Current status and bottle neck of octopod aquaculture: the case of American species

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Abstract

The increasing market demand for cephalopods and the experiences obtained with different species has boosted the interest in developing their culture in Latin America. In 2008, an international workshop was held in Puerto Montt, Chile, with 14 experts in experimental cephalopods aquaculture from Brazil, Chile, Spain and Mexico. Several topics were approach within the holobenthic species Octopus maya and the merobenthic species Enteroctopus megalocyathus, Octopus vulgaris and Robsonella fontaniana. Part of the conclusions demonstrated that the two greatest difficulties for their production were survival of paralarvae for merobenthic species, and survival of early juveniles for holobenthic species. Besides, there is a need to study the endogenous and exogenous factors affecting health and nutritional status of embryos, paralarvae and juveniles. These stages, which may limit the culture, should be extensively studied in order to develop the appropriate environmental conditions and culture systems for the physiological and behavioural requirements, from egg incubation up to juveniles to reach a grow-out phase.

Key words: octopus, larviculture, Octopus vulgaris, Octopus maya, Octopus mimus, Enteroctopus megalocyathus, Robsonella fontaniana, holobenthic/merobenthic species.

Introduction

From cephalopods, octopuses are considered economically interesting species for aquaculture. Their fishery has been steadily decreasing since the 90's, which has led to increase the demand for octopuses and thus the technological-scientific efforts to culture them from egg or paralarva up to a second stage (Boletzky and Hanlon 1983). Many cephalopods have been subject of several studies in captivity intended to investigate behavioural aspects (Hanlon and Wolterding 1989; Hochner et al. 2006), used as models in neurophysiology studies (Flores 1983; Wollesen et al. 2009), in predator-prey relationships (Villanueva 1993; Scheel 2002; Smith 2003) or to provide live specimens for aquariums (Summer and McMahon 1970; Bradley 1974; Anderson and Wood 2001). These have been the main reasons to explain why about 10% of cephalopod species (approximately 70 of the 700 known species) have been studied up to the 90's (Boletzky and Hanlon 1983). This is a high number compared to other marine invertebrates or fish species. The results from those studies have not been applied at a commercial scale.

Coastal octopuses have short life cycles (from 6 months to 3 years) and high growth rates, which imply a great potential to compete with fish in the market. However, octopuses are carnivorous and have a high demand for proteins during their entire life cycle (Houlihan et al. 1990; Giménez and García 2002; Iglesias et al. 2004; Domingues et al. 2007; Águila et al. 2007; Cerezo-Valverde et al. 2008) and apparently a high demand for highly unsaturated fatty acids during the reproductive conditioning, paralarval and early juvenile stages (Navarro and Villanueva 2000, 2003; Iglesias et al. 2007; Seixas et al. 2010;

Farías et al. 2011). On the other hand, paralarvae stage is sometimes long and depending on alive preys with an adequate nutritional composition (e.g. natural zooplankton), which makes their culture difficult. Other aspects that limit their culture is the wide growth rate variation (André et al. 2008, 2009) and low tolerance to salinity and oxygen concentrations (Hanlon 1987; Borer and Lane 1971; Boucher-Rodoni and Mangold 1985; Katsanevakis et al. 2005; Villanueva and Norman 2008).

Examples from a direct-development from holobenthic cephalopods with no planktonic stage are the Mexican red octopus Octopus maya and O. bimaculoides. *O maya*, species is endemic from the Yucatan Peninsula, its large size at hatching favours their feeding in captivity. Both species are considered as potential candidates for aquaculture. O. bimaculoides is a medium sized octopus (60 cm), distributed from central California (Santa Barbara), USA, to the west central coast of the Baja California Peninsula, Mexico. Grows to a maximum size of 800 g and has a lifespan of 1–1.5 years. It produces large eggs (~13 mm), with holobenthic development and has shown an easy adaptation to captivity (Solorzano et al. 2009). Whereas an indirect-developing merobenthic cephalopods with a planktonic (paralarval) stage is the common octopus Octopus vulgaris, a cosmopolitan species abundant in the Atlantic Ocean; O. mimus and Robsonella fontaniana, both found in the Southern Cone of South America; and Enteroctopus megalocyathus, an octopus native from the Patagonia. The aquaculture of merobenthic species is still experimental due to the feeding habits of paralarvae, which is the main problem (Vidal et al. 2002a, b; Villanueva 1994, 1995; Moxica et al. 2002; Iglesias et al. 2004; Carrasco et al. 2005).

In 2005, an International Workshop on O. vulgaris larviculture was held with experts from Spain, Brazil, Belgium, Japan and Norway, in both paralarval culturing and alive feeding practices for the different development stages from these species. In this workshop, the different rearing systems used in aquaculture were described, the main causes of larval mortality discussed, and the priorities in the future research lines established (Iglesias et al. 2007).

In the last decade, studies on nutrition (Águila et al. 2007, Domingues et al. 2007; Martínez et al. 2010, 2011; Quintana et al. 2010; Rosas et al. 2007, 2008, 2010; Solorzano et al. 2009; Seixas et al. 2010), culture conditions (Baltazar et al. 2000; Domingues et al. 2002; Vidal et al. 2002a, b; Carrasco et al. 2006; Pérez et al. 2006; Uriarte et al. 2010a, b), physiology (Rosas et al. 2007; André et al. 2008; Farías et al. 2009), growth (Doubleday et al. 2006; André et al. 2008, Briceño et al. 2010a,b; Uriarte et al. 2010a) and reproduction of cephalopods (Zúñiga et al. 1995; Santos-Valencia et al. 2000; Rocha et al. 2001; González et al. 2008; Uriarte et al. 2008; Farías et al. 2011) have proliferated. These investigations show that cephalopods, particularly juvenile and adult octopuses, can easily be maintained in culture conditions. However, they show high mortality rates during the first months of life (either as paralarvae or early juveniles) due to the lack of an appropriate food supply to meet their nutrition requirements. This is the great challenge besides the systems and technologies necessary for a mass culture (Solorzano et al. 2009;

Moguel et al. 2010; Uriarte et al. 2010b). Artemia is the most used and known prey in aquaculture, with strategies of enrichment to be used as food source for different shrimp and fish species (Navarro et al. 1999). While artemia has shown good results for octopuses, it is not the most suitable diet when has to be used at lower temperatures and high levels of n-3 unsaturated fatty acids (n-3 HUFA) enrichment is required.

In America some research groups have shown their interest to culture different octopus species. Until now several biological aspects has been investigated on O. bimaculoides, O. mimus, *O. maya,* O. vulgaris, Enteroctopus megalocyathus, Robsonella fontaniana showing that octopus aquaculture could be possible in the medium term. This work reviews the research results on octopus culture in America with an attempt to meet the actual knowledge about the biological aspects and highlight the future research needed to succeed the octopus aquaculture.

Current Status of the Octopod Culture in Different Species and/or Disciplines: A Perspective from South to North

Current Status of E. megalocyathus Production of Eggs and Paralarvae.

The Patagonian red octopus (E. megalocyathus) is a species native to the Patagonian coasts of Chile and Argentine (Ortiz et al. 2006). In 2008, E. megalocyathus represented the 63% from the total octopuses caught along the

Chilean coast (SERNAP 2008). This particular species has been banned for three years for fishery in Chile starting November 2008, reason that has lead their aquaculture as an important issue. At the UACH facilities in Puerto Montt, research has been undertaken with positive results like the culture conditions determined like controlled egg spawning, among others. Handling of broodstocks for acclimatization and achieving controlled spawning has not shown any problems in E. megalocyathus culture; for instances spawning of up to 3,000 eggs/female measuring between 7.5 and 11.5 mm (peduncle not included) have been obtained. Different diets have been tried in order to obtain the reproductive conditioning of Patagonian red octopus. According to Uriarte et al. (2008) the best diets is based on 100 to 70% crustaceans meat complemented with fish flesh. Crustaceans are being used for the broodstock female conditioning using the Chilean crab Cancer edwarsii and the ghost shrimp Callianassa garthi. When fish is used this can be offer either as fresh or frozen from the silverside fish Odontesthes regia. The fertilized eggs were obtained using the accumulated temperature method after 500 degrees in 120 days conditioning starting at 11 C, with and average yield of 3.697±758 eggs per female (n=9) with a 67% success in female conditioning. Females increased up to 85% of their initial body weight during the conditioning period. Moreover, Farías et al. (2011) working with conditioning diets found that egg quality given as proximate composition or fatty acid profile, were not affected neither by the type of diet, nor by the amount of food with diets based on fish and mixed with crustaceans, or under restricted diets. Nevertheless, females fed under feed restriction a significant lower fecundity was observed without affecting the egg quality.

Finally, the eggs can be incubated with parental care at 12 C, achieving a complete development within 150 days. Some microbiological aspects were critical during the embryonic development of E. megalocyathus eggs, being susceptible to microbial infections adhered to their bodies, resulting in death. At the CIEN Austral facilities (Puerto Montt, Chile) several bacteria present in the octopus eggs have been characterized to set up a health management (Uriarte et al. 2008). The most relevant bacteria found in those infection processes, are the following: Thalassomonas viridans, Colwellia piezophila, Marinosulfonomonas methylotropha, Pseudoalteromonas elyakovii, Sulfitobacter donghicola, Sulfitobacter mediterraneus, and Cobetia marina. Being found in infected eggs: Neptunomonas naphthovorans, Pseudomonas fulva, Pseudoalteromonas atlantica, Sulfitobacter donghicola, and Sulfitobacter mediterraneus. Morphologically, the infected eggs were characterized by changes in their colour (ranging from whitish to yellow), presence of swelling and decreased turgidity (Fig. 1A). Most eggs showed filaments on their surface (Fig. 1B). Through scanning electron microscopy, a high number of microorganisms were observed on their surface, revealing filaments containing cells grouped in chains, these filaments were similar to those observed with the optical microscope (Fig. 1C). No bacteria were found on the egg surface at spawning (Fig. 2A). At day 15 of incubation, the eggs were colonized by bacteria which formed a biofilm on their surface. When comparing healthy with infected eggs (Fig. 2B and 2C), it was clearly observed that the latter had a higher bacterial density with an evident presence of filamentous bacteria. The filaments appeared to be inserted into the pores from the egg surface, probably restraining the egg to exchange with the environment (Fig. 2D). The infected eggs also contaminated the healthy ones. As a health management measure, it is proposed that once the eggs have been spawned, the formation of bacterial biofilms should be avoided by exposing the eggs to a constant water flow.

E. megalocyathus wild juveniles have been reared in recirculation systems at Fundación Chile facilities (Quillaipe Chile). Growth rate of juveniles in the pre fattening period was evaluated using 10-500 g octopuses individually marked (with pit tag from equipment Trovan GR-250), fed with fresh fish and at 18±1 C and salinity of 33 ppm. During the first three months, the specific growth rate (SGR) of the juveniles varied between 0.33 and 1.25%/day, with an average of 0.92±0.1%/day (Fig. 3). The initial octopus size did not showed to have any effects on the SGR, which means that 10 g juveniles can reach 100 g in an average culture period of 8 months.

Nutrition studies conducted performed so far using wild juveniles show that E. megalocyathus grows better when fed fresh fish, associated to higher feed intake, as neither the digestibility nor the enzymatic activities from the hepatopancreas could be related to growth (Farías et al. 2010). A SGRs of 1.46 and 0.27%/day was observed in the Patagonian red octopuses fed fresh fish and crab paste, respectively. Values of 0.49 to 1.96%/day have been observed in the wild E. megalocyathus fed fresh crab within different rearing periods. When juveniles are fed a diet based on fresh mytilids resulted in the loss of growth –0.32%/day (Perez et al. 2006), an indication that the range of growth expected for juvenile Patagonian red octopuses could be close to 2%/day.

Current Status of the Larviculture of R. fontaniana with Emphasis on the Paralarval Culture Conditions.

R. fontaniana (D'Orbigny, 1834) is a small-sized octopus ("*baby-octopus"*) distributed along the Chilean and Peruvian Pacific coasts and part of the Argentine Atlantic Coast. R. fontaniana eggs are easy to collect from their natural environment. Great number of hatched paralarvae can be found in the reproductive season. Some studies on the reproductive biology of R. fontaniana indicate that the species can easily spawn up to 2500 eggs (Rocha et al. 2001; González et al. 2008) measuring from 2.4 to 4.7 mm (Uriarte et al. 2009). Adult organisms do not exceed 200 g, and its tolerance to farming conditions makes it a good candidate for cephalopod aquaculture (grouped with the "baby octopuses").

Embryonic development of R. fontaniana is faster than that of E. megalocyathus, where 68 to 71 days at 12 C can be observed (Uriarte et al. 2009). Similar to O. vulgaris, an exponential reduction in the yolk volume (Uriarte et al. 2009) inversely proportional to growth was observed during R. fontaniana embryonic development. On average, yolk weight of octopuses at hatching was 11.6±2.1% of the total egg weight, and hatched paralarvae was fed Lithodes santolla lecithotrophic larvae (3.1-5.6 mm).

Paralarvae completed their development in 70 days, reaching a survival rate of 33% with a critical mortality period at days 30-35. After 70 days reared at 12 C and 10 mm length (Fig. 4), the paralarvae showed a settlement behaviour, and 10 days after settlement, survival dropped to a critical value of 5% (Uriarte et al. 2010a). Paralarvae maintained an exponential growth from day 14 after hatching until reaching the size of a juvenile (29.7 mm) at 120 days after hatching (Fig. 4).

During the paralarval development of cephalopods, particularly from octopuses, changes are taken in the digestive physiology which is reflected in the structure of the digestive gland and the enzyme activity. Knowing how these changes occur will be the key to design the type of food and management that should be provided to animals under culture conditions. At the moment of hatching, yolk content of R. fontaniana paralarvae represents the 13% of their total body weight, which enables them to survive up to 5 days without food. The high acid phosphatase activity registered shows that paralarvae just after hatching uses their yolk reserves as the main source of energy (Pereda et al. 2009). Figure 5 shows the change of soluble protein content over time, which increases with the paralarva age. Similarly, an increase in trypsin could be observed, while acid phosphatase activity remained stable over time. This high proteolytic activity occurring during the paralarval development may indicate the digestive system maturation and an improved ability of paralarvae to digest increasingly complex preys, and preparing them to face the adult feeding activities.

Severa aspects regarding the feeding behaviour in nature of R. fontaniana paralarvae and their preys are unknown. Predatory activity of O. vulgaris paralarvae has previously been studied to establish how they react to the presence or absence of different preys (Villanueva et al. 1996; Villanueva and Norman 2008). From these studies, it is inferred that swimming speed of paralarvae ranges between 30 and 70 mm/s and drops to 12.8 mm/s during prey capture. It has been observed that swimming speed of Pagurus prideaux zoeae used as prey is slower than that observed on the paralarvae, which could facilitate their capture (Villanueva et al. 1996). As proposed by Iglesias et al. (2006), other preys such as artemia metanauplii/juveniles (1.4±0.44mm) have been used for feeding O. vulgaris paralarvae. The feeding studies of paralarvae of R. fontaniana with artemia metanauplii have showed a reduced suitability (González et al. 2008; Pereda et al. 2009; Uriarte et al. 2010b), whereas L. santolla zoea has shown good results (Uriarte et al. 2008, 2010a, b; Pereda et al. 2009).

It is possible to know whether larvae from other crustaceans, apart from L. santolla zoeae, could be used to feed them. L. santolla and Petrolistes laevigatus zoeae showed two different behaviors: P. laevigatus zoeae showed positive phototaxis and L. santolla zoeae showed positive geotaxis (Uriarte et al., 2008). Maximum swimming speed of zoeae from both species ranged from 20 to 30 mm/s; speed that was intermittent for L. santolla and continuous for P. laevigatus. Feed ingestion rate experiments showed that L. santolla and P. laevigatus zoeae were consumed at a ratio of one zoea paralarva/day during the first 16 days of life of R. fontaniana paralarvae. Despite the similarities in the ingestion rates, P. laevigatus resulted in a nutritional disadvantage than that observed with L. santolla. A low dry weight (80 µg/larva), along with a lower protein (20 µg/larva) and lipid (25 µg/larva) content plus the positive phototaxis and the size of their faces demonstrated why P. laevigatus zoeae are not an appropriate prey for R. fontaniana paralarvae. In contrast, a high proportion of the dry weight (1000 µg/larva), proteins (350 µg/larva) and lipids (220 µg/larva) of L. santolla zoea may seem the conditions that favour the nutrition and development of R. fontaniana paralarvae (Uriarte et al. 2010b).

Current Status of Octopus mimus Aquaculture

The importance of common octopus (Octopus mimus) in the Chilean fishery besides their biological attributes have stimulated to support the idea to name this species as potentially candidate for Chilean aquaculture, and idea that requires the development of an appropriated integral aquaculture from broodstock management to grow-out juvenile technology.

The O. mimus inhabits the Southeastern Pacific coast from the north of Peru to the San Vicente bay in Chile (Guerra et al. 1999), being an important resource for the artisanal benthonic fishing ground from both countries (Osorio 2002; Rocha and Vega, 2003; Cardoso et al. 2004).

The O. mimus reproduces throughout the year, with one or two seasonal peaks of mature females, being specific for each locality and without latitudinal gradient (Cortez et al. 1995; Olivares et al. 1996; Cardoso et al. 2004). The egg laying can be extended for 20 days due to asynchrony of the ovocytes development and the loss of the ovary function that predisposes their semelparous condition (Zamora and Olivares, 2004). The length of the embryonic development changes with the environmental temperature; in winter at 16 C it lasts 67 to 68 days whereas in summer at 20 C lasts between 38 to 43 days (Warnke 1999; Castro et al. 2002). However, the seasonal temperature effect on the morphometric and biochemical characteristics of the egg and paralarvae is not known. During the El Niño occurrence (“ENSO") when sea temperature reachs 24 C, the embryonic development lasts 25 days under laboratory conditions (Warnke 1999). This could explain that during ENSO 94-95 the fishery of common octopus increased significantly (Baltazar et al. 2000).

Under laboratory conditions, the sexual maturity of immature females can be controlled by means of photoperiod (Zúñiga et al. 1995) or the feeding, in any stage of sexual maturity.

The paralarvae of O. mimus during the first days are nourished by means of the nutritional yolk reserve, which lasts until day 5 or 6th after hatching. When paralarvae of common octopus is fed with artemia nauplii, or zoeas from Cancer setosus or Leptograsus variagatus, with temperatures around 19 to 26 C, the survival lasted only 12 days (Zuñiga and Olivares comm pers). The lack of knowledge of suitable paralarval feeding regime is then the bottleneck to obtain a successful juvenile production.

Even if the studies on aquaculture of O. mimus is an activity with increasing interest, the results are still at level of laboratory experiences (Olivares et al. 1996; Cortez et al. 1999; Baltazar et al. 2000, Pérez et al. 2006, Carrasco and Guisado 2010).

At the Universidad de Antofagasta facilities (Antofagasta, Chile) it has been determined the culture conditions during the grow-out of wild O. mimus juveniles, mainly focused to obtain optimal density conditions and an appropriated diet. Best SGRs were recorded in common octopuses fed with a paste made from clam with fish, or fresh clams, respectively, whereas only fish paste from salmon resulted in a decrease of weight. The fish that was successfully eaten was Cheilodactylus variegates, whereas the clams used corresponded to the common clam Protothaca thaca. The paste was prepared using gelatine as binder and extruded as sausages using lamb intestines as coat. The ingestion obtained with this paste was similar to that observed with fresh clam. The study of culture density effect on the growth rate during grow-out of wild juveniles, using densities of 5, 10 and 15 octopuses/m^2, showed that growth was maximized from 5 to 10 being significantly higher that than obtained with 15 octopuses/m^2, at least until day 60.

Current Status of O. vulgaris Paralarval Aquaculture from Brazil, with Emphasis on the Environmental Conditions Influences on the Embryonic Development and Yolk Reserves.

Environmental conditions are considered to play important roles to determine the eggs and larval quality in invertebrates and fish (Benzie 1998). However, much of the research done with O. vulgaris paralarvae aquaculture has been focused on the survival, growth and nutritional requirements (Imamura 1990; Villanueva 1994, 1995; Iglesias et al. 2002, 2004), with no much attention to the given factors affecting the embryos development and consequently, paralarval quality.

It has been documented that temperature has a dramatic impact on embryonic development of cephalopods (Boletzky 1987). Eggs incubated at lower temperatures yield larger hatchlings correlated as well to the yolk utilization rate into embryonic tissue and, therefore, the size of paralarvae at hatching (Vidal et al. 2002b). After the hatching, paralarvae rely on both endogenous (yolk) and exogenous (prey) food sources (Boletzky 1989; Vidal et al. 2002a, 2002b). Moreover it has been estimated that yolk content at the moment of hatching represents around 35-45% of the body dry weight and 10-15% of the body wet weight of squid paralarvae (Vidal et al. 2002b). In the same sense, paralarvae undergo a period where no growth is reported caused by the decrease in body mass due to yolk utilization, energy that it is being used as metabolism fuel during the first few days after hatching (DAH). As a result, the weight lost during yolk utilization is regained only through exogenous feeding resulting in a phase of not net growth. Vidal et al. (2002b) have proposed this life cycle characteristic, to be the cephalopod equivalent of the “critical period” phase found in larval fish and is mainly related to the high metabolic rate of paralarvae (Parra et al. 2000) and their high sensitivity to starvation (Vidal et al. 2006).

Observations under experimental conditions showed that the yolk content during hatching influences the survival of squid paralarvae during rearing (Vidal, 2002b, 2005). Both, the rate and the efficiency of yolk utilization are crucial for early development, growth and survival of paralarvae (Vidal et al. 2002b, 2005) and the same can also be expected for O. vulgaris paralarvae. Therefore it is important to understand the influence of environmental conditions on yolk utilization during embryonic development on the production of high quality paralarvae.

With the purpose of evaluating the influence of temperature on the conversion rates of yolk into tissue during embryonic development and on yolk utilization rates of paralarvae after hatching, studies were undertaken with O. vulgaris in southern Brazil. Eggs were incubated at 24±1 C with a salinity of 33 and samples of 50 eggs were obtained every 48 h from the first day of egg spawning until hatching. Embryos images and measurements were obtained using a light microscope coupled to a Zeizz camera. The yolk volume of eggs was estimated by superimposing standard geometric forms (ellipsis, cylinders and spheres) onto and then, volumes were converted into yolk weights (Fig. 6). Embryonic development lasted from 25 to 32 days. After hatching, paralarvae was maintained at two temperatures, 19 and 24 C, in the absence of food and their survival time recorded.

The preliminary results indicated that eggs incubated at 24±1 C yielded paralarvae with a mean yolk reserve of 23% of its body wet weight at hatching. Yolk weight decreased 48% during embryonic development, representing only 13% of the paralarvae dry weight at hatching. The yolk reserve allowed the paralarvae to survive up to eight days at 19 C and 10 days at 24 C in the absence of food; they were maintained exclusively on the energy derived from their yolk. However, the highest mortality rates were obtained earlier (day 3) for paralarvae maintained at the highest temperature. These results indicated that the yolk reserve is of vital importance for the survival of paralarvae during the first days after hatching, which corresponds to the critical period, where the highest mortality rates were observed (Villanueva, 1994, 1995; Vidal et al. 2002a, b, 2005; Iglesias et al. 2004). The development of effective technologies to obtain paralarvae of reliable quality for culturing will require high-quality research on fundamental aspects that environmental factors influence the embryonic development of cephalopods.

Current Status of Aquaculture of O. maya Juveniles in Mexico

The O. maya is a holobenthic species endemic to the Yucatán Peninsula. It is one of the most exploited species in Mexican fisheries and has an annual catch of over 10,000 tons (Santos-Valencia and Re-Regis 2000; Hernández-Flores et al. 2001). Studies conducted to date have shown that this species can be maintained under laboratory conditions for several generations and those juveniles can be fed alive, non-living, fresh or frozen food (Van Heukelem 1976, 1977, 1983; Solís 1998). At UNAM facilities (Yucatán Mexico), there is an experimental pilot unit for the production and rearing of *O. maya*. Between 2006 and 2010, 250 clutches were spawned with a total yield of 200,000 eggs with a wet weight of 0.13±0.001 g (N=553) at hatching from wild females (815±16g live weight). Females were conditioned for 30 days period, time they were fed a mixed diet made up from crab and mussel *Mytilus spp*. (75 and 25%, respectively) fed at 5% of their body weight/day. From the total spawns, 85% of females spawned fertilized eggs, 90% from them hatched after 45-60 days. With an average of 522±22 eggs per clutch were obtained, where only 15% of females yielded unfertilized eggs.

O. maya juveniles were reared in ten 8 m^2 tanks at densities of 25 to 125 animals/m^2. A maximum growth rate (6%/day) was observed when animals were maintained up to 60 days until they reach a wet weight of 2g at a density of 25 animals/m^2 (Mena et al, 2011.). This culture stage call as “pre-growout” was done in a semi-dark environment (90 lux/cm^2). Tanks were connected to a large-scale 4000 L recirculating seawater system maintained at 40 UPS. Using a squid paste as food a survival of 50% was obtained in such conditions. Survival can oscillate between 3 to 65%, depending on food type, intake, and feeding frequency. For the first two years *Artemia spp*. adults, are used as prey for juveniles during the first 15 days of age; afterwards, the animals are fed with crab paste bound with gelatine. A recent study showed that the use of gammarids (*Hyalle spp*) could be a better alive food option during the post embryonic stage of animals (Baeza-Rojano et al. 2011). During juvenile culture, squid paste is supplied three times a day in a proportion of 30% from the juvenile body weight (Rosas et al. 2007, 2008; Quintana et al. 2010). Using 7 and 19.6 m^2 outdoor tanks O. maya juveniles of 40 to 100 g can be obtained during 120 to 150 days period, with a survival of 50% when fed squid paste. Even if this data are under experimental conditions, will be necessary to perform further studies at commercial scale to obtain a better approach for production of O. maya.

Growth rate of O. maya juveniles is exponential and highly variable, with an average growth of 3%/day during the first 105 days (Fig. 7) (Briceño et al. 2010b). Briceño et al. (2010a) obtained other interesting data on physiology of hatchlings, as energy budget; when the energy needed was supply from the food intake (I) the energy needed for body mass as production (P) and respiration rate (R) as a function of weight and age during the exponential early growth stage from. In that study was highlight that when O. maya juveniles hatched, they have a greater requirement for respiratory metabolism (R) rather than for biomass production (P), suggesting a high metabolic cost associated with post-embrionic stage (Moguel et al. 2010). For this reason a high quality food should be provided during the first 15 days to satisfy the high energetic demands they have during this stage of their life. Gammarids has been used with high success to feed O. maya juveniles during this post embryonic stage (Baeza-Rojano et al. 2011)

Nutrition studies conducted to date show that digestive process of O. maya occurs in a slightly acid environment, with a pH ranging between 5 and 6 in the gastric juice through which food passes from the anterior stomach to the digestive gland (Martínez et al. 2010; Moguel et al. 2010). In the digestive gland it has been observed that juveniles use 8 h to complete the digestion process before the next feeding cycle (Martínez et al. 2011). In a more recent study was found that during starvation the juveniles of O. maya used preferentially Thr, Phe, Ile, Ala, Glu and Ser, suggesting a strong mobilization of both essential and non essential amino acids to maintain the homeostasis, amino acids that should be considered when a formulated diet is designed for O. maya.

Biological Aspects of Octopus bimaculoides Aquaculture

Since the paper of Pilson and Taylor (1961) describing how Octopus bimaculoides and O. bimaculatus can drill holes in the shells of their molluscan prey, through which they appear to inject a paralyzing venom, many aspects of biology from the O. bimaculoides has been studied in the last 50 years. Mainly, this research has been done at the Marine Biomedical Institute, of The University of Texas Medical Branch, (Galveston Texas, US), between the 70s and 90s. Hanlon and Forsythe (1985) found that O. bimaculoides showed “superior qualities for laboratory culture” and proposed this species as suitable for aquaculture due to their tolerance to rear at high densities with low cannibalism and no diseases at temperatures between 18 to 25 C. In a pilot scale using a large-scale 2600 L recirculating seawater system, O. bimaculoides juveniles were fed marine crustaceans alive, fish and other mollusks. In such conditions was observed that this species spawns from 250 to 750 eggs (10 to 17 mm long) per brood. The embryonic development was affected by temperature with 55 days for eggs maintained at 24 C and 85 days for eggs maintained at 18 C. Hatchlings have around 70mg wet weight and showed a growth rate between 4 to 7%/day during early exponential growth phase and 2 to 4%/day as late juveniles and adults. Conversion efficiency during growth stage was between 40 to 60% and an estimated life spam of 12 to 14 months was observed. A maximum size found in nature was 800g, observed while animals reached until 887g in laboratory. In other study, when O. bimaculoides juveniles were fed alive crabs (control diet), frozen shrimp (Penaeus spp) and marine worms (Nereis virens) showed that octopuses grew comparably to the control animals when fed frozen shrimp with growth rates between 2.6 and 2.8%/day (DeRusha et al. 1989). Although Hanlon and Forsythe (1985) observed that juveniles from this species are able to tolerate high densities, Cigliano (1993) observed that dominance factor between animals can lead to size variability due to behavioral factors. This characteristic prevents, in culture condition the use of enough shelters to avoid competition as it has been observed in other species (Domingues et al. 2011). O. bimaculoides late juveniles showed a high tolerance to complete anoxia for 4 (10C) to 8h (6 C) with no outward signs of stress or damage followed to a brief recovery (Seibel and Childress, 2000). Such characteristic could be an advantage under culture, when power failures or pump damage condition is common. According to Sinn (2008) O. bimaculoides juveniles under natural daylight conditions and constant food availability have the tendency to show a nocturnal activity, allowing the animals to avoid conspecific competition.

The most recent paper published on O. bimaculoides nutrition showed that the best diet for hatchlings 1 to 20 days age was Artemia salina adults enriched with AlgaMac (3050 flake, coarse flake particle 1.5 mm, Aquafauna Biomarine Inc., Hawthorne, CA, USA; crude protein: 17.6%, crude lipid: 56.2%, carbohydrates: 15.9, ash: 8.2). With this type of diet a growth rate of 4.05 5/day and 83.35 survival was registered. Also was observed that lysine and arginine were important amino acids for hatchlings suggesting that both amino acids should be considered when formulated diets are made (Solorzano et al. 2009).

Discussion

The existence of holobenthic and merobenthic species in America offers the opportunity to conduct research in areas of knowledge that allow researchers to solve the major problems in the development of octopus culture globally: rearing of paralarvae and early juveniles (Iglesias et al. 2006; Águila et al. 2007; Iglesias et al. 2007; Cerezo-Valverde et al. 2008).

According to the above mentioned results, it is possible to obtain 30-day-old O. vulgaris paralarvae using zooplankton and microalgae-enriched *Artemia* as food (Iglesias et al. 2007). O. vulgaris paralarvae have been massively reared in 1000 L tanks; however, mortality rates continue to be high, so further research is required to develop a transferable technology. On the other hand, the main problem for holobenthic cephalopods such as O. maya is the lack of a formulated diets to meets their nutritional requirements, which limits their mass culture. Up to date there is a diet for O. maya made on laboratory scale with high success, however, commercial diets will be necessary to support the commercial production of these species to obtain the production costs and the profitability of their aquaculture.

In this context, the following questions emerge regarding the paralarval rearing of octopuses: What conditions should be offered to paralarvae to obtain high survival rates until they reach the benthic juvenile stage using O. vulgaris, *E. megalociathus,* O. mimus *and* R. fontaniana*,* as models? Answering this question requires the research developing related to:

i) Characteristics of broodstock: The nutritional condition of females during the controlled culture (reproductive conditioning) and their effects on the quality and quantity of the offspring. This is a topic that should be considered in research for to obtain paralarvae and/or juveniles. This is particularly relevant when paralarvae are obtained from natural spawns as in the case of all American octopus species, since reproduction of several species are seasonal. This suggests that some months of the year may be more favourable than others for paralarvae to survive, both in an ecological-environmental context and in a female physiological condition context (Rosa et al. 2004, 2005; Otero et al. 2007; Leporati et al. 2008). Reproductive conditioning experiments with E. megalocyathus have shown that eggs can be obtained under controlled laboratory conditions in any season of the year. However, the reproductive behaviour that guaranties high egg fertilization by males is still unknown. The paralarvae production under controlled conditions does not show any problems for O. vulgaris (Iglesias et al. 2007); however, the information available on this matter has not yet been standardized in a secure technology for aspects such as type of tank, type and intensity of light, dissolved oxygen, optimal temperature, type of shelter, quality of the diet, etc. for broodstock conditioning. The aforementioned topics are required to be studied in a short and medium term, as well as the relationships between the characteristics of broodstock conditioning and the quality of paralarvae.

ii) Paralarval nutrition: Topics such as the type of alive food for paralarvae, the nutritional aspects of Artemia enrichment, and the nutritional physiology studies during the paralarval development (digestive capacity, enzyme activity, enzyme proteomics, use of vitelline reserves, essential fatty acid requirements, among others) should be included in future research programs focused on cephalopods. Recently, Villanueva and Norman (2008) reviewed the knowledge on biology and physiology of cephalopod paralarvae indicates that the new way to consolidate paralarval rearing is to meet their nutritional requirements, physiology, behaviour and environmental parameters. The results obtained for R. fontaniana show how the digestive capacity of paralarvae can be improved with an adequate diet (Pereda et al. 2009).

Culture system technology such as clear water versus green water, and conditions such as tank type, water flow and luminosity seem to be highly important to consolidate paralarval rearing (Villanueva and Norman 2008). In the case of R. fontaniana, which has an extended paralarval period of 70 days, juveniles were only obtained when using L. santolla zoeae as prey; this may indicate that other auxiliary cultures should be developed in order to complement the paralarvae diet. There is also evidences suggesting a relationship between the density of paralarvae and preys may be relevant for the final survival of paralarve (Villanueva et al. 1996; Iglesias et al. 2006).

This information leads us to speculate whether it would be possible and urgent to design a formulated diet for paralarvae. Considering their great voracity, it is worthwhile to formulate an artificial diet to obtain mass production at a commercial level. However, the limited knowledge of the digestive and nutritional physiology of paralarvae interferes with the achievement of this goal. At present, the major emphasis has been directed to manage the class of lipids, especially essential fatty acids, in live preys to optimize the protein/lipid ratio (Navarro and Villanueva 2000; Seixas et al. 2008, 2010). Other fundamental aspect that remain to be clarified are the free amino acid (essential and free AA ratio, among others) and trace element (such as copper) requirements of paralarvae.

Another important question is if the health status of paralarvae is conclusive when eggs have a long incubation period as in the case of E. megalocyathus. For this species, the bacteriological monitoring becomes important both during the egg incubation and the paralarval stage. This evaluation would not only permit to identify possible pathogenic agents but also to select possible beneficial bacteria that may be useful for the microbial management of paralarval cultures. Furthermore, it is necessary to determine the defence mechanisms that could be involved both in the embryonic and the paralarval development of octopuses. How do females contribute to this aspect during the embryonic development? Can the paralarval diet be improved to increase the defences during this difficult period? The use of artemia as a way to promote resistance to vibriosis in reared organisms is a fact (Rojas-García et al. 2009). This is why these questions should be first answer to achieve a sustainable paralarval culture.

What conditions should be offered to newly settled juveniles of merobenthic species in order to obtain high survival rates during the first stage of their benthic phase? Holobenthic species such as O. maya or O. bimaculoides could serve as excellent models to research the type of food and the nutritional requirements needed at this stage, in which replacing live or fresh food for formulated diets to meet their nutritional requirements seems to be reasonable. However, it is necessary to give a priority to research on the digestive capacity of these species and to determine when their digestive system is mature enough to start formulated diets. For O. maya and O. bimaculoides, both live and fresh frozen food have successfully been used, but this type of diet is expensive to bring their culture to a commercial level (Van Heukelem, 1977; Lee et al. 1991). Therefore, studies on artificial diets have become important in the last decade (O'Dor et al. 1983; García-García and Aguado-Giménez 2002; Aguado-Giménez and García-García 2003; Vaz-Pires et al. 2004; Miliou et al. 2005; Petza et al. 2006; Domingues et al. 2007; Aguila et al. 2007; Rosas et al. 2008; Cerezo-Valverde et al. 2008; Quintana et al. 2008; Farías et al. 2010), but unfortunately these studies have not found a type of food for cephalopods that is well consumed and allows an adequate production. The nutritional physiology of newly settled juveniles and of juveniles in a grow-out stage including digestive capacity, enzyme activity, enzyme proteomics, use and storage of reserves, fatty acid and essential amino acid requirements, digestibility of raw materials - should be studied to design sustainable diets for the octopus aquaculture.

What are the conditions to be standardized in the culture of newly settled juveniles and juveniles up to commercial size? Technology for the culture of early juveniles has not been developed yet; therefore, conditions such as the optimal type of tank (colour, size), shelter, water flow system, light intensity, density, feeding frequency and regimes, temperature, oxygen, salinity, pH, etc. are yet to be defined.

Although to date the experimental data indicates that octopuses can be reared worldwide, where pilot-scale technology is far from being an economic reality until research is fully developed to answer all the questions that have arisen from this review.

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FIGURE 1. E. megalocyathus eggs. A) morphololological differentiation between healthy egg (izq.) and infected egg (der.); B) filaments from infected egg under the stereoscopic microscope; C) filaments from infected egg under the optic microscope (100x).

FIG. 1

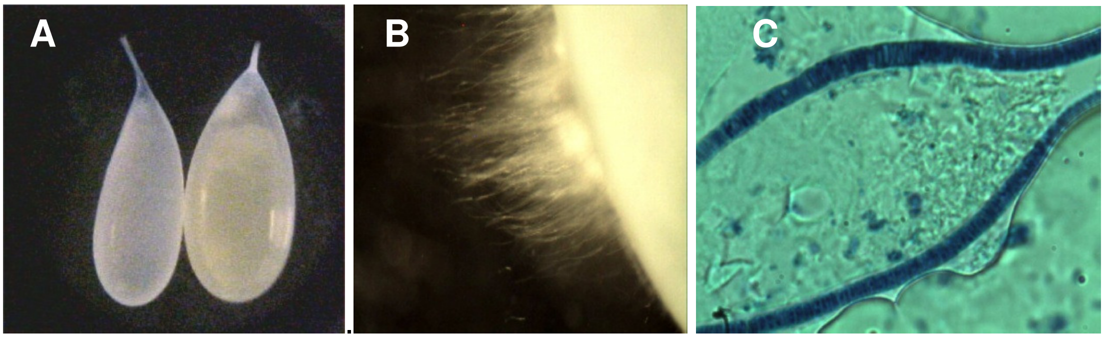


FIGURE 2. Pictures of the infected egg surface from E. megalocyathus under scanning electronic microscope: A) at spawning; B) non-infected egg of 15 days old after spawning; C) infected egg of 15 days old after spawning; D) infected egg of 25 days old after spawning.

FIG. 2

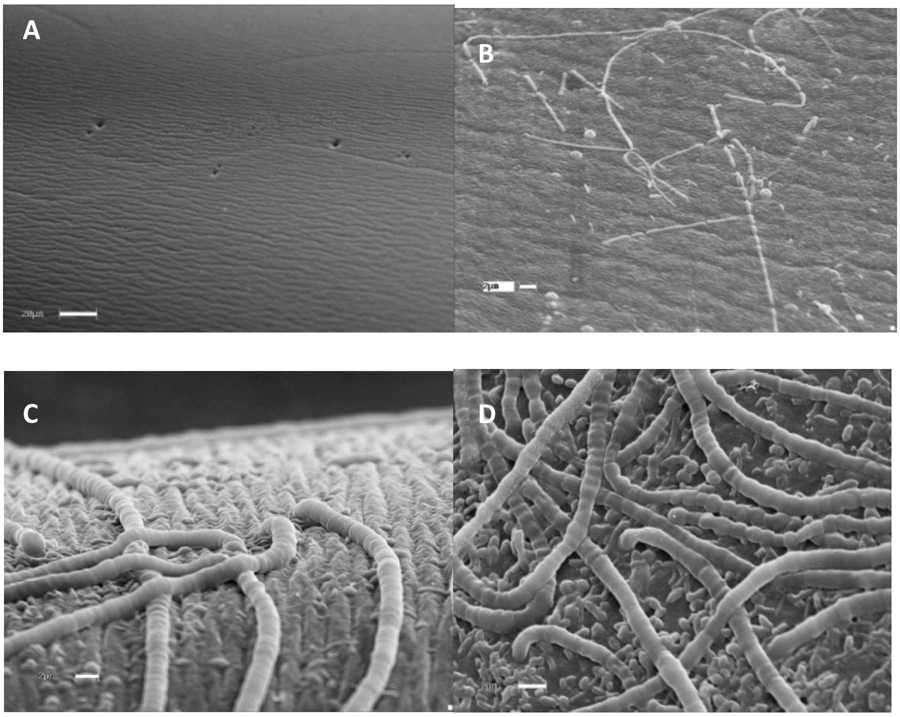


FIGURE 3. Specific growth rate (SGR) of E. megalocyathus wild juveniles reared in recirculation systems at 18 C and 33 ppm of salinity

FIG. 3

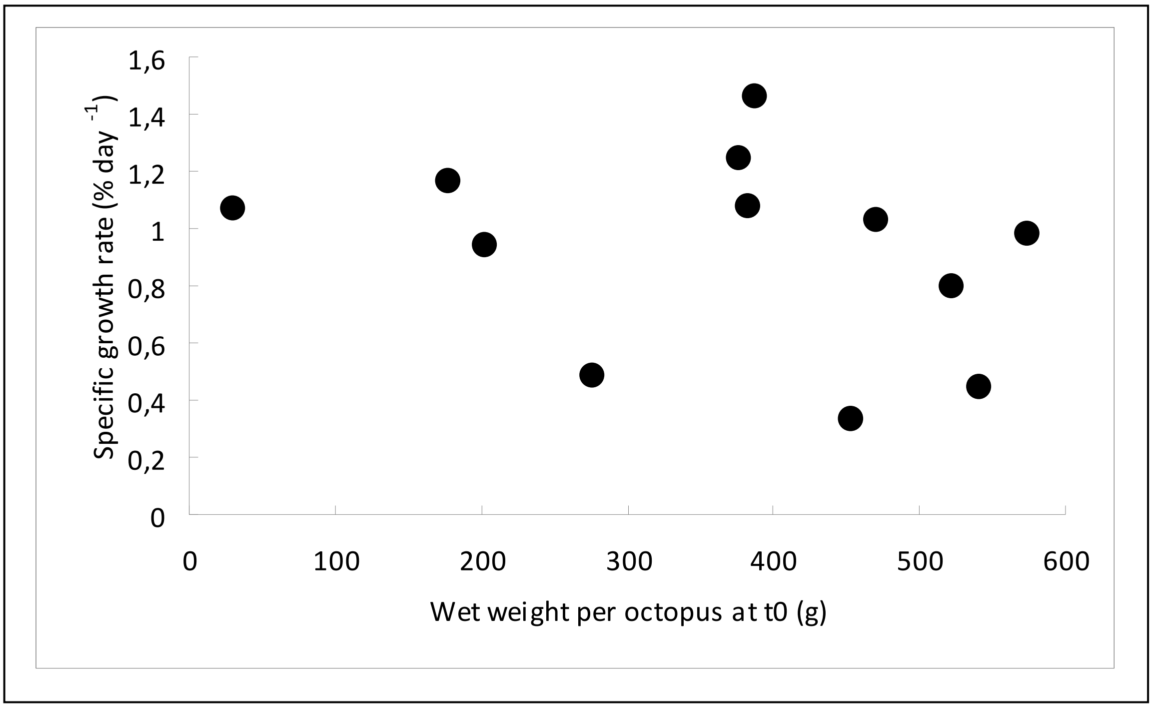

Specific growth rate (% day $^{-1}$)
0
0,2
0,4
0,6
0,8
1
1,2
1,4
1,6
0
100
200
300
400
500
600
Wet weight per octopus at t0 (g)

FIGURE 4. Curve of exponential growth in length (mm) for embryos, paralarva, and juveniles of R. fontaniana reared at 11 C and 30 ppm of salinity (data from Uriarte et al. 2009 and 2010).

FIG. 4

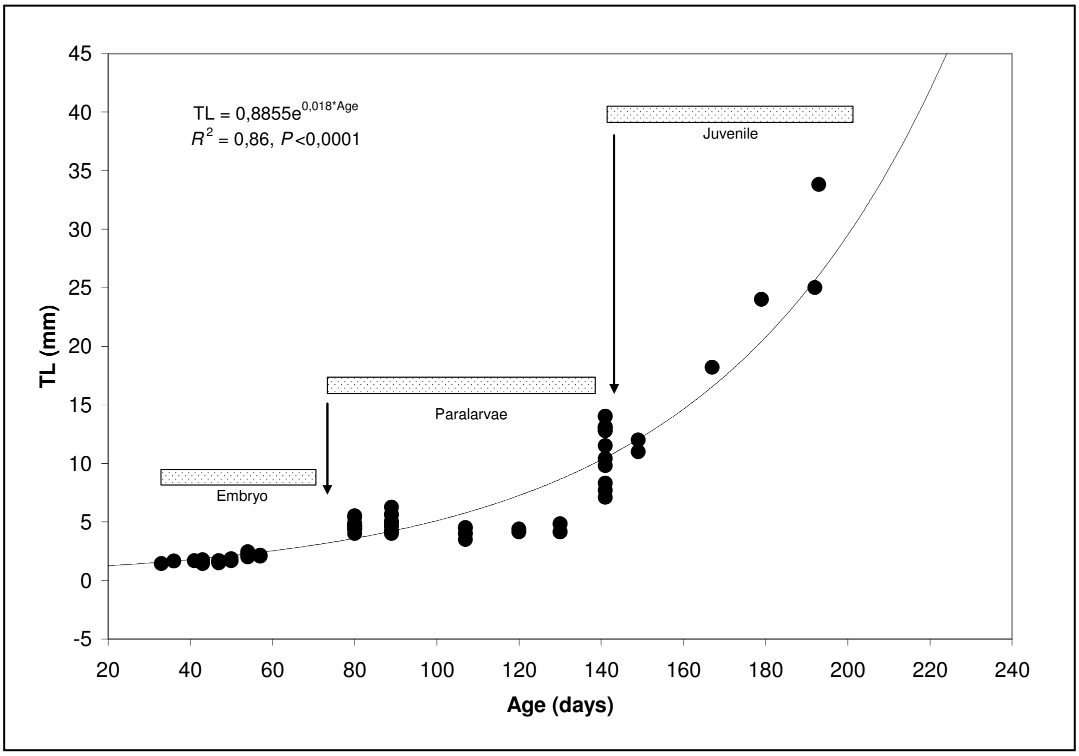
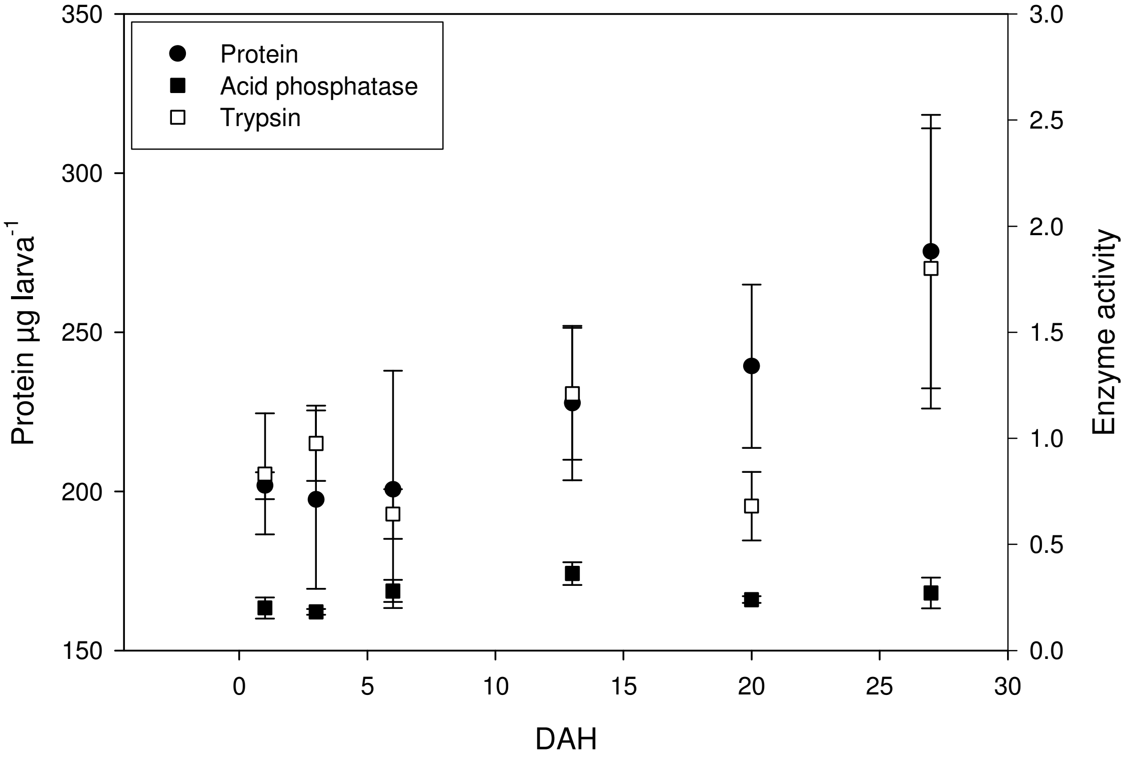

TL = 0,8855e^{0,018*Age}
R^2 = 0,86, P<0,0001
Embryo
Paralarvae
Juvenile
TL (mm)
Age (days)
45
40
35
30
25
20
15
10
5
0
-5
20
40
60
80
100
120
140
160
180
200
220
240

FIGURE 5. Protein content (µg/larva) and main enzyme activities (acid phosphatase and trypsin) in paralarvae of R. fontaniana fed on *L. santolla* zoea during development measured as days after hatching (DAH) (from data of Pereda et al. 2009).

FIG. 5



● Protein
■ Acid phosphatase
☐ Trypsin
Protein µg larva^{-1}
Enzyme activity
DAH
350
300
250
200
150
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30

FIGURE 6. Changes in yolk volume of O. vulgaris during embryonic development at 24 ± 1 C. (A) Day of egg laying (Eclipse), (B) between days 11 and 19 (cilinder) and, (C) day 21 (sphere).

FIG. 6

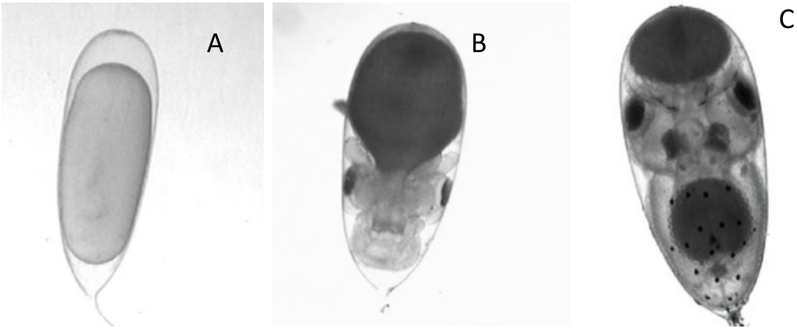
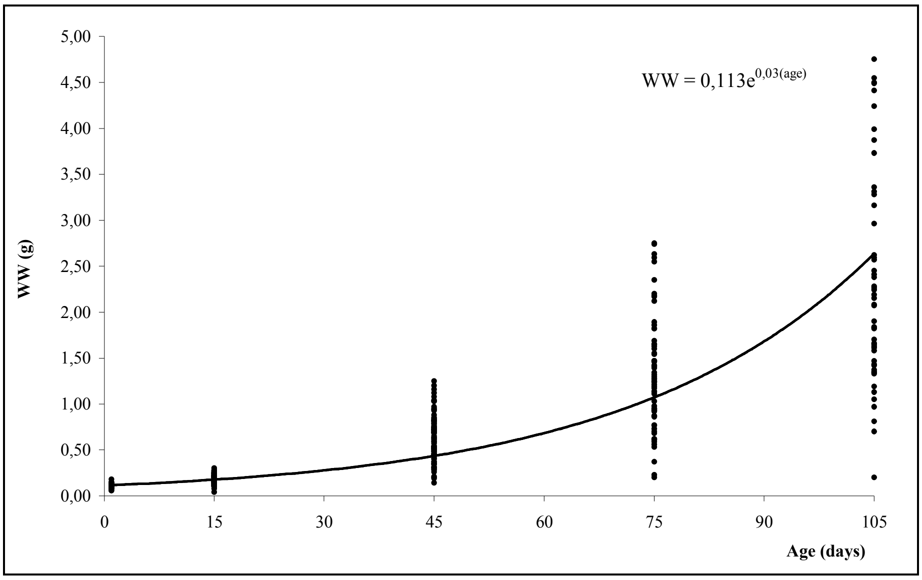


FIGURE 7. Exponential growth curve in wet weight (g) for juveniles of O. maya reared at 28 C and 32 ppm of salinity.

FIG. 7


WW = 0,113e^0,03(age)
WW (g)
5,00
4,50
4,00
3,50
3,00
2,50
2,00
1,50
1,00
0,50
0,00
0
15
30
45
60
75
90
105
Age (days)