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Response of newly hatched *Octopus bimaculoides* fed enriched *Artemia salina*: Growth performance, ontogeny of the digestive enzyme and tissue amino acid content

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ABSTRACT

The performance of *Octopus bimaculoides* juveniles from coastal areas of Baja California (Mexico) and reared in captivity was evaluated using *Artemia salina* as food source in three different treatments: *Artemia* enriched with either AlgaMac or *Spirulina maxima* and without enrichment. After 20 days, significant differences were found among treatments in terms of growth, which was significantly higher for juvenile fed AlgaMac-enriched *Artemia*, followed by those fed unenriched *Artemia*. Moreover, far higher growth rates (0.74–0.88 mg day^{-1}) were obtained than those reported for other octopus species of the same size. Digestive enzyme activity during the experimental period (20 days) showed an oscillatory behavior, with a tendency to stabilize after day 15. Trypsin was the most important protease, though lipases and amylases were also present. The whole-body lipid content of the juvenile was apparently influenced by the lipid content in the food. The amino acid profile remained unaffected after juvenile were fed the different treatments; however, differences were found between the initial and final whole-body content of the juvenile, with relatively lower amounts of isoleucine, leucine and tyrosine, and relatively higher amounts of threonine, alanine and glycine after 20 days of feeding. The *Artemia* amino acid content of phenylalanine, isoleucine, leucine and valine was limited, and growth would likely be further promoted with a more suitable diet. Thus, *O. bimaculoides* is a promising species for commercial culture, and even though good results were obtained when fed *Artemia*, a more appropriate food source should be sought to obtain an amino acid profile that will maximize growth.

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1. Introduction

In recent years the global demand for cephalopods, especially *Octopus* sp., has resulted in increasing interest in culturing these species. Several studies have established that cephalopods adapt relatively easily to captivity, have a high growth rate and high feed conversion ratios (Nixon, 1969; Mangold, 1983; Navarro and Villanueva, 2003). These characteristics have encouraged researchers around the world to further study these species. Though it has been shown that *Octopus vulgaris* has an easy reproduction process with an elevated number of hatched eggs (Iglesias et al., 2000), the long and complicated paralarval phase is generally a limiting step in its culture, both with respect to time and feeding concerns. In Mexico, *Octopus maya* and *Octopus bimaculoides* are recognized as commercially important species and have been described as having a unique life cycle. Both undergo direct development without a paralarval phase, conserving all other attributes as any other cephalopod of commercial interest (Aguila et al., 2007; Domingues et al., 2007; Rosas et al., 2007, 2008).

O. bimaculoides (Pickford and McConnaughey, 1949) is a medium sized octopus (60 cm). It is distributed from central California (Santa Barbara), USA, to the west central coast of the Baja California peninsula, Mexico, and prefers sand and mud habitats generally less than 30 m deep. This species grows to a maximum size of 800 g and has a lifespan of 1–1.5 years. It produces large eggs (~13 mm), with direct development to juvenile (a paralarval phase is not observed), and has shown an easy adaptation to captivity. As a sub-tropical species the preferred temperatures are from 12 to 25 °C, with 18 °C as ideal for reproduction (Forsythe and Hanlon, 1988a,b).

Though *O. bimaculoides* has attracted interest as a potential species for culture, general aspects of its life cycle are still poorly understood. It is known that it preferentially feeds on several species of

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Table 1
Initial and final weight (mg), growth rate (mg d^{-1}), specific growth rate (SGR, % day^{-1}) and total growth increment (%) of *Octopus bimaculoides* juveniles fed during 20 days on *Artemia salina* enriched with either AlgaMac-3050 (AE) or *Spirulina* (SE) and without enrichment (WE)

	Treatments		
	SE	AE	WE
Initial weight (mg)	89±0.01	89±0.01	99±0.02
Final weight (mg)	163±0.01[b]	200±0.03[a]	188±0.02[b]
Growth rate (mg day^{-1})	3.7±0.12[c]	5.6±0.11[a]	4.4±0.19[b]
SGR (% day^{-1})	3.02±0.13[b]	4.05±0.13[a]	3.20±0.15[b]
Growth increase (%)	183.1	224.7	189.9
Mortality (%)	27.78±1.5	16.67±1	0.0

Mean values in the same row with different superscript letters are significantly different.

crustaceans and mollusks (Rocha, 2003; González Acevedo, unpublished). In fact, its predatory behavior (Sinn et al., 2001; Sinn, 2008) can result in the reduction or elimination of local populations of several species of gastropods and bivalves (Ambrose, 1982). For a closely related species, *O. bimaculatus*, Ambrose (1984) concluded that feeding depends on the availability of food in its habitat, organisms allowed to feed on chitons, limpets, crabs, bivalves and gastropods over several weeks showed a preference for crabs and bivalves over gastropods. In addition to food and feeding habits, if captive rearing is to be developed it is of the utmost importance to determine the nutritional requirements for proper feeding regimes in captivity, including the possibility of formulating complete diets for a healthy and efficient development of all stages up to commercial size organisms.

A few studies have been reported on the rearing of *O. vulgaris* paralarvae using suitable live prey as food (Iglesias et al., 2007; Seixas et al., 2008). Another approach is co-feeding, where natural food like *Artemia* is combined with formulated diets (Villanueva, 1995; Navarro and Villanueva, 2000, 2003; Villanueva et al., 2004).

Artemia salina is the most nutritious living organism used as live prey in aquaculture for many marine species. Preliminary results obtained with *O. maya* have shown that *Artemia* adults improve the growth of newly hatched organisms, reducing cannibalism and, consequently, enhancing survival (Rosas et al., unpublished). Domingues et al. (2004) reported that one-day-old cuttlefish fed eagerly on several live food sources, including *Artemia*, grass shrimp or fish larvae, although they showed a preference for mysids, indicating once more the importance of live food in the development of cephalopods. It should be noted that *Artemia* can be easily enriched by adding nutrients that will carry essential elements either incorporated or inside the digestive system to improve its quality as feed. Its use, therefore, warrants further research.

In order to contribute to the knowledge of octopus culture and husbandry, this study aimed to measure the effect of using *Artemia* enriched with either *Spirulina* or AlgaMac-3050 as food source (during the first 20 days of life) on the survival, growth rate, ontogeny of digestive enzymes, and tissue amino acid content of *O. bimaculoides*, compared with the effect of unenriched *Artemia*.

2. Materials and methods

2.1. Artemia culture

A. salina was cultured at 27 °C as follows: after hydrating for 1 h, the cysts with seawater were washed in a sodium hypochlorite solution, and then transferred and kept in an 80-L column with constant air bubbling and fed on a mixture of live *Tetraselmis suecica* and *Isochrysis galbana* until adult size (15 days old). Adult *Artemia* were enriched, resulting in three experimental treatments: a) *Artemia* enriched with freeze-dried commercial *Spirulina maxima*; b) *Artemia* enriched every 24 h 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); and c) unenriched *Artemia*. *S. maxima* and AlgaMac were thoroughly emulsified using an electric blender for 2 min with the seawater before adding as feed to *Artemia* at a rate of 0.081 g L^{-1} and 0.2 g L^{-1} for *Spirulina* and AlgaMac, respectively.

2.2. Experimental conditions

One hundred and twenty laboratory-hatched specimens (Fish Culture and Biotechnology Unit of the Marine Science School, University of Baja California) of *O. bimaculoides* were used for this experiment. The specimens were distributed in nine 4-L plastic beakers (N=15 animals/beaker) to achieve three treatments in triplicate. All experimental units were connected to open flow by a seawater system and temperature was maintained at 21±2 °C with a photoperiod of 12:12 h light/dark. All animals were individually weighed on an electronic balance (±0.1 mg) at the beginning of the experiment and randomly distributed. Each experimental unit was supplied with sufficient 2-cm-long gastropod shells as shelters (30 per unit) to avoid stress and covered with lids to prevent the octopuses from escaping. Tanks were aerated continuously to maintain oxygen levels in each chamber above 6 mg L^{-1}.

Treatments were assigned randomly and juveniles were fed three times a day. One or two juveniles from each experimental unit were sampled on days 0, 2, 6, 10, 15 and 20, and stored at −80 °C for further analysis.

2.3. Growth parameters

At the end of the experiment organisms were weighed and the growth rate (mg day^{-1}) was determined as the difference between the initial and final weight.

$$\text{Specific growth rate}\left(\text{SGR}, \%\text{day}^{-1}\right)\ \text{was determined as:}$$
$$\text{SGR} = [(\text{Ln}W_2 - \text{Ln}W_1)/t]*100$$

where W_2 and W_1 are the final and initial wet weights of the octopus, Ln the natural logarithm, and t the number of experimental days (20).

2.4. Chemical analysis

2.4.1. Enzyme activity assays

The frozen whole bodies of the juveniles from days 0, 2, 6, 10, 15 and 20 were thawed and the whole organisms homogenized in distilled water (1:3 w/v) and centrifuged to obtain a crude extract. Protein concentration was then analyzed according to Bradford (1976)

Table 2
Lipid content (% dry weight) of the experimental treatments (*Artemia salina*) used as feed

	Lipids (%)
Artemia salina	
Sp	6.72±0.03
Alg	6.55±0.22
Unenriched	6.02±0.80
Treatments: whole body tissue samples	
Initial	4.22±0.90[b]
SE	6.94±0.33[a]
AE	6.72±0.50[a]
WE	5.75±0.20[ab]

Whole tissue of *Octopus bimaculoides* juveniles before and after being fed during 20 days on *Artemia salina* enriched with either *Spirulina* (SE) or AlgaMac-3050 (AE) compared to unenriched *A. salina* (WE).
Sp=*Artemia salina* enriched with freeze dried commercial *Spirulina*; Alg=*Artemia salina* enriched with AlgaMac-3050; Unenriched=*Artemia salina* without enrichment.