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Health status of post-spawning *Octopus maya* (Cephalopoda: Octopodidae) females from Yucatan Peninsula, Mexico

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Abstract The present study aimed to evaluate the health status of *Octopus maya* females on different days after spawning (days 0, 10, 20, 30, and 40). A total of 25 *O. maya* females were examined in terms of physiological (i.e., weight loss, hepatosomatic and gonadosomatic indexes, and hemocyanin, protein, glucose, cholesterol, and acylglycerides concentrations in plasma) and immunological variables (i.e., total hemocyte count, hemagglutination, and phenoloxidase activity). We hypothesized that *O. maya* females should maintain their physiological integrity throughout the post-spawning period until the hatching of the offspring. Results showed that the physiological and immunological indicators measured in post-spawning females significantly changed with time. Loss of body weight over time and a decrease in the hepatosomatic and gonadosomatic indexes were observed. Hemolymph components showed variations that reflect the consumption of reserves and coincide with an increased immune process of hemagglutination and phenoloxidase activity in hemocytes. Our results demonstrate that *O. maya* females are adapted to maintain an adequate state of health to care for their spawn despite the long period of starvation and contribute to the identification of the mechanisms involved in maintaining the integrity of these animals during one of the most critical phases of their life cycle.

Keywords Cephalopod · Hemolymph · Physiology · Metabolism · Immunology

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Introduction

Octopus maya Voss & Solis, 1966 is an endemic species of the Yucatan Peninsula, occurring in the states of Campeche, Yucatan, and Quintana Roo, Mexico. This species is one of the most important fishery resources in Mexico (Rosas et al., 2014). The life cycle of *O. maya* is short, around 8–12 months

(Hanlon & Forsythe, 1985) and in general the females are already functionally mature at around 350 g of body weight (Solis, 1998; Avila-Poveda et al., 2016). Females of this species may lay from 1500 to 2000 large eggs of up to 17 mm long, which produce large benthic hatchlings around 6–7 mm mantle length (Roper et al., 1984).

As other incirrate octopods, after spawning *O. maya* females decrease and stop feeding during incubation of the eggs (Wodinsky, 1977), and focus exclusively on their care until the hatching of the juveniles. Maternal care generally includes the protection of the egg mass from potential predators, ventilation by flushing water through the eggs, cleaning the surface of the eggs, and removing dead embryos (Vidal et al., 2014).

In the laboratory, artificial incubation of *O. maya* eggs has been successfully used to obtain high survival rates (>85%) of the juveniles at hatching (Caamal-Monsreal et al., 2015, 2016; Juárez et al., 2015), with the advantage that the artificial incubation system uses less space and water (Rosas et al., 2014). In this system, the total embryonic development lasts between 40 and 50 days at 24°C (Rosas et al., 2014; Vidal et al., 2014; Tercero et al., 2015). In the wild, many incirrate octopod females lay their eggs in dens, blocking up the opening with stones to reduce vulnerability and avoid predation of the embryos (Rocha et al., 2001; Garci et al., 2016). Under such conditions, it is difficult for scientists to find nesting females and consequently study this critical part of their life cycle (Anderson et al., 2001; Garci et al., 2016).

During brooding, female octopuses direct their metabolic efforts to increase post-reproductive survival (Calow, 1987). The metabolism of female octopuses is sustained by endogenous reserves, which may cause physiological wear that results in death after the hatching of the offspring (Rosa et al., 2004). This could compromise the health status of the female, thereby the safety of embryos. Little information is available on the general condition and the health status of post-spawning female octopuses.

The aim of the present study was to evaluate the health status of *O. maya* females after spawning and to increase knowledge of the physiological and immunological processes during this period. We hypothesized that female octopuses should maintain their physiological integrity throughout the post-spawning period until the hatching of the offspring, since the survival of the next generation depends partially on maternal care.

Materials and methods

Animals

Specimens of *O. maya* were captured using artisanal fishery lines and crab as bait, in the coast in front of the Sisal harbor (Yucatan, Mexico) and transported in a 120 l circular tank with seawater to the facilities of the National Autonomous University of Mexico (UNAM/Sisal, Yucatan, Mexico). In the laboratory, male and female octopuses (1:1) were placed in 12,000 l flow-through circular tanks with aerated seawater for two weeks to acclimate and to ensure that mating had occurred. PVC tubes (4″ in diameter) were placed in the tanks as refuges and the octopuses were fed twice a day with frozen crabs *Callinectes sapidus* Rathbun 1896 as suggested by Rosas et al. (2014). Food not ingested and feces were removed daily.

Afterwards, females were acclimated in 160 l flow-through dark rectangular tanks with aerated seawater until spawning. In these conditions, females were fed with diets based on crab *C. sapidus* and squid *Dosidicus gigas* (d'Orbigny, 1835) with the addition of a commercial vitamin mix, minerals, and high quality oils (Tercero et al., 2015). After spawning, the eggs were removed to avoid any interference associated with the presence of bacteria or fungi that can occur in some strings of eggs in captive conditions (C. Rosas, pers. comm.).

The females were weighed and subsequently returned to the tanks, where they remained until sampling. Seawater in these tanks was maintained at 24.6 ± 0.8°C, salinity 35–38 UPS, dissolved oxygen 6.34 ± 0.25 mg l^{-1}, pH 7.42 ± 0.2, and a photoperiod of 10:14 h red light–dark was used. Every day tanks were cleaned to remove feces. A total of 25 female octopuses were analyzed ($n = 5$ females per sample period) for physiological and immunological variables at 0, 10, 20, 30, and 40 days after spawning (DAS).

Hemolymph sampling and preparation

To sample hemolymph, octopuses were anesthetized with hypothermia at 8°C for several minutes as suggested by Linares et al. (2015). After this period, it was possible to observe a reduction of the breathing rate (indicated by the mantle contractions) and locomotor activity, paler color of the mantle and the arms, and loss of normal posture. The animal was removed

from the cold water, the arms were positioned at rest and covered with a clean piece of cloth, and the mantle was exposed for surgery. Hemolymph was withdrawn from the cephalic aorta using a pre-chilled catheter connected to a 5 ml Falcon tube and used immediately or kept refrigerated (2–8°C) until use (Lópes, 2010).

Hemolymph was centrifuged at 800×*g* for five min at 4°C to separate the plasma, which was used to evaluate plasmatic metabolites, phenoloxidase (PO), and hemagglutination activity. The cellular pellet from each sample was washed twice with isotonic solution (IS: 0.45 M NaCl, 10 mM KCl, 10 mM HEPES, 7.3 pH, and 10 mM EDTA–Na_2) and centrifuged as described above. Then, the cellular pellet was re-suspended several times with cacodylate buffer (10 mM cacodilic acid, 10 mM CaCl, pH 7.0) in equal volume of hemolymph and centrifuged at 13,000×*g* for 5 min at 4°C. The supernatant was used to evaluate the PO activity from degranulated hemocytes.

Physiological variables

After sampling hemolymph, confirmation of death was carried out by destruction of the brain (Fiorito et al., 2015). The animals were weighed (total weight) and the gonad and the digestive gland were removed and weighed separately to obtain the gonadosomatic (GSI) and hepatosomatic indexes (HSI), respectively (Cortez et al., 1995).

The glucose, cholesterol, and acylglyceride concentrations in plasma were determined in triplicate in 96-well flat bottom plates by specific commercial chromogenic kits (ELITech Group®), by adding 10 µl of plasma to 200 µl of the appropriate enzyme reagent for each sample. The protein concentrations were also determined in triplicate in 96-well flat bottom plates. However, in this case, the plasma was previously diluted in sterile water (100x) and then 10 µl of this solution was mixed with 200 µl of the commercial solution (Bio-rad Protein assay 500-0006) according to Bradford method (1976). Bovine serum albumin was used as a standard. Absorbance values of all metabolites were recorded in a microplate reader (Bio-rad model 550) and the concentrations (mg ml^{-1}) were calculated using standard curves considering the dilution factor for each sample. All plasmatic metabolites were determined in triplicate.

Hemocyanin concentration was measured by placing 10 µl of hemolymph diluted in 990 µl of Tris 0.1 M, pH 8.0, in a 10-mm cuvette. The absorbance was measured at 335 nm (UV-SENSE; SLM AMINCO Mod DW). Hemocyanin concentration was calculated using an extinction coefficient of 17.26 calculated on the basis of the functional subunit of 74 kDa (Chen & Cheng, 1993a, b).

Immunological variables

To avoid immune system activation by endotoxins, all glassware was washed with E-toxa-clean prior to use and solutions were prepared using pyrogen-free water and filtered with an Acrodisc of 0.2 µm.

Total hemocyte count was performed in duplicate yielding a minimum area count of 0.04 mm^3, cells were counted in a Neubauer chamber from a hemolymph aliquot fixed with 4% formaldehyde in Alsever's solution (115 mM $C_6H_{12}O_6$, 30 mM Na_3-$C_6H_5O_7$, 338 mM NaCl, 10 mM EDTA.Na_2, pH 7.0) with a 1:3 dilution. Samples were kept at 2–8°C, for a maximum period of 24 h until the analysis.

Hemagglutination activity was measured using human blood (type O+) obtained from a local blood bank. Prior to use, the erythrocytes were washed three times with 0.9% saline solution, centrifuged at 380×*g* at 25°C for five min, and then adjusted to a final volume of 2%. Samples of 50 µl of octopus plasma were added to a U-shaped 96-well microliter plate and a two-fold serial dilution was prepared using 0.9% saline solution as the diluent. An equal volume of the erythrocyte solution was added to each well and incubated for 3 hours at room temperature. In the controls, the plasma was replaced by 0.9% saline solution. The plasma hemagglutination titer was expressed as the reciprocal of the highest dilution showing a positive visible pattern of agglutination (Pascual et al., 2012).

Phenoloxidase system activity was measured by spectrophotometry in triplicate in 96-well flat bottom plates (Hernández-López et al., 1996; Le Pabic et al., 2014). The technique was adjusted for *O. maya*: 50 µl of plasma and degranulated hemocytes were incubated for 10 min at 37°C to transform the proPO into PO without using exogenous trypsin. To evaluate phenoloxidase activity in the degranulated hemocytes, the plasma was replaced by 50 µl of trypsin (bovine pancreatic 0.1 mg ml^{-1}; Sigma T8003). Then, 180 µl

of L-3,4-dihydroxyphenylalanine (L-DOPA, 3 mg ml^{-1}; Sigma D9628) was added to each well and the microplate was incubated for more than 10 min at 37°C. Absorbance was measured at 490 nm in an ELISA microplate reader (Benchmark Bio-Rad model 550). Results were expressed as the increment of 0.001 in optical density (Pascual et al., 2012).

Statistical analysis

The physiological and immunological indicators of female *O. maya* measured immediately after spawning and at subsequent moments (days 10, 20, 30, and 40) were analyzed using Principal Coordinate Analysis (PCoA). Physiological indicators were weight loss (g), hepatosomatic index, gonadosomatic index, glucose (mg ml^{-1}), acylglycerides (mg ml^{-1}), cholesterol (mg ml^{-1}), plasmatic proteins (mg ml^{-1}), and hemocyanin concentration (mM); whereas immunological indicators were total hemocyte count (cells mm^{-3}), hemagglutination activity (titer), and phenoloxidase system activity (OD 490 nm). A total of $n = 5$ independent females were measured each day. Dissimilarity measures between female samples were obtained using the reciprocal of Gower's index (S_{17}; Legendre & Legendre, 1998) on the otherwise untransformed data ($n = 25$ samples; $k = 11$ descriptors).

To analyze changes in the physiological and immunological indicators in post-spawning female *O. maya* through time, a permutational MANOVA was used on the dissimilarity matrix (Anderson, 2001). The underlying experimental design was a one-way model with the number of days since spawning as a fixed factor with five levels. A total of 9999 unrestricted permutations of raw data were used to obtain the empirical distribution of pseudo-F values (Anderson, 2001; McArdle & Anderson, 2001). Paired comparisons between centroids were carried out following a similar procedure by calculating empirical pseudo-t values.

The final configuration of samples obtained through PCoA was represented in a 2-dimensional figure with the first and second principal coordinates for the interpretation of general patterns through time. Additionally, box plots were done to show daily values of the physiological and immunological descriptors separately.

Results

Multivariate analysis of physiological and immunological indicators showed that the first three principal coordinates accounted for 67% of the total variation (Table 1). The PCoA configuration showed how samples representing females from different days were effectively ordered in the first and second axes: samples corresponding to day 0 were located to the left and opposite to those corresponding to days 20, 30, and 40 (PCoA 1); while samples from day 0 were clearly above those of day 10 (PCoA 2; Fig. 1). A third axis (PCoA 3) only separated samples from day 20 with those from day 40 (not shown).

The indicators that were positively and most highly correlated to PCoA 1 were GSI and HSI (Table 1), showing that the weight of gonads and digestive gland relative to total body size were higher on samples from day 0 and decreased through time (Fig. 1). Correspondingly, weight loss was highly but negatively correlated to PCoA 1, showing that the amount of biomass lost by females was small immediately after spawning and increased in time (i.e., total body mass decreased in a non-linear manner as time went by; Fig. 2A). Hemagglutination activity was also negatively correlated to PCoA 1, suggesting that females

Table 1 Results of a PCoA on seven physiological and four immunological descriptors measured in female *O. maya* immediately after spawning (Day 0), and after 10, 20, 30, and 40 days

	PCoA 1	PCoA 2	PCoA 3
Variation (%)	35	21	11
Weight loss	−0.80	0.05	0.20
Hepatosomatic Index	0.71	0.15	0.18
Gonadosomatic Index	0.85	−0.26	0.01
Proteins	−0.15	0.47	−0.79
Cholesterol	0.57	0.48	−0.02
Acylglycerides	0.36	0.71	0.19
Glucose	−0.33	0.69	−0.10
Hemocyanin	0.29	0.69	0.00
Hemocytes	0.16	0.38	0.55
Hemagglutination	−0.63	0.14	0.31
Phenoloxidase system	0.20	0.12	−0.09

The amount of variation (%) explained by the first three principal coordinates, as well as Spearman correlation coefficients between descriptors and these coordinates are shown

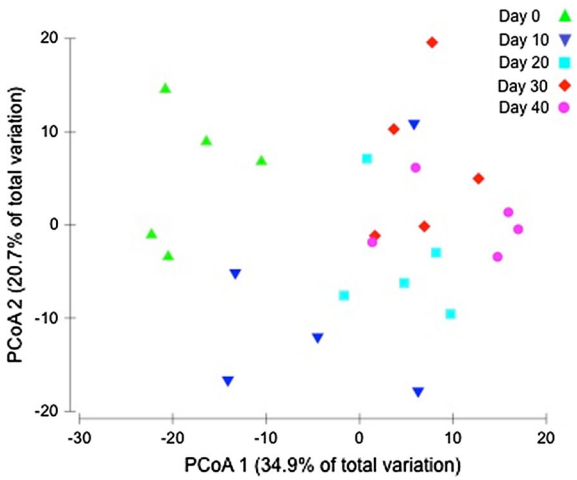

Fig. 1 Principal coordinate analysis (PCoA) of seven physiological and four immunological indicators measured in female *O. maya* immediately after spawning (Day 0), and after 10, 20, 30, and 40 days. A total of $n = 5$ independent females were measured each day

sampled on day 0 had less activity than those from day 20 and onwards (Fig. 2J). Correlation values of acylglycerides, glucose, and hemocyanin concentrations were positive with respect to PCoA 2 (Table 1), indicating that samples from females on day 10 had higher concentrations of these metabolites than those immediately after spawning (Fig. 2B–G).

The permutational MANOVA showed that the physiological and immunological indicators measured on post-spawning females significantly changed with time (pseudo-$F = 3.81$; $P < 0.0001$, 9921 unique permutations). Pairwise comparisons between centroids showed that samples from females on day 0 were significantly different from the rest (Table 2), suggesting that most changes in these descriptors occur immediately after spawning and before 10 days have elapsed (Fig. 2). Significant differences were also found between centroids from days 10 and 20 compared to those on day 40, but centroids representing samples from day 30 and 40 were statistically similar (Table 2). These results suggest that the physiological and immunological condition of post-spawning *O. maya* females remains relatively similar from day 10 to 20, and a second moment of relevant changes takes place somewhere between day 20 and 40 after spawning (Fig. 2).

Discussion

Here, we presented the first information about the physiological and immunological conditions of post-spawning *O. maya* females, to increase the understanding of the mechanisms involved in maintaining of homeostasis during one of the most critical phases of their life cycle.

Studies on reproductive and spawning behavior of cephalopods are scarce and most of the information currently available comes from research carried out under laboratory conditions (Hanlon & Messenger, 1996; Mather et al., 2010). Although field observations are difficult to perform, they can contribute with valuable information to this important part of their life cycle (Garci et al., 2016). According to Van Heukelem (1976), brooding behavior does not depend on the presence of eggs. In his observations, under laboratory conditions, *Octopus cyanea* Gray, 1849 females whose eggs were separated continued brooding behavior even when placed in another aquarium with no access to the former nest site and died after about the same time interval as animals allowed to brood their eggs.

Cephalopods have developed a wide array of reproductive strategies showing a high adaptive flexibility (Rocha et al., 2001). It is well known that the female octopuses spawn once in their life, eat less or stop feeding during the care of the eggs, and die soon after the hatching of the offspring. A study with female *Octopus hummelincki* Adam, 1936 indicates that death is due in part to the secretions of the optical glands (Wodinsky, 1977). When these glands are removed after spawning, the female stops spinning eggs, started eating again, gained weight, and prolongs her life span (four months or more). These results suggest that secretions from the optic glands perform different functions, including copulation control, broodiness, food intake, and longevity. Several neuropeptides, neurotransmitters, and hormones have been detected in the nervous lobes and endocrine glands controlling reproduction in *Octopus vulgaris* Cuvier, 1797 (Di Cosmo & Polese, 2013; Polese et al., 2015). A recently proposed model indicates that the nervous lobes are at the center of a dense network of molecules that provides connection to the nervous system and the endocrine glands, regulating the on–off switch between energy storage and reproduction (Di Cosmo

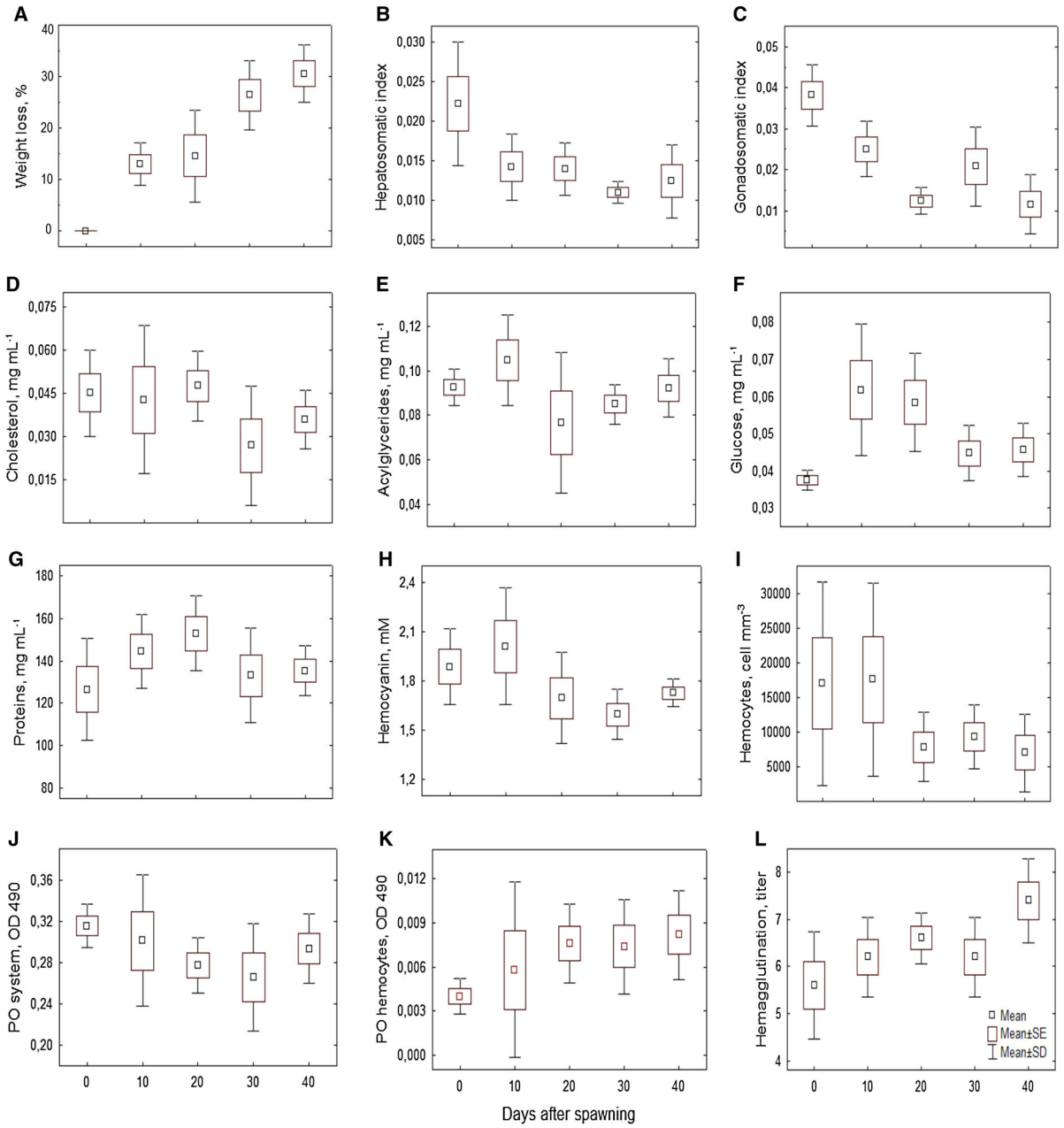

Fig. 2 Box plots of immunological and physiological indicators measured in female *O. maya* immediately after spawning (Day 0), and after 10, 20, 30, and 40 days. A total of $n = 5$ independent females were measured each day. PO: phenoloxidase optical density at 490 nm. Interpretation of univariate statistical features depicted in these figures must be done with caution since they assume that all response variables are uncorrelated and independent of each other

& Polese, 2016). Considering that the females used in this study were found under the same neuroendocrine regulation, in post-spawning conditions, the separation of the eggs could represent a lower energetic demand associated with ventilation and care. However, in general terms the physiological response (immune regulation, feeding, and behavior) can be considered similar to that of females in the presence of eggs.

Table 2 Pseudo-*t* values resulting from paired comparisons among centroids in each level of factor "Days" in the permutational MANOVA on seven physiological and four immunological descriptors measured in post-spawning female *O. maya*; * indicates significant pseudo-*t* values at $P < 0.05$

Pseudo-*t* values					Groups			
Days	0	10	20	30	Days			
0					0	a		
10	1.8*				10		b	
20	2.6*	1.1			20		b	
30	2.6*	1.5	1.5		30		b	c
40	3.3*	1.9*	1.6*	1.2	40			c

Different letters (a–c) assigned to levels of factor "Days" indicate distinct or indistinct groups formed by similar/different centroids

Nutritional status is considered one of the most important factors determining the ability of animals to use ingested and stocked nutrients. In previous studies, the metabolites in the hemolymph, digestive gland, and arm muscles were determined in an attempt to relate diet quality with the nutritional condition of *O. maya* (Aguila et al., 2007; Moguel et al., 2010; Martinez et al., 2014). The results of these studies demonstrated that the metabolites are related to the general health status of the animals, which helped define what metabolic routes and immune mechanisms are used under critical conditions, as observed in other invertebrate species [*Litopenaeus setiferus* (Linnaeus, 1767), Sánchez et al., 2001; *Litopenaeus vannamei* (Boone, 1931), Pascual et al., 2004; *Panulirus argus* (Latreille, 1804), Pascual et al., 2012].

After spawning, weight loss was observed in the wild and under laboratory/aquarium conditions in several octopus species. In the wild, *Enteroctopus dofleini* (Wülker, 1910) females lost 50–71% of their total body weight while brooding (Cosgrove, 1993). In laboratory conditions, Cortez et al. (1995) observed a body weight loss of 25% in *Octopus mimus* Gould, 1852 females, while Van Heukelem (1976) found that *O. cyanea* lost 36%. O'Dor & Wells (1987) and Hernández-Garcia et al. (2002) showed that *O. vulgaris* spawn females can lose 50% of their biomass during maternal care. Similarly, Anderson et al. (2002) showed that, in aquarium conditions, *E. dofleini* had a mean of 49.5% of body weight loss, whereas *Octopus rubescens* Berry, 1953 females lost a mean of 50.3% of their body weight before dying. In the present study, post-spawning *O. maya* females presented gradual loss of their total body weight, reaching 30.7 ± 5.33% lower weight after 40 DAS when compared with animals observed at 0 DAS. However, taking into consideration that the hatching of the juveniles occurs 45–50 DAS at 24°C and that females usually die 45–61 DAS (Van Heukelem, 1976), it is possible that these animals can lose more biomass before the end of their life cycles.

Considering that female octopuses can lose up to 50% of their biomass during maternal care, the nutritional conditions in the pre-spawning period are crucial to their physiological integrity and consequent reproductive success (Otero et al., 2007). During reproduction, resources were mobilized from somatic to gametic tissues in *O. vulgaris*, and final maturation was partially reached at the expense of the body muscles and the digestive gland, suggesting that the nutritional reserves are essential during this phase (O'Dor & Wells, 1978; Tait, 1986). In this sense, Rosa et al. (2002, 2004) and Otero et al. (2007) found that final maturation is reached directly from food rather than from stored products, suggesting that as a complement of nutritional reserves, octopuses need energy for both reproduction and growth (Moltschaniwskyj, 2004).

Previous to spawning, *O. maya* females of the present study were fed with high quality diets mainly composed of crab and squid protein, with the addition of a commercial vitamin mix, minerals, and high quality oils (Tercero et al., 2015). With these diets, total number of eggs spawned (1,374 ± 527 S.D. $n = 40$) was similar to that observed in wild females (Roper et al., 1984) indicating that they met the nutritional requirements for reproduction. Although the nutritional condition of the *O. maya* females from the present study could be different from the nutritional condition of wild females, the mobilization of nutrients and the immune condition of post-spawning females can be useful to understand the physiological mechanisms that are involved during maternal care in this species.

A decrease in both hepatosomatic and gonadosomatic indexes in *O. maya* females starting from 10 DAS was observed in the present study, reaching its lowest value at the end of the sampling period. A reduction in the digestive gland weight and a deterioration of general physiological condition associated with starvation during maternal care in other cephalopod species were also observed (e.g., *O. vulgaris*,

Tait, 1986; Estefanell et al., 2010; *O. mimus*, Cortez et al., 1995; Zamora & Olivares, 2004; *Octopus tehuelchus* d'Orbigny, 1834, Pollero & Iribarne, 1988; *Sepia officinalis* Linnaeus, 1758, Castro et al., 1992). During this process, a decrease in proteins, carbohydrates, and lipids in the digestive gland and muscle were also registered, indicating that during maternal care there are irreversible biochemical and structural changes that lead to female death (Pollero & Iribarne, 1988). But what kind of physiological mechanisms are operating that maintain the female strong enough to care for her spawn?

Results in the present study showed that the plasmatic concentration of proteins, cholesterol, and acylglycerides were similar in *O. maya* females on the different DAS, indicating that the muscle, gonad, and digestive gland reserves were used as a source of energy and molecules to maintain the physiological integrity of post-spawning *O. maya* females. Although the biochemical pathways in octopuses remain to be investigated, the results obtained here help to explain how post-spawning females could use their reserves to maintain the energy demand of the post-spawn condition. Previous studies demonstrated that in *O. maya* proteins are used through a gluconeogenesis pathway to synthesize muscle glycogen and plasmatic glucose (Linares et al., 2015). If, as in other invertebrates, glycogen is the main source of glucose (Rosas et al., 2012), the increased glucose levels observed in post-spawning *O. maya* females 10 and 20 DAS most likely resulted from the breakdown of the muscle glycogen reserves, as was observed in other octopus species during periods of fasting (García-Garrido et al., 2010).

In cephalopods, the digestive gland is not only a site to digest food but it is also a site of nutrient reserves (Rosa et al., 2005a, b). Therefore, it is possible that the constant concentrations of plasma metabolites during the post-spawning period were the product of the catabolism of reserves in the digestive gland and gonad, used as a source of energy to maintain female octopuses in this period. In starved shrimps *L. vannamei*, one of the main mechanisms used for energy is the catabolism of the free amino acids and lipids (Comoglio et al., 2004; Pascual et al., 2006). The results obtained in the present study suggest that stored triglycerides were released from complex lipids to be transported, together with cholesterol by phospholipids to be used as a source of energy.

Heras & Pollero (1989) postulated that hemocyanin and lipoproteins could be the primary transport of fatty acid and other lipids in cephalopods. When gonadosomatic development begins, these fatty acids are specifically transported to the nucleus of the inactive cells of the oocyte, where they are transformed into long chain fatty acids (ARA, EPA, DHA) at the start of vitellogenesis. Once fertilized, the formation of plasma membranes begins, mainly from the nervous system (Budelmann et al., 1997). This mechanism suggests that an inverse process could be occurring during maternal care, with the digestive gland and ovary being the main source of lipids, while proteins are obtained directly from the muscles.

Most studies on nitrogen metabolism of marine invertebrates during starvation indicate that they are well adapted to use protein as a source of energy (Claybrook, 1983). Results obtained from various species of starved crustaceans (e.g., *L. vannamei*, *Penaeus japonicus* Bate, 1888, *Penaeus esculentus* Haswell, 1879) have suggested that the main mechanism used to obtain energy is through the catabolism of the free amino acid pool (FAAP) (Cuzon et al., 1980; Dall & Smith, 1986; Comoglio et al., 2004). This type of process was also observed in fasting *O. maya* juveniles (George-Zamora et al., 2011). If the catabolism of the free amino acid pool is working in post-spawning females, these could explain why the osmoregulatory capacity and osmotic pressure were maintained during 40 DAS (not shown).

In addition to plasma metabolites, hemocyanin levels are also directly related to the nutritional status of shrimps *L. vannamei* and *L. setiferus* (Rosas et al., 2001, 2002). Hemocyanin is a copper-containing protein that represents more than 60% of the total protein in octopus hemolymph (Malham et al., 1998). Immune components have a solid protein base and hemocyanin plays an important role in its function (Rosas et al., 2011). Recent studies have demonstrated that in addition to its multifunctional role (oxygen transporter, storage protein, carotenoids carrier, osmolite, ecdysone transporter) hemocyanin has a fungistatic (Destoumieux-Garzon et al., 2001), and proPO-like function (Adachi et al., 2003). In the present study, the concentration of hemocyanin remained stable during the 40 DAS, and may partly explain the observed stability in PO activity. The multifunctionality of this molecule is linked to physiological plasticity, and indicates that females possess

mechanisms allowing them to maintain an adequate state of health to care for their spawn, despite the long period of starvation.

The immune system is involved in maintaining biological integrity of living beings, allowing recognition and neutralization of harmful microorganisms or molecules from the environment or that are the result of metabolic processes. Molluscan defense mechanisms involve interrelated cell-mediated and humoral reactions (Gueguen et al., 2003), but remain poorly studied in cephalopods (Gestal & Castellanos-Martínez, 2015).

Changes in the number, morphology, and/or viability of hemocytes can be used as health indicators (Ellis et al., 2011), since such changes can occur in animals exposed to stress or that are parasitized. Malham et al. (1998) exposed *Eledone cirrhosa* Lamarck, 1798 to repeated hemolymph sampling (0, 2, and 4 h) and observed a significant increase in the number of hemocytes 2 h after the first sampling, decreasing after 4 h. Variations in the number of hemocytes were also observed in *E. cirrhosa* exposed to air for 5 min (Malham et al., 2002).

In *O. vulgaris*, a significant increase in the number of hemocytes was observed 4 h after injection with lipopolysaccharide of *Escherichia coli* Castellani and Chalmers, 1919 when compared with animals injected only with buffered phosphate saline solution (Locatello et al., 2013). In our study, the total number of circulating hemocytes of *O. maya* females decreased from 20 to 40 DAS, indicating metabolic wear. Similar values were reported for *O. vulgaris* collected from the wild and acclimated in the laboratory for one (Rodríguez-Domínguez et al., 2006) and 24 h before hemolymph extraction (Castellanos-Martínez et al., 2014).

Defense reactions are often accompanied by melanization cascade (proPO activating), which is intimately associated with the appearance of factors stimulating cellular defense by aiding phagocytosis and encapsulation reactions. Prophenoloxidase-activating system, mediated by hemocytes, is a zymogen of PO enzyme that catalyzes both o-hydroxylation of monophenols and oxidation of phenols to quinones leading to the synthesis of melanin (Sritunyalucksana & Söderhall, 2000). Because of their immune functions, highest PO levels are usually found in association with epithelial barriers, respiratory, and circulatory systems in *S. officinalis* (Le Pabic et al., 2014). The hemocytes of cephalopods have phagocytic capacity, antibacterial activity, encapsulation, and also participate in the processes of inflammation, and regeneration of wounds (Beuerlein et al., 2002; Castillo et al., 2015).

Interactions between cellular and humoral factors play an important role in the immune system of invertebrates. The stability in hemocyanin, phenoloxidase activity, and the increase in hemagglutination activity observed in the present study could be reflecting the immunological compensation of *O. maya* females, to ensure the care of the eggs until they hatch. Adjustments in these immune effectors may indicate the ability to decrease susceptibility to opportunistic pathogens, thus avoiding infection of the eggs.

The endocrine and immune systems represent the major internal correlation systems within the organisms. Although acting independently of one another, these systems communicate in an integrated way to coordinate a set of appropriate physiological and behavioral responses (Di Cosmo & Polese, 2016). The immune and physiological responses analyzed show that *O. maya* females are well adapted to tolerate food deprivation during the post-spawning period, which explains their ability to maintain maternal care activities, including the protection and caring of the egg mass from potential predators. The simple physiological constraint of oxygen provision in marine invertebrates may have important ecological and evolutionary consequences (Baeza & Fernández, 2002), so it is important to improve understanding of the main processes involved in maintaining the health of octopus. This information can be useful for monitoring wild populations or to improve management of aquaculture production systems.

Conclusion

Results herein indicate that *O. maya* females are adapted to maintain an adequate post-spawning health condition using energy reserves and compensating immunologically by increasing humoral and cellular processes, this represents an advantage for the next generation.

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References

Adachi, K., T. Hirata, T. Nishioka & M. Sakaguchi, 2003. Hemocyte components in crustaceans convert hemocyanin into a phenoloxidase-like enzyme. Comparative Biochemistry and Physiology—Part B 134: 135–141.

Aguila, J., G. Cuzon, C. Pascual, P. M. Domingues, G. Gaxiola, A. Sánchez, T. Maldonado & C. Rosas, 2007. The effects of fish hydrolysate (CPSP) level on *Octopus maya* (Voss and Solis) diet: digestive enzyme activity, blood metabolites, and energy balance. Aquaculture 273: 641–655.

Anderson, M. J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32–46.

Anderson, R. C., J. B. Wood & R. A. Byrne, 2002. Octopus senescence: the beginning of the end. Journal of Applied Animal Welfare Sciences 5: 275–283.

Avila-Poveda, O. H., N. Koueta, F. Benítez-Villalobos, J. Santos-Valencia & C. Rosas, 2016. Reproductive traits of *Octopus maya* (Cephalopoda: Octopoda) with implications forfisheries management. Molluscan Research 36: 29–44.

Baeza, J. & M. Fernández, 2002. Active brood care in Cancer setosus (Crustacea: Decapoda): the relationship between female behaviour, embryo oxygen consumption and the cost of brooding. Functional Ecology 16: 241–251.

Beuerlein, K., S. Lohr, B. Westermann, P. Ruth & R. Schipp, 2002. Components of the cellular defense and detoxification system of the common cuttlefish *Sepia officinalis* (Mollusca, Cephalopoda). Tissue Cell 34: 390–396.

Bradford, M. M., 1976. A refined and sensitive method for the quantification of microgram quantities of protein utilizing the principal protein-dye binding. Analytical Biochemistry 72: 248–254.

Budelmann, B. U., R. Schipp & S. V. Boletzky, 1997. Cephalopoda: Microscopic Anatomy of Invertebrates. Wiley-Liss, New York.

Caamal-Monsreal, C., M. Mascaró, P. Gallardo, S. Rodríguez, E. Noreña-Barroso, P. Domingues & C. Rosas, 2015. Effects of maternal diet on reproductive performance of *O. maya* and its consequences on biochemical characteristics of the yolk, morphology of embryos and hatchling quality. Aquaculture 441: 84–94.

Caamal-Monsreal, C., I. Uriarte, A. Farias, F. Díaz, A. Sánchez, A. D. Re & C. Rosas, 2016. Effects of temperature on embryo development and metabolism of *O. maya*. Aquaculture 451: 156–162.

Calow, P., 1987. Fact and theory—an overview. In Boyle, P. R. (ed.), Cephalopod life cycles. Academic Press, London: 351–365.

Castellanos-Martínez, S., M. Prado-Alvarez, A. Lobo-da-Cunha, C. Azevedo & C. Gestal, 2014. Morphologic, cytometric and functional characterization of the common octopus (*Octopus vulgaris*) hemocytes. Developmental & Comparative Immunology 44: 50–58.

Castillo, M. G., K. A. Salazar & N. R. Joffe, 2015. The immune response of cephalopods from head to foot. Fish and Shellfish Immunology 46: 145–160.

Castro, B. G., J. L. Garrido & C. G. Sotelo, 1992. Changes in composition of digestive gland and mantle muscle of the cuttlefish *Sepia officinalis* during starvation. Marine Biology 114: 11–20.

Chen, C. & S. Y. Cheng, 1993a. Hemolymph PCO_2, hemocyanin, protein level and urea excretions of *Penaeus monodon* exposed to ambient ammonia. Aquatic Toxicology 27: 281–292.

Chen, C. & S. Y. Cheng, 1993b. Studies on hemocyanin and hemolymph protein levels of *Penaeus japonicus* based on sex, size, and moulting cycle. Comparative Biochemistry and Physiology—Part B 106: 293–296.

Claybrook, D. L., 1983. Nitrogen metabolism. In Mante, L. H. (ed.), The biology of Crustacea. Academic Press, New York: 163–213.

Comoglio, L., G. Gaxiola, A. Roque, G. Cuzon & O. Amin, 2004. The effect of starvation on refeeding, digestive enzyme activity, oxygen consumption and ammonia excretion in juvenile white shrimp *Litopenaeus vannamei*. Journal of Shellfish Research 23: 243–249.

Cortez, T., B. G. Castro & A. Guerra, 1995. Reproduction and condition of female *Octopus mimus* (Mollusca: Cephalopoda). Marine Biology 123: 505–510.

Cosgrove, J. A., 1993. In situ observations of nesting female *Octopus dofleini* (Wülker, 1910). Journal of Cephalopod Biology 2: 33–45.

Cuzon, G., J. Cahu, J. F. Aldrin, J. L. Messager, G. Stéphan & M. Mével, 1980. Starvation effect on metabolism of *Penaus japonicus*. Proceedings of the World Mariculture Society 11: 410–423.

Dall, W. & D. M. Smith, 1986. Oxygen consuption and ammonia-N excretion in fed and starved tiger prawns, *Penaeus esculentus* Hanswell. Aquaculture 55: 23–33.

Destoumieux-Garzon, D., D. Saulnier, J. Garnier, C. Jouffrey, P. Bulet & E. Bachère, 2001. Crustacean immunity. Antifungal peptides are generated from the *C terminus* of shrimp hemocyanin in response to microbial challenge. Journal of Biological Chemistry 276: 47070–47077.

Di Cosmo, A. & G. Polese, 2013. Molluscan bioactive peptide. In Kastin, A. J. (ed.), Handbook of biologically active peptides. Elsevier, Amsterdam: 276–286.

Di Cosmo, A. & G. Polese, 2016. Neuroendocrine-immune systems response to environmental stressors in the cephalopod *Octopus vulgaris*. Frontiers in Physiology 7: 434.

Ellis, R. P., H. Parry, J. I. Spicer, T. H. Hutchinson, R. K. Pipe & S. Widdicombe, 2011. Immunological function in marine invertebrates: responses to environmental perturbation. Fish and Shellfish Immunology 30: 1209–1222.

Estefanell, J., J. Socorro, F. J. Roo, H. Fernández-Palacios & M. Izquierdo, 2010. Gonad maturation in *Octopus vulgaris* during ongrowing, under different conditions of sex ratio. ICES Journal of Marine Science 67: 1487–1493.

Fiorito, G., A. Affuso, J. Basil, A. Cole, P. Girolamo, L. D'Angelo, L. Dickel, C. Gestal, F. Grasso, M. Kuba, F. Mark, D. Melillo, D. Osorio, K. Perkins, G. Ponte, N. Shashar, D. Smith, J. Smith & P. L. R. Andrews, 2015. Guidelines for the Care and Welfare of Cephalopods in Research – A consensus based on an initiative by CephRes, FELASA and the Boyd Group. Laboratory Animals 49: 1–90.

Garci, M. E., J. Hernández-Urcera, M. Gilcoto, R. Fernández-Gago, A. F. González & Á. Guerra, 2016. From brooding to hatching: new insights from a female *Octopus vulgaris* in the wild. Journal of the Marine Biological Association of the United Kingdom 96: 1341–1346.

García-Garrido, S., I. Hachero-Cruzado, D. Garrido, C. Rosas & P. Domingues, 2010. Lipid composition of the mantle and digestive gland of *Octopus vulgaris* juveniles (Cuvier, 1797) exposed to prolonged starvation. Aquaculture International 18: 1223–1241.

George-Zamora, A., T. Viana, S. Rodriguez, G. Espinoza & C. Rosas, 2011. Amino acid mobilization and growth of juvenile *Octopus maya* (Mollusca: Cephalopoda) under inanition and re-feeding. Aquaculture 314: 121–124.

Gestal, C. & S. Castellanos-Martínez, 2015. Understanding the cephalopod immune system based on functional and molecular evidence. Fish & Shellfish Immunology 46: 120–130.

Gueguen, Y., J. P. Cadore, D. Flament, C. Barreau-Roumiguière, A. L. Girardot, J. Garnier, A. Hoareau, E. Bachère & J. M. Escoubas, 2003. Immune gene discovery by expressed sequence tags generated from hemocytes of the bacteria-challenged oyster, *Crassostrea gigas*. Gene 303: 139–145.

Hanlon, R. T. & J. W. Forsythe, 1985. Advances in the laboratory culture of Octopuses for biomedical research. Laboratory Animal Science 35: 33–40.

Hanlon, R. T. & J. B. Messenger, 1996. Cephalopod Behaviour. Cambridge University Press, Cambridge.

Heras, H. & R. J. Pollero, 1989. Blood lipids of the small octopus *Octopus tehuelchus* (Mollusca, Cephalopoda) at different stage of sexual maturation. Comparative Biochemistry and Physiology 92: 571–579.

Hernández-García, V., J. L. Hernández-López & J. J. Castro-Hdez, 2002. On the reproduction of *Octopus vulgaris* off the coast of the Canary Islands. Fisheries Research 57: 197–203.

Hernández-López, J., T. Gollas-Galván & F. Vargas-Albores, 1996. Activation of the prophenoloxidase system of brown shrimp (*Penaeus californiensis* Holmes). Comparative Biochemistry and Physiology—Part C 13: 61–66.

Juárez, O., C. E. Galindo, F. Díaz, A. D. Re, A. M. Sanchez-García, C. Caamal-Monsreal & C. Rosas, 2015. Is temperature conditioning *Octopus maya* fitness? Journal of Experimental Marine Biology and Ecology 467: 71–76.

Le Pabic, C., C. Safi, A. Serpentini, J. Lebel, J. Robin & N. Koueta, 2014. Prophenoloxidase system, lysozyme and protease inhibitor distribution in the common cuttlefish *Sepia officinalis*. Comparative Biochemistry and Physiology—Part B 172–173: 96–104.

Legendre, P. & L. Legendre, 1998. Numerical Ecology. Elsevier, Amsterdam.

Linares, M., S. Rodríguez, C. Caamal-Monsreal, A. Olivares, O. Zuñiga, A. Sanchez, C. Pascual, P. Gallardo & C. Rosas, 2015. Timing of digestion, absorption and assimilation of octopus species living in tropical (*Octopus maya*) and subtropical-temperate (*O. mimus*) ecosystems. Aquatic Biology 24: 127–140.

Locatello, L., G. Fiorito, L. Finos & M. B. Rasotto, 2013. Behavioural and immunological responses to an immune challenge in *Octopus vulgaris*. Physiology & Behavavior 122: 93–99.

Lópes, H. C. 2010. Caracterización estacional de la condición fisiológica de la población Silvestre del pulpo rojo *Octopus maya* (Voss y Solís-Ramírez, 1966) en la localidad de Sisal, Yucatán-México. Thesis, Universidad Autónoma de Guerrero, Acapulco, México.

Malham, S. K., C. L. Coulson & N. W. Runham, 1998. Effects of repeated sampling on the haemocytes and hemolymph of *Eledone cirrhosa* (Lam.). Comparative Biochemistry and Physiology—Part A 121: 431–440.

Malham, S. K., A. Lacoste, F. Gélébart, A. Cueff & S. A. Poulet, 2002. A first insight stress-induced neuroendocrine and immune changes in the octopus *Eledone cirrhosa*. Aquatic Living Resources 15: 187–192.

Martinez, R., P. Gallardo, C. Pascual, J. C. Navarro, A. Sánchez, C. Caamal-Monsreal & C. Rosas, 2014. Growth, survival and physiological condition of *Octopus maya* when fed a successful formulated diet. Aquaculture 426–427: 310–317.

Mather, J. A., R. C. Anderson & J. B. Wood, 2010. Octopus: The Ocean's Intelligent Invertebrate. Timber Press, Portland, OR.

McArdle, B. H. & M. J. Anderson, 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82: 290–297.

Moguel, C., M. Mascaró, O. Avila-Poveda, C. Caamal-Monsreal, A. Sánchez, C. Pascual & C. Rosas, 2010. Morphological, physiological, and behavioural changes during post-hatching development of *Octopus maya* (Mollusca: Cephalopoda) with special focus on digestive system. Aquatic Biology 9: 35–48.

Moltschaniwskyj, N. A., 2004. Understanding the process of growth in cephalopods. Marine and Freshwater Research 55: 379–386.

O'Dor, R. K. & M. J. Wells, 1987. Energy and nutrient flow. In Boyle, P. R. (ed.), Cephalopod life cycles. Academic Press, London: 109–133.

O'Dor, R. K. & M. J. Wells, 1978. Reproduction versus somatic growth: hormonal control in *Octopus vulgaris*. Journal of Experimental Biology 77: 529–540.

Otero, J., A. González, M. P. Sieiro & A. Guerra, 2007. Reproductive cycle and energy allocation of *Octopus vulgaris* in Galician waters, NE Atlantic. Fisheries Research 85: 122–129.

Pascual, C., L. Arena, G. Cuzon, G. Gaxiola, G. Taboada, M. Valenzuela & C. Rosas, 2004. Effect of a size-based selection program on blood metabolites and immune

response of *Litopenaeus vannamei* juveniles fed different dietary carbohydrate levels. Aquaculture 230: 405–416.

Pascual, C., A. Sánchez, E. Zenteno, G. Cuzon, G. Gabriela, R. Brito, R. Gelabert, E. Hidalgo & C. Rosas, 2006. Biochemical, physiological, and immunological changes during starvation in juveniles of *Litopenaeus vannamei*. Aquaculture 251: 416–429.

Pascual, C., J. P. Huchin-Mian, N. Simoes, P. Briones-Fourzan, E. Lozano-Alvarez, A. Sanchez, J. A. Pérez-Vega, R. Simá-Alvarez, C. Rosas & R. Rodriguez-Canul, 2012. Physiological and immunological characterization of Caribbean spiny lobsters *Panulirus argus* naturally infected with Panulirus argus Virus 1 (PaV1). Diseases of Aquatic Organisms 100: 113–124.

Polese, G., C. Bertapelle & A. Di Cosmo, 2015. Role of olfaction in *Octopus vulgaris* reproduction. General and Comparative Endocrinology 210: 55–62.

Pollero, R. J. & O. O. Iribarne, 1988. Biochemical changes during the reproductive cycle of the small Patagonian octopus, *Octopus tehuelchus*, D'Orb. Comparative Biochemistry and Physiology - Part B 90: 317–320.

Rocha, F., A. Guerra & A. Gonzáles, 2001. A review of reproductive strategies in cephalopods. Biological Reviews 76: 291–304.

Rodríguez-Domínguez, H., M. Soto-Búa, R. Iglesias-Blanco, C. Crespo-González, C. Arias-Fernández & J. García-Estévez, 2006. Preliminary study on the phagocytic ability of *Octopus vulgaris* Cuvier, 1797 (Mollusca: Cephalopoda) haemocytes in vitro. Aquaculture 254: 563–570.

Roper, C. F. E., M. J. Sweeney & C. E. Nauen, 1984. FAO Species Catalogue. 3. Cephalopods of the World. An Annotated and Illustrated Catalogue of Species of Interest to Fisheries. In FAO Fisheries Synopsis No. 125, vol. 3. FAO, Rome.

Rosa, R., M. L. Nunes & C. Sousa Reis, 2002. Seasonal changes in the biochemical composition of *Octopus vulgaris*, Cuvier, 1797, from three areas of the Portuguese coast. Bulletin of Marine Science 71: 739–751.

Rosa, R., P. R. Costa & M. L. Nunes, 2004. Effect of sexual maturation on the tissue biochemical composition of *Octopus vulgaris* and *O. defilippi* (Mollusca: Cephalopoda). Marine Biology 145: 563–574.

Rosa, R., P. R. Costa, N. Bandarra & A. J. P. Nunes, 2005a. Changes in tissue biochemical composition and energy reserves associated with sexual maturation in the ommastrephid squids *Illex coindetii* and *Todaropsis eblanae*. The Biological Bulletin 208: 100–113.

Rosa, R., J. Pereira & M. L. Nunes, 2005b. Biochemical composition of cephalopods with different life strategies, with special reference to a giant squid, *Architeuthis* sp. Marine Biology 146: 739–759.

Rosa, R., P. R. Costa, N. Bandarra & A. J. P. Nunes, 2005c. Changes in tissue biochemical composition and energy reserves associated with sexual maturation in the ommastrephid squids *Illex coindetii* and *Todaropsis eblanae*. The Biological Bulletin 208: 100–113.

Rosas, C., G. Cuzon, G. Taboada, C. Pascual, G. Gaxiola & A. Van Wormhoudt, 2001. Effect of dietary protein and energy levels (P/E) on growth, oxygen consumption, hemolymph and digestive gland carbohydrates, nitrogen excretion and osmotic pressure of *Litopenaeus vannamei* and *L. setiferus* juveniles (Crustacea, Decapoda; Penaeidae). Aquaculture Research 32: 1–20.

Rosas, C., G. Cuzon, G. Gaxiola, C. Pascual, G. Taboada, L. Arena & A. Van Wormhoudt, 2002. An energetic and conceptual model of the physiological role of dietary carbohydrates and salinity on *Litopenaeus vannamei* juveniles. Journal of Experimental Marine Biology and Ecology 268: 47–67.

Rosas, C., C. Pascual, M. Mascaró, P. Gebauer, A. Farias, K. Pascke & I. Uriarte, 2011. Applied Ecophysiology: an integrative form to know how culture environment modulates the performance of aquatic species from an energetic point of view. Aquaculture/Book 3. Intech, Rijeka: 161–194.

Rosas, C., A. Valero, C. Caamal-Monsreal, I. Uriarte, A. Farias, P. Gallardo, A. Sánchez & P. Domingues, 2012. Effects of dietary protein sources on growth, survival and digestive capacity of *Octopus maya* juveniles (Mollusca: Cephalopoda). Aquaculture Research 44: 1029–1044.

Rosas, C., P. Gallardo, M. Mascaró, C. Caamal-Monsreal & C. Pascual, 2014. *Octopus maya*. In Iglesias, J., L. Fuentes & R. Villanueva (eds), Cephalopod culture. Springer, Dordrecht: 383–396.

Sánchez, A., C. Pascual, A. Sánchez, F. Vargas-Albores, G. Le Moullac & C. Rosas, 2001. Hemolymph metabolic variables y immune response in *Litopenaeus setiferus* adult males: the effect of acclimation. Aquaculture 198: 13–28.

Solis, M., 1998. Aspectos biológicos del pulpo *Octopus maya* Voss y Solis. Contribuciones de investigación pesquera. Inst Nac de la Pesca 7: 1–38.

Sritunyalucksana, K. & K. Söderhall, 2000. The proPO and clotting system in crustaceans. Aquaculture 191: 53–69.

Tait, R. W., 1986. Aspects physiologiques de la senescence post reproductive chez *Octopus vulgaris*, PhD thesis, University of Paris, Paris.

Tercero, J. F., C. Rosas, M. Mascaro, G. Poot, P. Domingues, E. Noreña, C. Caamal-Monsreal, C. Pascual, J. Estefanell & P. Gallardo, 2015. Effects of parental diets supplemented with different lipid sources on *Octopus maya* embryo and hatching quality. Aquaculture 448: 234–242.

Van Heukelem, W. F., 1976. Growth, bioenergetics and lifespan in *Octopus cyanea* and *Octopus maya*. PhD thesis, University of Hawaii, Hawaii.

Vidal, E. A. G., R. Villanueva, J. P. Andrade, I. G. Gleadall, J. Iglesias, N. Koueta, C. Rosas, S. Segawa, B. Grasse, R. M. Franco-Santos, C. B. Albertin, C. Caamal-Monsreal, M. E. Chimal, E. Edsinger-Gonzales, P. Gallardo, C. Le Pabic, C. Pascual, K. Roumbedakis & J. Wood, 2014. Cephalopod culture: current status of main biological models and research priorities. In Vidal, E. A. G. (ed.). Advances in Marine Biology. Advances in Cephalopod Science: Biology, Ecology, Cultivation and Fisheries. Elsevier, Amsterdam: 1–98.

Wodinsky, J., 1977. Hormonal inhibition of feeding and death in octopus-control by optic gland secretion. Science 198: 948–951.

Zamora, C. M. & P. A. Olivares, 2004. Variaciones bioquímicas e histológicas asociadas al evento reproductivo de la hembra de *Octopus mimus* (Mollusca: Cephalopoda). International Journal of Morphology 22: 207–216.