), the quality of food supplied to the broodstock also plays an important role affecting egg quality and paralarvae size.

The critical factor determining early survival rate of paralarvae is the starvation during the first days of life (Vidal et al., 2002), thus, the effects of tank volume and prey availability are vital. An increase in prey density will result in a higher predator-prey encounter rate, therefore higher prey density enhances the consumption rate and feeding success. As prey density increases the number of prey captures rises and the predator searching time and energy expenditure is gradually reduced by time taken during the prey handling processes. The probability of early starvation increases with the lower prey density leading the reduction of mean consumption rate (Marques et al., 2006). The results obtained from this study showed that the diets and tank volumes used had a clear influence on growth and survival of paralarvae. In the 100 L experiment, the best results of growth performance were obtained with the diet based on *G. adscensionis*. After 15 days of rearing, these paralarvae gained more than twice of its initial weight and were bigger than paralarvae fed *Artemia* spp. The high growth rates was already reported by Villanueva & Norman, (2008) for paralarvae fed decapod crustacean zoeae and in the studies of Reis (2011) similar results of IGR 6.29 ± 1.10% were obtained with *G. adscensionis* diet.

With increased tank volume (500 L) and *G. adscensionis* as first prey, even better growth results were obtained, the size of paralarvae at 16th day of culture was significantly bigger than those obtained for 100 L experiment. However, the bigger paralarvae size in this experiment could be the consequence that hatchlings were obtained from another broodstock female and were initially bigger (1.68 ± 0.09 mm) compare to 100 L experiment (1.57 ± 0.09 mm).

Despite, in previous studies, the amount of days of paralarvae rearing and prey offered is different; the survival rates still remained low. In the present study, the survival rate of *Artemia* spp. juveniles fed paralarvae was 9.61% after 15 days of culture, and were bigger than obtained from *Artemia* spp. *nauplii* (5.61%) but no statistical difference between these groups were detected (Table 8). The best survival rates until now were obtained by Hamazaki et al. (1991), who achieved 28.9% of survival at day 25, and Seixas et al. (2010), who attained survival rates of 35 to 53% after 15 days of rearing and 7 to 20% at day 25 by feeding paralarvae with *Artemia* juveniles enriched with a mixture of microalgae and commercial products rich in several fatty acids.

Significantly improved survival rates were obtained in studies where decapod crustacean zoeae were used. The present study survival result using *G. adscensionis* zoeae as sole prey during first 15 days of paralarval life in 100 L was 28.67%, which is significantly bigger than that obtained in paralarvae fed *Artemia*, while in 500 L tank survival reached 2.5% at 77 day and 0.1% at 160th day of culture. In the work of Reis, (2011) the highest SR was also obtained with the same prey – *G. adscensionis* zoeae (64.83 ± 23.62) although, the rearing lasted for 9 days. Iglesias et al., 2007 reviewed the unpublished data from J. Roo (ICCM, Canary Islands) where they reported the improved survival rate of paralarvae fed with the same crab species but combined with enriched *Artemia*, and achieved 27% at 28 days of culture. For instance, Itami et al. (1963) used zoeae of Palaemon serrifer and obtained benthic juveniles with survival of 5% at the 60th day of rearing. Iglesias et al. (2002) fed octopus paralarvae with *Maja brachydactyla* zoeae combined with *Artemia* spp. and achieved 31.5% of survival at day 40 and for the first time closed the *O. vulgaris* life cycle. Moxica et al. (2002) and Carrasco et al. (2003, 2005) reported survival of 8.3% at one month of rearing, and 3.4% at 60 days, respectively, using the same prey.

The three diets used in this study were different in their moisture and total lipid content (Table 8). The crab zoeae were characterized by lower moisture and total lipid than those reported for both *Artemia nauplii* and juveniles. Our results for *Grapsus* zoeae are similar with these reported by Reis (2011). The highest total lipid content was found in *Artemia nauplii*.

The variation in lipid content throughout paralarval growth in rearing experiments seems to be related to diet (Navarro & Villanueva 2000, 2003; Moxica et al. 2002; Okumura et al. 2005). In present study the influence of prey on the lipid composition of cultured octopus paralarvae was observed. Within lipid classes, the *Grapsus* zoeae presented higher content in neutral than in polar lipids (Table 9). TAG – the primary class for lipid storage and energy provision (Tocher et al., 2008) constituted a major class of neutral lipids. According to Navarro and Villanueva (2000, 2003), Villanueva et al. (2004) and Iglesias et al. (2007), octopus paralarvae need a prey with high content of cholesterol – the most important simple lipid. The same authors also point that paralarvae also require phospholipids and PUFA. Phospholipids are the source of the substrate for the formation of eicosanoids, a range of bioactive derivatives of HUFA, especially ARA and EPA (Tocher et al., 2008). Within the tested diets *Artemia* juveniles represented the higher percentage of these lipid classes.

Octopuses and cephalopods in general are characterized by low lipid contents, with relatively large phospholipid and sterol fractions, and triacyglycerides as minor components (Nash et al., 1978; Hayashi and Yamamoto, 1987; Navarro and Villanueva, 2000). In present study hatchlings presented low content in neutral lipids but, during the rearing period, paralarvae shifted towards its higher content. The main changes occurred among neutral lipids were due to the increase in TAG and SE, and related to the lipid deposition. Within polar lipids, a significant reduction in PE was detected in all reared paralarvae and could be related to the use of lipids for energy.

Palmitic acid (16:0), stearic acid (18:0), DHA (22:6n-3) and EPA (20:5n-3) were the most abundant fatty acids found in the lipids of newly born *O. vulgaris* paralarvae. As has been reported for many cephalopod species, the dietary requirements for n–3 PUFA, particular DHA is critical in early developmental stages (Miliou et al., 2007). DHA plays a multifunctional role in a wide variety of adaptive processes, maintaining the structural and functional integrity of cell membranes in fish (Sargent et al., 1995) and may be important for the correct development and survival of fast growing phospholipid-rich cephalopods (Navarro and Villanueva, 2000, 2003). Among the diets used, the crab zoeae were the prey with the highest content in DHA and ARA and high content of EPA. The *Artemia nauplii* used in this study presented a low content in EPA, but its level in *Artemia* juveniles was the highest. The ARA content of both *Artemia* diets was also very low and DHA was totally absent. Therefore, these diets seem unsuitable, as they don't resemble the natural lipid profile of paralarvae.

Dietary lipids are important as a source of essential fatty acids (Tocher, 1995) and the changes in paralarval lipid composition were visible after 15 days of rearing period. The fatty acid composition of the paralarvae at the end of the feeding treatments reflected the FA composition of their experimental diets. Paralarvae fed *G. adscensionis* zoeae were highly polyunsaturated, characterized by high levels of n-3 HUFA, predominantly 22:6 n-3 and 20:5 n-3, with 20:4 n-6 as the major n-6 PUFA, and with 16:0 followed by 18:0 as the predominant saturated fatty acids. The high EPA content of *Artemia* diets followed by its rose in paralarvae and the low content of the DHA and ARA in these preys, explains its reduction after rearing although, they still remained the most abundant PUFAs. All the reared paralarvae presented a higher absolute amount of n-3 and n-6 PUFA compared with newly hatched animals. The crab zoeae diet was the highest in PUFA and n-3 HUFA but showed the lowest value of total n-3 FA. The total n-6 FA in this group was also higher, mainly due to the high ARA content. EPA competes with ARA in eicosanoid production, thus eicosanoids actions are determined by the ratio of EPA/ARA (Tocher, 2003). Arachidonic acid has been proved effective in improving egg quality (Sargent et al., 1995) and survival at the early life stages of fish (Castell et al., 1994; Bessonart et al., 1999; Koven et al., 2001), the ARA content in octopus hatchlings of the present work was 5.02 ± 0.04%. Despite the level of ARA in octopuses appears to be an inherited characteristic and does not correlate with the dietary input (Navarro and Villanueva, 2000) in this study, the elevated level of ARA was observed in the fastest growing paralarvae and showing the best survival rate in P-GR group, Miliou et al. (2006) refers that the high levels in ARA seem to be associated with an improved growth of *O. vulgaris*. In addition, the lowest survival and growth was observed in paralarvae fed with both *Artemia* diets, where the amount of ARA was poor.

Okumura et al. (2005) suggested that a DHA/EPA ratio equal to 1.5 in common octopus is a necessary condition for the normal growth and development. High mortality and poor growth associated with nutritional imbalance in fatty acid profiles has been observed when DHA/EPA is below 1.5 (Navarro & Villanueva 2000, 2003, Okumura et al. 2005). Newly hatched paralarvae of this study had 1.43 ± 0.01 DHA/EPA and this ratio raised to 1.50 ± 0.03 in paralarvae fed *Grapsus* zoeae, while a reduction in the DHA/EPA ratio was observed in paralarvae fed with *Artemia nauplii* and juveniles. The reduction in the DHA/EPA ratio for paralarvae fed both stages of *Artemia* was mostly due to the absence of DHA in *Artemia*, therefore showing a correlation of this FA content and prey contribution.

Among the appropriate nutrient needs during the first period of live, paralarvae require a live prey of a critical size and suitable swimming behavior (Villanueva, 1994) available in big amounts. The lack of a sufficient quantity of suitable food is one of the main impediments for developing experimental and mass culture of the delicate paralarval stage of cephalopods. A wide variety of live and inert prey has been tested in laboratory experiments, where the most successful used decapod zoeae (Itami et al. 1963; Forsythe & Toll, 1991; Villanueva, 1994, 1995; Shiraki, 1997; Carrasco et al. 2003, 2005; Iglesias et al. 2004) and natural zooplankton collected from a sea (Turk et al. 1986; Hanlon et al. 1989). In the present work a simple method of obtaining decapod crustacean zoeae for rearing planktonic paralarval and juvenile cephalopods was developed.

G. adscensionis zoeae are being experimentally used as life prey for commercially-valued species, such as the octopus (Carro, 2004), and were chosen for this study as the most abundant and easy available decapod crab distributed along the coasts of Canary Islands. Ovigerous females occur throughout the year, and egg batches are estimated to be laid every 24 days (Hartnoll, 2009). To determine the necessary amount of crabs for the broodstock, sex ratio and individual fecundity of females were studied and simple calculations were made.

Assuming, that the amount of zoeae required for a 15 days experiment is 0.2 zoeae/mL per day, then for 1800 paralarvae (6 replicates of 100 L tanks) it will be 120 000 zoeae/day. The mean fecundity of 1 female is 70 000 zoeae, which implies that it's necessary to have 2 females ready to spawn for each day. However a study of monthly variation in sex ratio for this species (Shcherbakova et al. 2011) estimated that, during the experimental setup, the sex ratio of crab population will be 1:1, and from the total amount of females only half will carry eggs. Thus, to achieve 2 females with eggs, it is necessary to obtain at the least 8 crabs, where 4 will be males, 2 females with eggs and 2 without. Therefore for a 15 days experiment 120 broodstock crabs are required.

This scheme of zoeae production could help to provide the necessary live prey for the first paralarval stage during the rearing period. The excess zoeae are easily maintained during several days with rotifers. The broodstock crabs adapt easily to captivity, keep reproducing continuously, allowing their use for further experiments.

Final considerations

It is believed that the nutritional aspects together with rearing conditions are the most important factors influencing octopus paralarval mortality. An appropriate culture-rearing condition for this species must promote low mortality, this being of extreme importance when commercial-scale culture is the objective. In this study 500 L tank volume with moderate paralarval and life prey distribution showed better results of growth and survival than in 100 L. According to visual observations these paralarvae were more active and mobile, distributing freely in water column and developing hunting instincts after life prey.

From their lipid composition at birth and that of their natural prey, we may conclude that *O. vulgaris* paralarvae require a food rich in PUFA, especially DHA, EPA and ARA. The diet, which could meet these requirements and be available for the large-scale production is still unresolved problem. As many studies previously showed, *Artemia* spp. as a sole prey has limited success (Hamazaki et al. 1991), and does not seem to be the right choice for feeding the early stages of *O. vulgaris* (Iglesias et al. 1997; Villanueva et al. 2002; Seixas et al. 2008). This is probably due to *Artemia* low content in essential fatty acids, mainly DHA and ARA. Decapod crustaceans zoeae, such as *Liocarcinus depurator, Pagurus prideaux, Maja brachydactyla, Grapsus adscensionis* (Villanueva, 1994, 1995; Carrasco et al. 2003, 2005; Iglesias et al. 2004; Socorro et al., unpublished data) were verified as providing better rearing results and received more attention recently.

The present results showed that octopus paralarvae first feeding and growth can be successfully stimulated using as food zoeae of *G. adscensionis* rich in PUFA, especially DHA and ARA, and adequate amount of EPA. The use of *Grapsus* to obtain a regular supply of zoeae as food for rearing the octopus paralarvae have several advantages: a) females occur all the year round, they have a high fertility rate and spawn several times between moults; b) the size of zoeae vary within the limits 50-100% of the mantle length of paralarvae (Villanueva, 1994) and excess of zoeae can be frozen and used for feeding older paralarvae; c) rearing of a *Grapsus* broodstock is simple in captivity. Therefore these advantages could make them serious candidates for future use in aquaculture and experimental studies on octopus paralarvae.

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Annex I. Fatty acids (FA) composition (µg.g DW^{-1}) of prey supplied to *O. vulgaris* paralarvae.

FA/Prey	G. adscensionis	Artemia spp. nauplii	Artemia spp. juvenile
C14:0	0.28 ± 0.03^{b}	0.43 ± 0.03^{a}	0.23 ± 0.06^{b}
C15:0	0.17 ± 0.02^{a}	0.14 ± 0.01^{ab}	0.11 ± 0.03^{b}
C16:0	4.76 ± 0.68^{b}	6.44 ± 0.42^{a}	3.68 ± 0.81^{b}
C16:1(n-7)	0.77 ± 0.08^{c}	1.51 ± 0.10^{a}	1.14 ± 0.25^{b}
C17:0	0.31 ± 0.03^{b}	0.52 ± 0.04^{a}	0.30 ± 0.04^{b}
C17:1(n-7)	0.13 ± 0.01^{b}	0.32 ± 0.03^{a}	0.15 ± 0.04^{b}
DMA C18:0	0.37 ± 0.03^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
C18:0	1.89 ± 0.18^{b}	4.03 ± 0.28^{a}	3.32 ± 0.71^{a}
C18:1(n-9)	4.54 ± 0.91^{c}	10.43 ± 0.64^{a}	6.65 ± 1.37^{b}
C18:1(n-7)	1.23 ± 0.10^{b}	3.90 ± 0.28^{a}	4.09 ± 0.86^{a}
C18:2(n-6)cis	1.04 ± 0.25^{b}	3.60 ± 1.62^{a}	0.96 ± 0.20^{b}
C18:3(n-3)	0.31 ± 0.05^{c}	13.89 ± 1.12^{a}	2.98 ± 0.67^{b}
C18:4(n-3)	0.04 ± 0.01^{c}	2.27 ± 0.19^{a}	0.55 ± 0.13^{b}
C20:1(n-9)	0.22 ± 0.06^{b}	0.41 ± 0.05^{a}	0.27 ± 0.06^{b}
C20:2(n-6)	0.33 ± 0.08^{a}	0.15 ± 0.01^{b}	0.06 ± 0.01^{c}
C20:4(n-6)	2.22 ± 0.28^{a}	0.40 ± 0.04^{b}	0.50 ± 0.12^{b}
C20:3(n-3)	0.08 ± 0.01^{b}	0.58 ± 0.05^{a}	0.13 ± 0.03^{b}
C20:5(n-3)	2.00 ± 0.28^{b}	1.60 ± 0.13^{b}	3.27 ± 0.76^{a}
C22:6(n-3)	1.17 ± 0.37^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
UK	0.15 ± 0.03^{c}	1.55 ± 0.16^{a}	0.60 ± 0.12^{b}
SUM	23.00 ± 0.11^{b}	54.21 ± 0.15^{a}	29.60 ± 0.17^{b}
SFA	8.16 ± 0.93^{b}	11.86 ± 0.80^{a}	7.90 ± 1.70^{b}
MUFA	7.13 ± 0.98^{c}	17.00 ± 1.12^{a}	12.50 ± 2.61^{b}
PUFA	7.56 ± 1.28^{b}	23.81 ± 2.55^{a}	8.60 ± 1.96^{b}
n-3 HUFA	3.44 ± 0.69	2.57 ± 0.21	3.44 ± 0.80
n-3	3.79 ± 0.66^{c}	19.11 ± 1.57^{a}	6.99 ± 1.61^{b}
n-6	3.77 ± 0.62^{a}	4.44 ± 1.70^{a}	1.61 ± 0.35^{b}
n-9	4.81 ± 0.97^{c}	11.30 ± 0.71^{a}	7.02 ± 1.46^{b}
n-3/n-6	1.00 ± 0.01^{b}	4.66 ± 1.27^{a}	4.34 ± 0.14^{a}
DHA/EPA	0.58 ± 0.11^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
EPA/ARA	0.90 ± 0.02^{c}	4.02 ± 0.35^{b}	6.51 ± 0.12^{a}
DHA/ARA	0.52 ± 0.11^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}

Data is presented in µg/g of sample in dry weight ± SD. **SFA** – saturated fatty acids; **MUFA** – monounsaturated fatty acids; **PUFA** – polyunsaturated fatty acids; **n-3 HUFA** – sum of ω 3 highly unsaturated fatty acids; **n-3** sum of ω 3 fatty acids; **n-6** sum of ω 6 fatty acids; **n-9** sum of ω 9 fatty acids; **DHA** – 22:6 n-3; **EPA** – 20:5 n-3; **ARA** – 20:4 n-6; Different letters in superscript within the same row indicate statistical differences (P<0.05).

Annex II. Fatty acids (FA) composition (% of total FA content) of prey supplied to O. vulgaris paralarvae.

FA/Prey	*G. adscensionis*	*Artemia* spp. *nauplii*	*Artemia* spp. juvenile
C14:0	1.24 ± 0.06^{a}	0.79 ± 0.04^{b}	0.76 ± 0.06^{b}
C15:0	0.73 ± 0.13^{a}	0.25 ± 0.01^{c}	0.37 ± 0.02^{b}
C16:0	20.67 ± 0.21^{a}	11.89 ± 0.39^{b}	12.40 ± 0.38^{b}
C16:1(n-7)	3.40 ± 0.61^{a}	2.79 ± 0.09^{b}	3.84 ± 0.09^{a}
C17:0	1.36 ± 0.10^{a}	0.96 ± 0.03^{b}	1.05 ± 0.13^{b}
C17:1(n-7)	0.58 ± 0.08	0.60 ± 0.02	0.50 ± 0.03
DMA C18:0	1.64 ± 0.17^{a}	0.00 ± 0,.00^{b}	0.00 ± 0.00^{b}
C18:0	8.27 ± 0.55^{b}	7.43 ± 0.25^{c}	11.21 ± 0.35^{a}
C18:1(n-9)	19.62 ± 1.47^{b}	19.27 ± 0.65^{b}	22.50 ± 0.56^{a}
C18:1(n-7)	5.41 ± 0.71^{c}	7.20 ± 0.21^{b}	13.83 ± 0.18^{a}
C18:2(n-6)cis	4.48 ± 0.50^{ab}	6.57 ± 2.64^{a}	3.25 ± 0.07^{b}
C18:3(n-3)	1.36 ± 0,.32^{c}	25.62 ± 0.73^{a}	10.06 ± 0.36^{b}
C18:4(n-3)	0.18 ± 0.05^{c}	4.19 ± 0.13^{a}	1.85 ± 0.11^{b}
C20:1(n-9)	0.95 ± 0.13^{a}	0.76 ± 0.08^{b}	0.93 ± 0.02^{a}
C20:2(n-6)	1.43 ± 0.14^{a}	0.28 ± 0.01^{b}	0.20 ± 0.01^{c}
C20:4(n-6)	9.64 ± 0.20^{a}	0.74 ± 0.08^{c}	1.70 ± 0.10^{b}
C20:3(n-3)	0.35 ± 0.07^{c}	1.07 ± 0.03^{a}	0.44 ± 0.03^{b}
C20:5(n-3)	8.68 ± 0.14^{b}	2.94 ± 0.10^{c}	11.05 ± 0.81^{a}
C22:6(n-3)	5.02 ± 0.94^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
UK	0.67 ± 0.17^{c}	2.85 ± 0.13^{a}	2.04 ± 0.07^{b}
SFA	35.55 ± 1.02^{a}	21.91 ± 0.73^{c}	26.70 ± 0.79^{b}
MUFA	31.00 ± 0.19^{b}	31.39 ± 1.16^{b}	42.25 ± 0.75^{a}
PUFA	32.77 ± 1.18^{b}	43.85 ± 1.78^{a}	29.02 ± 1.42^{c}
n-3 HUFA	14.89 ± 0.99^{a}	4.74 ± 0.15^{c}	11.62 ± 0.84^{b}
n-3	16.42 ± 0.65^{c}	35.25 ± 1.03^{a}	23.59 ± 1.30^{b}
n-6	16.34 ± 0.54^{a}	8.13 ± 2.73^{b}	5.44 ± 0.14^{c}
n-9	20.79 ± 1.59^{b}	20.87 ± 0.73^{b}	23.75 ± 0.52^{a}
n-3/n-6	1.00 ± 0.01^{b}	4.66 ± 1.27^{a}	4.34 ± 0.14^{a}
DHA/EPA	0.58 ± 0.11^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
EPA/ARA	0.90 ± 0.02^{c}	4.02 ± 0.35^{b}	6.51 ± 0.12^{a}
DHA/ARA	0.52 ± 0.11^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}

Data presented in mean percentage of total FA content ± SD; **SFA** – saturated fatty acids; **MUFA** – monounsaturated fatty acids; **PUFA** – polyunsaturated fatty acids; **n-3 HUFA** – sum of ω 3 highly unsaturated fatty acids; **n-3** sum of ω 3 fatty acids; **n-6** sum of ω 6 fatty acids; **n-9** sum of ω 9 fatty acids; **DHA** – 22:6 n-3; **EPA** – 20:5 n-3; **ARA** – 20:4 n-6; Different letters in superscript within the same row indicate statistical differences ($P<0.05$).

Annex III. Lipid classes (LC) of *O. vulgaris* paralarvae at hatching and after 15 days of rearing with different diets.

LC/Paralarvae	P-0DAH		P-GR		P-AN		P-AJ	
SM	0.42 ± 0.19^{ab}		0.62 ± 0.16^{a}		0.19 ± 0.01^{b}	NG	0.33 ± 0.11^{ab}	
PC	19.24 ± 0.42^{a}	J	16.07 ± 1.15^{b}	NG	14.41 ± 1.90^{b}	G	15.28 ± 1.40^{b}	NG
PS+PI	17.18 ± 0.55^{a}		12.21 ± 0.35^{c}	J	13.44 ± 0.43^{bc}		14.16 ± 1.68^{b}	
PG	2.12 ± 0.18		1.91 ± 0.51		2.89 ± 0.41	G	3.20 ± 1.57	G
PE	21.61 ± 0.69^{a}		16.87 ± 0.10^{c}		19.55 ± 1.32^{b}		18.85 ± 0.95^{b}	
MAG	0.00 ± 0.00^{b}	NG	0.73 ± 0.09^{a}		0.81 ± 0.38^{a}		1.45 ± 0.76^{a}	
DAG	0.00 ± 0.00		0.00 ± 0.00		0.00 ± 0.00		0.00 ± 0.00	
CHO	31.99 ± 0.75^{a}		20.38 ± 1.24^{c}	N	21.35 ± 2.31^{bc}	N	23.94 ± 1.31^{b}	
FFA	0.00 ± 0.00^{c}		0.00 ± 0.00^{c}		1.07 ± 0.20^{a}	N	0.78 ± 0.19^{b}	
TAG	4.59 ± 0.69^{b}		7.88 ± 2.86^{ab}		11.97 ± 1.23^{a}		12.32 ± 3.52^{a}	
SE	1.36 ± 0.33^{d}		20.67 ± 2.17^{a}		12.35 ± 0.75^{b}		8.29 ± 1.47^{c}	
Pigments	0.00 ± 0.00^{c}	J	1.05 ± 0.11^{a}		0.35 ± 0.02^{b}		0.00 ± 0.00^{c}	J
UK	1.48 ± 0.14	N	1.60 ± 0.11	N	1.40 ± 0.25	N	1.38 ± 0.37	N
TPL	62.05 ± 1.21^{a}	J	50.01 ± 1.54^{b}		52.92 ± 2.47^{b}		54.67 ± 4.18^{b}	
TNL	37.95 ± 1.21^{b}	J	48.94 ± 1.57^{a}		46.73 ± 2.48^{a}		45.33 ± 4.18^{a}	

Data is presented as percentage of total lipids ± SD; **SM** – sphingomyelin; **PC** – phosphatidylcholine; **PS+PI** - phosphatidylserine+phosphatidylinositol; **PG** – phosphatidylglycerol; **PE** – phosphatidylethanolamine; **MAG** – monoacylglycerol; **DAG** – diacylglycerol; **CHO** – cholesterol; **FFA** – free fatty acids; **TAG** – triacylglycerol, **SE** – sterol ester; **UK** – unknown; **TPL** – total polar lipids; **TNL** – total neutral lipids. Different letters in superscript within the same row indicate statistical differences at the $P<0.05$ level. Capital letters in the right column of data represent: J – statistical similarity between paralarvae and *Artemia* juvenile ($P>0.05$); N - statistical similarity between paralarvae and *Artemia nauplii* ($P>0.05$); G - statistical similarity between paralarvae and *G. adscensionis* zoeae ($P>0.05$).

Annex IV. Fatty acid composition (µg.g DW^{-1}) of hatchlings and 15 days paralarvae fed *G. adscensionis* zoeae, *Artemia* spp. *nauplii* or *Artemia* spp. juveniles.

Fatty acid/ paralarvae	P - 0 DAH		P - GR		P - AN		P - AJ	
C14:0	0.28 ± 0.06	GJ	0.25 ± 0.05	GJ	0.26 ± 0.07	GJ	0.24 ± 0.03	GJ
C16:0	4.32 ± 0.70^{b}	GJ	9.39 ± 1.26^{a}		7.41 ± 1.80^{a}	N	7.93 ± 0.76^{a}	N
C16:1(n-7)	0.15 ± 0.06^{b}		0.46 ± 0.13^{a}	G	0.39 ± 0.13^{ab}		0.53 ± 0.12^{a}	G
C17:0	0.40 ± 0.07^{b}	GJ	0.72 ± 0.06^{a}		0.84 ± 0.19^{a}		0.87 ± 0.14^{a}	
DMA C18:0	1.17 ± 0.13^{b}		3.23 ± 0.27^{a}		1.43 ± 0.28^{b}		1.04 ± 0.17^{b}	
C18:0	2.23 ± 0.38^{b}	G	6.94 ± 0.59^{a}		6.47 ± 1.53^{a}		6.76 ± 0.53^{a}	
C18:1(n-9)	0.65 ± 0.13^{c}		3.93 ± 0.83^{b}	G	6.05 ± 1.66^{a}	GJ	4.45 ± 0.56^{ab}	G
C18:1(n-7)	0.34 ± 0.06^{c}	G	1.51 ± 0.30^{b}	G	2.51 ± 0.62^{a}		2.87 ± 0.39^{a}	N
C18:2(n-6)cis	0.16 ± 0.03^{b}	GJ	1.01 ± 0.24^{a}	GJ	1.59 ± 0.46^{a}	GJ	1.44 ± 0.40^{a}	GJ
C18:3(n-3)	0.00 ± 0.01^{b}	G	0.17 ± 0.02^{b}	G	4.02 ± 1.30^{a}	J	2.93 ± 0.96^{a}	J
C18:4(n-3)	0.00 ± 0.00	G	0.00 ± 0.00	G	1.20 ± 1.29	GNJ	0.56 ± 0.69	GJ
C20:1(n-9)	0.81 ± 0.14^{b}		1.27 ± 0.11^{a}		1.35 ± 0.27^{a}		1.33 ± 0.04^{a}	
C20:2(n-6)	0.13 ± 0.02^{c}	NJ	0.77 ± 0.12^{a}		0.44 ± 0.10^{b}	G	0.33 ± 0.03^{b}	G
C20:4(n-6)	1.21 ± 0.20^{b}		7.22 ± 0.71^{a}		1.73 ± 0.36^{b}	G	1.96 ± 0.01^{b}	G
C20:3(n-3)	0.43 ± 0.07^{c}		0.81 ± 0.07^{b}		1.08 ± 0.21^{a}		0.95 ± 0.10^{ab}	
C20:5(n-3)	4.19 ± 0.65^{b}	J	6.41 ± 0.67^{b}		7.07 ± 1.38^{ab}		8.31 ± 0.14^{a}	
C22:5(n-3)	0.31 ± 0.05^{b}		0.66 ± 0.06^{a}		0.33 ± 0.06^{b}		0.37 ± 0.02^{b}	
C22:6(n-3)	5.99 ± 0.94^{b}		9.62 ± 1.01^{a}		5.09 ± 0.92^{b}		4.43 ± 0.61^{b}	
UK	0.56 ± 0.09^{b}	J	0.56 ± 0.10^{b}	J	0.82 ± 0.64^{ab}	J	1.32 ± 0.33^{a}	J
SUM	24.05 ± 0.10^{b}	GJ	57.32 ± 0.18^{a}	N	54.57 ± 0.38^{a}	N	54.44 ± 0.20^{a}	J
SFA	8.62 ± 1.36^{b}	GJ	21.34 ± 2.21^{a}		16.90 ± 3.96^{a}		17.47 ± 0.85^{a}	
MUFA	2.35 ± 0.45^{c}		8.04 ± 1.36^{b}	G	11.44 ± 2.71^{a}	GJ	10.50 ± 0.76^{ab}	GJ
PUFA	12.52 ± 1.98^{b}	J	27.38 ± 2.78^{a}	N	25.40 ± 5.80^{a}	N	25.15 ± 0.61^{a}	N
n-3 HUFA	10.92 ± 1.71^{b}		17.57 ± 1.79^{a}		13.86 ± 2.58^{ab}		14.44 ± 0.84^{ab}	
n-3	10.93 ± 1.71^{b}		17.75 ± 1.80^{a}	N	19.67 ± 4.50^{a}	N	18.26 ± 0.87^{a}	N
n-6	1.56 ± 0.26^{c}	J	9.60 ± 1.02^{a}		5.15 ± 1.24^{b}	GN	6.34 ± 0.75^{b}	N
n-9	1.60 ± 0.28^{c}		5.47 ± 0.93^{b}	GJ	8.09 ± 1.85^{a}	J	6.64 ± 0.34^{ab}	GJ
n-3/n-6	7.03 ± 0.10^{a}		1.85 ± 0.04^{d}	G	3.83 ± 0.33^{b}	NJ	2.92 ± 0.49^{c}	J
DHA/EPA	1.43 ± 0.01^{a}		1.50 ± 0.03^{a}		0.72 ± 0.06^{b}		0.53 ± 0.08^{c}	G
EPA/ARA	3.48 ± 0.04^{b}		0.89 ± 0.01^{c}	G	4.10 ± 0.24^{a}	N	4.24 ± 0.07^{a}	N
DHA/ARA	4.96 ± 0.06^{a}		1.33 ± 0.05^{d}		2.97 ± 0.42^{b}		2.26 ± 0.31^{c}	

Data are presented in µg.g DW^{-1} ± SD. **SFA** – saturated fatty acids; **MUFA** – monounsaturated fatty acids; **PUFA** – polyunsaturated fatty acids; **n-3 HUFA** – sum of ω 3 highly unsaturated fatty acids; **n-3** sum of ω 3 fatty acids; **n-6** sum of ω 6 fatty acids; **n-9** sum of ω 9 fatty acids; **DHA** – 22:6 n-3; **EPA** – 20:5 n-3; **ARA** – 20:4 n-6; Different superscript letters within the same row indicate statistical differences ($P<0.05$); Capital letters in the right column of data represent: J – statistical similarity between paralarvae and *Artemia* juvenile ($P>0.05$); N - statistical similarity between paralarvae and *Artemia nauplii* ($P>0.05$); G - statistical similarity between paralarvae and *G. adscensionis* zoeae ($P>0.05$).

Annex V. Fatty acid (FA) composition (% of total lipid content) of hatchlings and 15 days paralarvae fed with *G. adscensionis*, *Artemia nauplii* or *Artemia* juveniles.

FA/ paralarvae	P - 0 DAH	P - GR	P - AN	P - AJ
C14:0	1.18 ± 0.06^{a} G	0.44 ± 0.04^{b}	0.49 ± 0.03^{b}	0.48 ± 0.04^{b}
C16:0	17.97 ± 0.33^{a}	16.35 ± 0.69^{b}	14.28 ± 0.68^{c}	15.82 ± 0.88^{b}
C16:1(n-7)	0.62 ± 0.16^{b}	0.81 ± 0.17^{ab}	0.75 ± 0.08^{ab}	1.05 ± 0.21^{a}
C17:0	1.66 ± 0.02^{a}	1.25 ± 0.05^{b} G	1.62 ± 0.07^{a}	1.75 ± 0.35^{a} G
DMA C18:0	4.89 ± 0.30^{a}	5.65 ± 0.28^{a}	2.77 ± 0.14^{b}	2.09 ± 0.44^{c}
C18:0	9.26 ± 0.11^{c}	12.13 ± 0.34^{b}	12.48 ± 0.63^{b}	13.50 ± 0.51^{a}
C18:1(n-9)	2.69 ± 0.16^{d}	6.83 ± 0.88^{c}	11.60 ± 0.76^{a}	8.88 ± 0.83^{b}
C18:1(n-7)	1.40 ± 0.06^{d}	2.63 ± 0.32^{c}	4.83 ± 0.22^{b} G	5.72 ± 0.59^{a} G
C18:2(n-6)cis	0.68 ± 0.03^{c}	1.75 ± 0.31^{b} J	3.04 ± 0.21^{a} JG	2.86 ± 0.70^{a} JG
C18:3(n-3)	0.06 ± 0.00^{c}	0.30 ± 0.04^{b}	7.66 ± 0.87^{a}	5.82 ± 1.71^{a}
C18:4(n-3)	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	2.23 ± 2.30^{a} JN	1.15 ± 1.45^{ab} JG
C20:1(n-9)	3.36 ± 0.10^{a}	2.22 ± 0.08^{c}	2.62 ± 0.12^{b}	2.67 ± 0.07^{b}
C20:2(n-6)	0.53 ± 0.03^{d}	1.34 ± 0.13^{a} G	0.85 ± 0.03^{b}	0.67 ± 0.03^{c}
C20:4(n-6)	5.02 ± 0.04^{b}	12.60 ± 0.26^{a}	3.36 ± 0.30^{d}	3.92 ± 0.18^{c}
C20:3(n-3)	1.80 ± 0.07^{a}	1.41 ± 0.08^{b}	2.10 ± 0.13^{a}	1.91 ± 0.27^{a}
C20:5(n-3)	17.45 ± 0.10^{a}	11.19 ± 0.31^{c} J	13.71 ± 0.66^{b}	16.62 ± 0.76^{a}
C22:5(n-3)	1.30 ± 0.02^{a}	1.16 ± 0.05^{b}	0.64 ± 0.04^{d}	0.74 ± 0.07^{c}
C22:6(n-3)	24.89 ± 0.16^{a}	16.80 ± 0.74^{b}	9.91 ± 0.91^{c}	8.89 ± 1.58^{c}
UK	2.32 ± 0.03^{a} J	0.97 ± 0.07^{b}	1.56 ± 1.11^{ab} JG	2.66 ± 0.78^{a} JN
SFA	35.86 ± 0.35^{ab} G	37.24 ± 0.46^{a} G	32.63 ± 1.58^{c}	34.53 ± 0.81^{b} G
MUFA	9.75 ± 0.35^{c}	13.98 ± 1.17^{b}	21.32 ± 1.22^{a}	19.86 ± 1.59^{a}
PUFA	52.06 ± 0.26^{a}	47.80 ± 1.31^{b}	44.49 ± 1.60^{c} N	42.95 ± 1.49^{c} N
n-3 HUFA	45.44 ± 0.23^{a}	30.68 ± 1.10^{b}	26.92 ± 1.41^{c}	28.19 ± 2.45^{bc}
n-3	45.45 ± 0.24^{a}	30.98 ± 1.09^{c}	36.82 ± 1.91^{b} N	35.44 ± 1.94^{b} N
n-6	6.47 ± 0.09^{c} JN	16.74 ± 0.21^{a} G	7.68 ± 0.31^{b} JN	7.50 ± 0.45^{b} JN
n-9	6.66 ± 0.11^{d}	9.50 ± 0.80^{c}	14.88 ± 0.90^{a}	12.18 ± 0.83^{b}
n-3/n-6	7.03 ± 0.10^{a}	1.85 ± 0.04^{c}	4.81 ± 0.45^{b} JN	4.75 ± 0.56^{b} JN
DHA/EPA	1.43 ± 0.01^{a}	1.50 ± 0.03^{a}	0.72 ± 0.06^{b}	0.53 ± 0.08^{c} G
EPA/ARA	3.48 ± 0.04^{b}	0.89 ± 0.01^{c} G	4.10 ± 0.24^{a} N	4.24 ± 0.07^{a} N
DHA/ARA	4.96 ± 0.06^{a}	1.33 ± 0.05^{d}	2.97 ± 0.42^{b}	2.26 ± 0.31^{c}

Data presents in µg/g of sample in dry weight ± SD. **SFA** – saturated fatty acids; **MUFA** – monounsaturated fatty acids; **PUFA** – polyunsaturated fatty acids; **n-3 HUFA** – sum of ω 3 highly unsaturated fatty acids; **n-3** sum of ω 3 fatty acids; **n-6** sum of ω 6 fatty acids; **n-9** sum of ω 9 fatty acids; **DHA** – 22:6 n-3; **EPA** – 20:5 n-3; **ARA** – 20:4 n-6; Different letters in superscript within the same row indicate statistical differences at the P<0.05 level; Capital letters in the right column of data represent: J – statistical similarity between paralarvae and *Artemia* juvenile (P>0.05); N - statistical similarity between paralarvae and *Artemia nauplii* (P>0.05); G - statistical similarity between paralarvae and *G. adscensionis* zoeae (P>0.05).