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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.

Specific growth rate $\left(\mathrm{SGR}, \%\mathrm{day}^{-1}\right)$ was determined as:

$$\mathrm{SGR} = [(\mathrm{Ln}W_2 - \mathrm{Ln}W_1)/t]*100$$

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.

using bovine serum albumin (BSA) as standard to report the activities per g of protein.

Alkaline protease activity was determined according to a modified method described by Sarath et al. (1989). Briefly, the incubation mixtures consisted of 80 µL of 0.05 M Tris–HCl/10 mM $CaCl_2$ (pH 8.1) buffer, 20 µL crude extracts and 150 µL 2% azocasein as substrate. The reaction was performed at 37 °C for 1 h and stopped by adding 750 µL 10% TCA. The optical density of the supernatant was measured at 360 nm.

Trypsin activity was determined following the modified method described by Erlanger et al. (1961), using BAPNA (Nα-benzoyl-L-arginine-4-*p*-nitroanilide hydrochloride) as substrate, and the amount of p-nitroaniline liberated from BAPNA (pH 8.2; 25 °C) measuring absorbance at 410 nm.

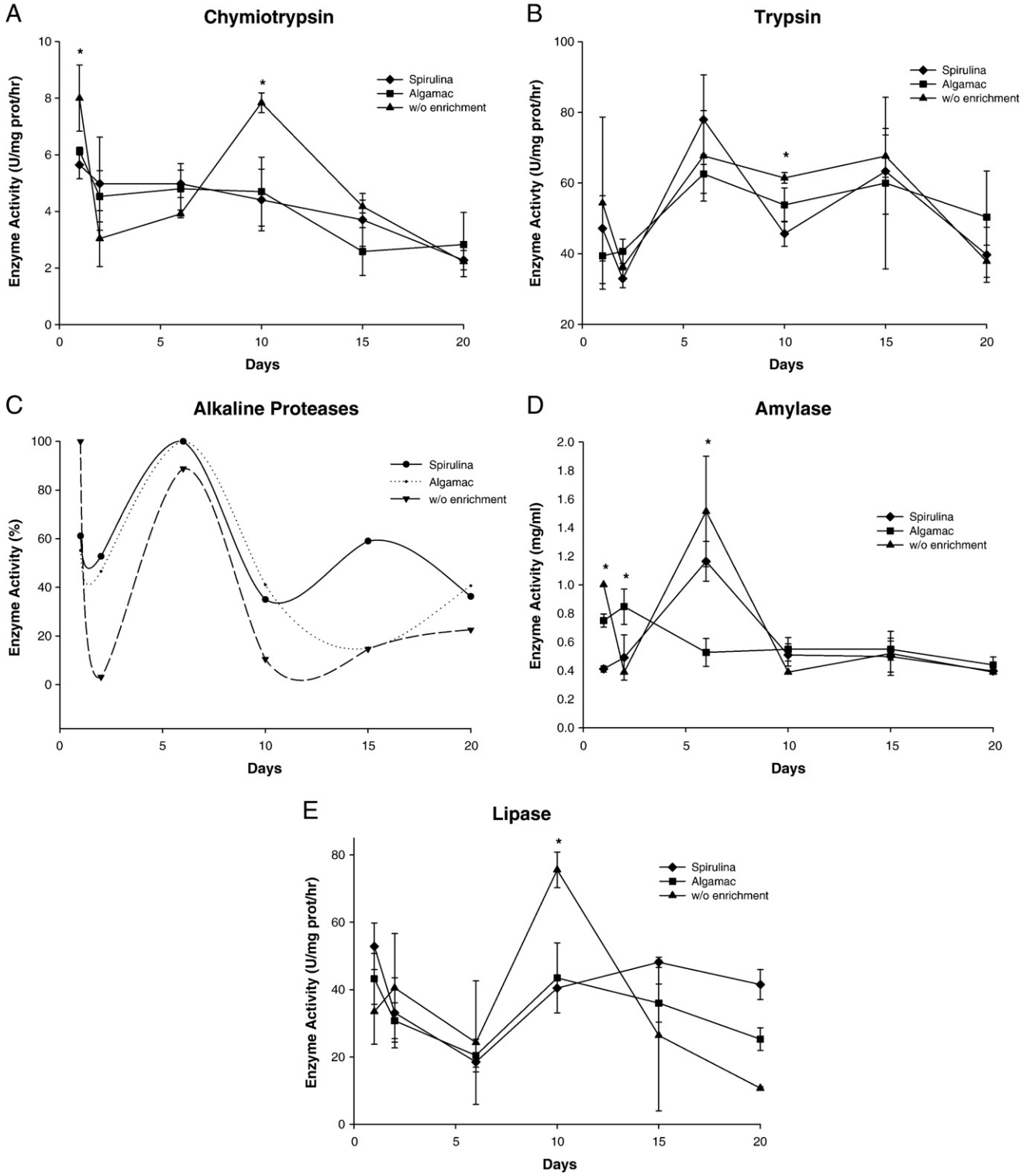

Fig. 1. (A) Chymotrypsin, (B) trypsin, (C) alkaline protease, (D) amylase, and (E) lipase activity of *Octopus bimaculoides* juveniles fed during 20 days on enriched (*Spirulina* and AlgaMac-3050) and unenriched *Artemia salina*. Standard deviations are indicated with bars and asterisks indicate significant differences at $P<0.005$.

Chymotrypsin activity was assayed according to Hummel (1959), using BTEE (N-benzoyl-L-tyrosine ethyl ester) as substrate. The increase in absorbance at 256 nm resulting from the hydrolysis of BTEE at pH 8.1 at 25 °C was recorded every 2 min for 6 min.

Lipase activity was determined by the hydrolysis of 4-nitrophenyl-caproate (4-NPC) according to the modified method described by Gjellesvik et al. (1992). Briefly, the reaction was initiated by adding the enzyme extract to 0.5 M Tris–HCl (pH 7.4) buffer, 4-NPC (100 mM in ethanol, with a final concentration 0.35 mM in assay mixture). Temperature was maintained at 25 °C and the increase in absorbance was recorded at 400 nm.

The α-amylase activity was determined according to Bernfeld (1955), using a blend containing 1% starch as substrate. A solution of 3.5-dinitrosalicylic acid was used as the acid reagent to determine the amount of reducing sugar (maltose) produced by measuring absorbance at 540 nm.

One unit (U) of activity was defined as the amount of enzyme liberating 1 μmol of product per min under the conditions described above for each enzymatic assay.

2.4.2. Amino acid analysis

The amino acid content of individual samples from the beginning and end of the experiment was determined. Amino acid profiles from total tissue were determined after lipid was extracted according to Folch et al. (1957). Tissue samples were hydrolyzed with 200 μL of 6 N HCl acid and 0.06% phenol in a closed vial and heated to 110 °C for 24 h. The hydrolyzed samples were dried with nitrogen and re-hydrated with 1 mL water. Samples were then filtered (0.45 μm) and refrigerated until used. Samples were chromatographed through a reverse phase column (3.9×150 mm) 4 μm Nova Pak™ C_{-18}, using the water-acetonitrile gradient recommended by the Waters AccQ•Tag™ system (Milford, MA, USA), to an Agilent HPLC (Santa Clara, CA, USA) equipped with a florescence detector (Agilent, 1100 series). A fluorescence detector was set up for an excitation wavelength of 250 nm and emission wavelength of 395 nm. Analyses were conducted at a constant temperature of 40 °C. HPLC signal calibration and standard curves were obtained by using an amino acid standard solution containing from 18.75 to 150 pmol of each amino acid.

2.4.3. Lipid determination

Lipids were extracted according to Folch et al. (1957) and the amount of total lipid quantified gravimetrically.

2.5. Statistical analyses

Mean values are reported ±standard deviation. After confirming the normality and homogeneity of variance, data were analyzed by one-way ANOVA. Multiple comparisons were performed using Tukey's test. Differences were considered significant at $P<0.05$. For percentage values, an arcsen transformation was performed prior to analysis.

3. Results

During the experimental period (0–20 days), no significant difference in mortalities was observed. The *Spirulina*-enriched (SE) treatment showed the highest mortality (27.78±1.5%), followed by the AlgaMac-enriched (AE) treatment (16.67±1.0%), while no mortality was observed in the treatment without enrichment (WE).

Daily growth rate (mg day^{-1}) and SGR showed significant differences ($P<0.05$). Highest values were recorded for animals fed AE, followed by WE, and least growth was observed in the SE treatment (Table 1). The total growth increase was 224% for the treatment showing the highest growth rate in only 20 days, corresponding to AE, whereas the lowest growth rate corresponded to the SE treatment, with 183% total increase (Table 1).

No difference in total lipid content was observed for enriched or unenriched *Artemia* adults (Table 2). Also, there were no differences in total lipid content of whole body octopuses fed the experimental diets during 20 days, although these values were higher than those observed at the beginning of the experiment (Table 2).

Enzyme activity in the octopus tissue extract showed a nonlinear behavior during the experimental period. Enzyme activity peaks were observed on day 5 (alkaline proteases, trypsin and amylase) and on day 10 (chymotrypsin, trypsin and lipase). After day 15, the enzyme activity began to stabilize and reached steady state around day 20 of the experimental period (Fig. 1).

Table 3
Amino acid (AA) composition (mg of AA/g protein) of *Artemia salina* and *Octopus bimaculoides* juveniles fed during 20 days on *Artemia salina* enriched with either *Spirulina* (SE) or AlgaMac-3050 (AE) compared to *A. salina* without enrichment (WE)

	Artemia salina[a]			*Octopus bimaculoides*			
	Sp	Alg	Unenriched	Final			
				Initial	SE	AE	WE
Essential AA (mg/g)							
Arginine	45.3	45.7	39.5	47.5±0.49	47.7±0.20	47.0±0.30	44.4±0.03
Phenylalanine	106.1	104.7	106.6	91.8±0.31	99.8±0.50	96.2±0.17	95.9±0.08
Histidine	17.8	17.7	18.2	19.8±0.05	19.5±0.06	19.2±0.05	19.9±0.02
Isoleucine	122.3	119.1	125.6	127.4±0.13[a]	117.3±0.32[b]	119.4±0.31[b]	118.9±0.19[b]
Lysine	48.3	48.5	47.7	51.7 ±0.08	51.5±0.66	48.7±0.42	51.0±0.33
Leucine	175.4	172.3	179.5	188.3±0.24[a]	168.9±0.30[b]	171.6±0.02[b]	173.7±0.10[b]
Methionine	12	13.2	16.9	11.2±0.79	14.4±0.90	11.4±0.08	14.1±0.33
Threonine	53.7	51.8	44.5	51.9±0.35 [b]	60.8±0.06[a]	60.0±0.02[a]	59.2±0.07[a]
Valine	99	97.9	99.1	97.4±0.11[a]	87.2±0.15 [b]	86.8±0.13 [b]	87.1±0.04 [b]
Nonessential AA (mg/g)							
Aspartic acid	49.4	50.1	48.8	48.1±0.07	50.1±0.24	50.5±0.09	51.0±0.08
Proline	20.1	21	20.2	19.2±0.03	19.3±0.05	19.2±0.01	19.4±0.02
Tyrosine	29.8	28.6	29.3	28.8±0.003[a]	27.1±0.05[b]	26.2±0.04[b]	26.5±0.07[b]
Alanine	71.4	71.1	67.2	59.4±0.06[a]	67.8±0.32[b]	70.2±0.01[b]	68.6±0.02[b]
Glutamic acid	68.9	73.9	73.2	69.1±0.42	66.8±0.14	75.4±0.09	70.7±0.41
Glycine	43.1	45.7	44.3	44.7±0.31[b]	57.9±0.12[a]	55.2±0.27[a]	56.3±0.11[a]
Taurine	1.9	2.8	1.9	5.6±0.06	5.7±0.07	5.7±0.06	5.9±0.09
Serine	35.3	35.8	37.8	38.2±0.05	38.1±0.05	37.4±0.19	37.6±0.10

Mean values in the same row with different superscript letters are significantly different.
[a] Sp=*Artemia salina* enriched with *Spirulina maxima,* Alg=*Artemia salina* enriched with AlgaMac 3050, Unenriched=*Artemia salina* without enrichment.

The *Artemia* samples used to determine the amino acid content were taken as a single sample since they came from the same batch, so no replicate groups were analyzed and statistical analyses could not be performed. Octopus amino acid content failed to show significant differences among experimental treatments at the end of the experiment, though differences were observed in tissue amino acid content in the initial samples as shown in Table 3. For instance, isoleucine, leucine, and tyrosine were higher in *O. bimaculoides* at the beginning of the experiment than after 20 days, whereas threonine, alanine, and glycine were higher at the end of the experimental period than just after hatching.

4. Discussion

In the present study *O. bimaculoides* juveniles were able to grow and develop using *Artemia* as the sole food source, showing higher growth rates than those previously reported for other octopus species fed *Artemia* (Iglesias et al., 1996) or other food sources (Moxica et al., 2002).

Highest mortality occurred in the SE treatment, whereas no mortality was observed in the control treatment (WE) during the experimental period; however, no significant difference was observed among treatments, probably because of the number of organisms used per treatment. All mortalities were observed after day 15, probably due to cannibalism, a behavior commonly observed in octopus. This cannibalistic behavior can be attributed to nutritional requirements, stress (Iglesias et al., 2000; Rosas-Vázquez et al., 2005) or due to competition for space and dominance, based on the size of the organisms (Cigliano,1993). In this study, cannibalism was higher in the treatment with the lowest growth rate, so it was probably not provoked by a nutritional requirement or at least one that could have influenced their growth rate. Octopuses fed unenriched *Artemia* had a medium growth rate during the present study (3.2±0.2, mg day^{-1}) and there was no evidence of cannibalism. The growth rate was still higher than that reported for other species of similar age, indicating that unenriched *Artemia* is an alternative food source that can be used to grow out this species during the first 20 days of rearing. Actually, in our laboratory we are testing small gastropods (*Littorina planaxis*, *Littorina scutulata* and *Tegula funebralis*) as preferred food, and the results are showing that these gastropods produce similar growth rates (González et al., unpublished) than that obtained with *Artemia* in the present study.

All treatments at the end of the experiment showed significant differences in growth. The AE treatment resulted in the highest growth rate, followed by WE, while SE had the lowest. AlgaMac, which was used in the AE treatment, is a commercial product containing a high percentage of lipid and DHA, whereas *Spirulina*, used in the SE treatment, is known for its fatty acid and amino acid content (Belay et al., 1996). Contrary to what was expected, the unenriched *Artemia* (WE) resulted in a better growth rate than that observed for SE. Rosas et al. (2007) observed that a diet with high lipid content had a detrimental effect on *O. maya*. Hence, there is no apparent reason to enrich *Artemia* with products with a high PUFA content; however, even if it is not necessary to provide a high lipid level (Navarro and Villanueva, 2000), the quality of lipid (fatty acid profile) could still be of importance to meet the nutritional requirements of *O. bimaculoides*, albeit during this study, the fatty acid profile was not analyzed either for the enriched *Artemia* or the juvenile octopuses.

Nevertheless, an impact on the total lipid content was observed between the initial and final stages. This difference of more than 2% between the initial and final content did not affect the experimental treatments at the end of the experiment. As stated before, it is difficult to compare the treatments or explain the differences observed since some mortality due to cannibalism was observed, acting as a source of confusion for the possible effect of the diets.

It should be noted that in all treatments *Artemia* produced better results than those reported for *O. vulgaris*. Iglesias et al. (1996) used *Artemia nauplii* followed by metanauplii (1–4 mm cultured at 20 °C) to feed *O. vulgaris* paralarvae (up to 30 days old) and obtained a lower growth rate (0.03 mg dry weight day^{-1}) than that found here. The growth rates observed in the present study (3.0 to 4.5% day^{-1}, 3.7 to 4.4 mg day^{-1} wet weight) are similar to those reported for *O. maya* hatchlings fed *Artemia* (4 to 5% day^{-1}; Rosas et al., 2008), suggesting that both *O. bimaculoides* and *O. maya* have a good aquaculture potential. During the exponential growth phase, instantaneous relative growth rates (% increase in body weight or length per day) remain constant and generally fall within a range of 5 to 10% increase in body weight per day. The animals double their weight at a very steady rate (for example, every 14 days at 50% day^{-1} or every 7 days at 10% day^{-1}). The larger the ultimate size a species grows to, the longer the exponential growth phase tends to be, and thus more weight doublings are possible during this phase (Forsythe, 1984; Forsythe and Van Heukelem, 1987). *O. maya* attains an adult size at ~1 kg of body weight and achieves a growth rate of about 9% day^{-1} doubling its weight during the first 100 days of the exponential growth phase (Van Heukelem, 1976), while *O. digueti*, which grows to a maximum size of less than 100 g, only achieves 6.5% day^{-1} doubling during its 70-day exponential phase (DeRusha et al., 1989). Like other cephalopod species, *O. bimaculoides* fed *Artemia* can reach the double of living weight, suggesting also an exponential growth phase.

The reason why *O. bimaculoides* could be fed *Artemia* is according to that reported by Hanlon and Forsythe (1985), who found that octopus hatchlings readily attack and capture prey of a wide size range, anywhere from 1 to 2 times their own mantle length. During the course of our experiment we also determined that newly hatched *O. bimaculoides* started to capture prey 10 to 12 h after hatching. Likewise, it has been observed that *O. maya* easily prey on *Artemia* adults due to their slow swimming behavior, capturing them as they swim toward the tank bottom. The data obtained for *O. maya* show that during the first 10 to 15 days hatchlings had an epipelagic behavior, indicating an ability to prey on epibenthic prey like mysid shrimp or *Artemia* adults (Moguel et al., in press).

The amino acid profile obtained in the *Artemia* treatments, although performed on a single sample, indicated that arginine and threonine were lower in the WE treatment than in the SE and AE treatments, whereas methionine was higher. Moreover, octopuses from the different treatments showed differences in some amino acids at the beginning and end of the experiment; however, no differences were found between treatments.

The *Artemia* essential amino acid (EAA) content of phenylalanine, isoleucine, leucine, and valine was limited. When the *Artemia* EAA profiles are compared with *O. bimaculoides* fed during 20 days, those EAA resulted in a higher concentration than the whole body composition. However, the levels of the rest of the EAA (arginine, histidine, lysine, methionine, and threonine) were higher at the beginning and after octopuses were fed *Artemia*. This result suggests that *O. bimaculoides* had the capacity to grow at such a rate even when the diet was deficient in some EAA. It is therefore possible that growth could be promoted even to a higher degree with a more suitable diet. Moreover, although the role of these EAA in *O. bimaculoides* is still undefined, the results obtained for *O. vulgaris* paralarvae suggest that lysine, arginine, and isoleucine could play a protagonist role in protein metabolism of cephalopods (Villanueva et al., 2004). For instance, arginine is vigorously metabolized in cephalopods (Hochachka et al., 1983). During anaerobic work, arginine phosphate is hydrolyzed, leading to increased availability of arginine for condensation with glucose-derived pyruvate to form octopine. Octopine is the main anaerobic end product that accumulates in adult cephalopods during periods of exercise and stress (Hochachka et al., 1976; Storey and Storey, 1978). On the other hand, lysine has been identified as a growth promoter in *S. officinalis* subadults (Domingues, 1999), suggesting that

not only is a high concentration required but that it may also have a metabolic role. This could explain why *O. bimaculoides* from this study preserved its lysine and arginine levels even though *Artemia* was deficient in them.

Enzyme activity ontogeny registered during this experiment for the different treatment groups shows typical enzyme stimulation where the comparison between total alkaline activity and the other proteases presents evidence that trypsin plays a principal role in enzyme activity among alkaline proteases. Besides, lipase activity tends to stabilize after day 15, with a peak in activity on day 10. Morote et al. (2005) found low trypsin activities in new born paralarvas *O. vulgaris*, however, after feeding with zoeas of *Maja squinado* and *Palaemon serratus*, *A. salina* and eggs and larvae of *Solea senegalensis*, the trypsin levels were variable, with the highest activities in paralarvaes fed *Artemia* after day 7. Villanueva et al. (2002) report high trypsin activities on days 10, 15 and 20 after feeding *O. vulgaris Artemia*.

Digestive enzyme induction has been observed in *O. vulgaris* paralarvae and juveniles, and *O. maya* adults (Villanueva et al., 2004; Rosas et al., 2008). Although it is difficult to relate digestive enzyme induction to growth rate or survival, alkaline proteases, chymotrypsin and lipases showed greater activity in *O. bimaculoides* fed unenriched *Artemia*, suggesting that those *Artemia* may be better digested, enhancing the digestibility and at the end the biomass accumulation. It is interesting to note that in animals fed enriched *Artemia*, the digestive enzyme activity was not inducted, suggesting that lipid in the *Artemia* could have inhibited the digestibility of those diets. A similar behavior was observed when pre-adult *O. maya* were fed high lipid diets (Domingues et al., 2007).

O. bimaculoides is a promising species for commercial culture, since the growth rates are comparable to those of other important commercial species. Even though good results were obtained when fed *Artemia*, further experimentation is recommended using other natural and balanced food sources in order to obtain an amino acid profile for *O. bimaculoides* that will maximize growth performance.

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