Fisheries Research 152 (2014) 62–65

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Characterization of deformed hatchlings of *Octopus vulgaris* obtained under captivity from a small female

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ARTICLE INFO

Article history:
Received 1 December 2012
Received in revised form 30 July 2013
Accepted 20 August 2013

Keywords:
Octopus vulgaris
Reproduction
Hatchlings
Deformed
Captivity

ABSTRACT

The common octopus (*Octopus vulgaris*), a promising species for aquaculture, spawns easily under captivity from mature females (usually above 1 kg wet weight). Octopus juveniles and adults are collected from nature to obtain eggs and paralarvae for aquaculture development trials. In July 2011, a very small female (150 g wet weight but with an age estimation of 300 days-old) spawned almost 77,000 paralarvae. Malformations of paralarvae were noticed in the first spawning days, namely the absence of arms. Despite not being lethal, these abnormalities might be derived from the physiological condition of the breeding specimen (the female's lower weight to the estimated amount of living days), which might be eventually related to nutritional unbalances or genetic parameters that were transferred to the eggs.

1. Introduction

The common octopus (*Octopus vulgaris*) is considered a promising species for aquaculture since it displays a rapid and easy adaptation to captivity, a short life cycle, rapid growth (3–15% body weight/day), high food conversion rate (40–60%), high fecundity (100,000–500,000 eggs/female) and high market prices (Fuentes, 2005; Iglesias et al., 2000). Males and females are normally captured in the wild and mate in captivity, being spawns easily obtained in females above 1 kg (Iglesias et al., 2000). Female octopuses display egg parental care, guarding the spawn throughout the embryonic development, during which they stop feeding and continuously aerate the egg masses with water flushes from the funnel (Iglesias et al., 2004). Eggs undergo direct embryonic development to planktonic hatchlings (paralarvae), which are used in aquaculture technology development studies. In 2011, a small female (150 g wet weight) spawned several egg strings that produced approximately 77,000 paralarvae, some of which displayed malformations such as the absence of arms. Hence, the aims of the present study were: (i) to morphologically characterize the deformed hatchlings in terms of external and internal deformations; (ii) to determine the percentage of deformations along the hatching days; (iii) to establish paralarvae changes in size and body composition; and (iv) to characterize the female that laid these eggs in terms of age (by using the daily increments of the beaks) and size.

2. Material and methods

The capture of the *O. vulgaris* female (150 g) in April 2011, by artisanal fishermen on Tenerife coast was performed using pots. After being captured, the female was transported to the IEO facilities in Tenerife, and isolated in a 1000 L round glass fibre tank, which was half covered by a shadowing net and subjected to a natural geographic photoperiod (from 10 light hours and 14 dark hours to 11 light hours and 13 darks hours), under natural sunlight (ranging from 90 lux at 8:00 and 2000 lux at 14:00). This tank was part of an open seawater system, with a water flow of 6 L/min. The mean water temperature was 21.01 ± 0.69 °C, salinity of 36.8 ± 0.14 PSU and dissolved oxygen concentrations kept close to saturation. The female was fed *ad libitum* on a mixed frozen diet of squid, shrimp and mussels (1/3 of each food item daily).

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0165-7836/$ – see front matter
http://dx.doi.org/10.1016/j.fishres.2013.08.017

After 31 days in captivity, the female spawned. Egg masses were handled under the standard methods used during embryonic development, at similar water parameters (Iglesias et al., 2000). The female remained in the tank until its death (weighing 110 g), which occurred simultaneously with the last hatching. Newly hatchlings were collected each day and concentrated in a 5 L jar. Paralarvae number was estimated through the average counting of 10 samples of 10 mL. Malformations of the resulting octopus hatchlings were detected from the first day. Samples of newly hatched paralarvae were collected from subsequent hatching days of the egg masses, namely on the 2nd day, 5th day and 7th day. These samples were used to characterize groups of paralarvae by means of: (i) size and total number of paralarvae, (ii) type and percentage of deformed paralarvae, (iii) lipid composition of paralarvae, (iv) paralarvae tissue histology, and (v) paralarvae beak morphology. In addition, the age of the female was estimated by counting the daily increments of the beak.

Regarding histology, samples were preserved in formaldehyde at 20:1 (volume by sample), followed by dehydration in ethanol (70%, 90% and 95%). Then, pre-infiltration, infiltration and polymerization was performed with Heraeus Technovit 7100 (Heraeus Kulzer GmbH, Germany). Longitudinal and transverse sections of 3 μm were cut in a Microm HM 340 E microtome (Microm International GmbH, Germany) and stained with toluidine blue O, or Ehrlich's hematoxylin solution and eosin Y, or periodic acid Schiff (PAS) solution. Staining solutions were acquired from Carl Roth GmbH + Co. KG (Germany).

Moisture contents were determined from paralarvae samples using the method of Horwitz (1980). Total lipid (TL) contents (percentage dry weight; % DW) were obtained after extraction according to Christie (1982). Lipid classes (LC) were quantified according to Olsen and Henderson (1989) by scanning densitometry and using a Shimadzu CS-9001PC dual wavelength flying spot scanner. Fatty acid methyl esters (FAME) were obtained according to Christie (1982) and were separated and quantified by using a Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector (250 °C) and a fused silica capillary column Supelcowax^{TM} 10 (30 m × 0.32 mm I.D.). Individual FAMEs were identified by reference to authentic standards and well characterized fish oils (PUFA n° 3, SUPELCO PARK, Bellefonte, USA).

The age estimation was carried out according to the methodology described in Perales-Raya et al. (2010), by counting daily increments in the inner lateral wall surfaces (LWS) of the upper beaks. At present, daily deposition of increments in LWS of *O. vulgaris* has been confirmed for paralarvae ages of up to 26 days (Hernández-López et al., 2001) and for adult weights of 160–610 g (Canali et al., 2011). Beaks were extracted, cleaned with water, and preserved in distilled water at ≈4 °C. The beak LWS were analyzed by using a Nikon Microscope Multizoom AZ100 with a magnification of 100×. Daily increments were visible under vertical reflected (episcopic) light because they are opaque structures. Since paralarvae beaks are almost transparent, its morphology and microstructure were analyzed at 400× magnification under transmitted light, by using Nomarski differential interference contrast that creates a 3D image of the beak surface.

Results are shown as means ± standard deviation (SD). Data were checked for normal distribution with the one-sample Kolmogorov–Smirnoff test (Zar, 1999) as well as for homogeneity of the variances with the Levene test (Zar, 1999). A Student-*t* test (Zar, 1999) was performed to compare differences between hatchlings. In all statistical tests, $p < 0.05$ was considered statistically significant. The statistical analysis was performed using the SPSS package (version 13.0) from SPSS Inc.

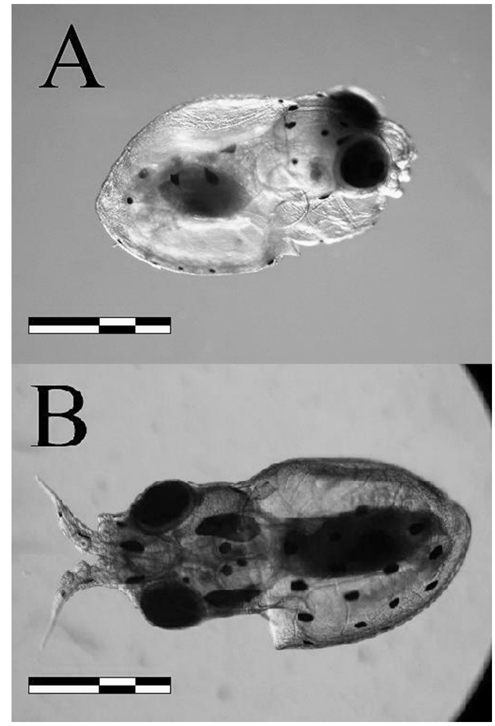

Fig. 1. Deformed hatchlings of the common octopus (*Octopus vulgaris*), reared in captivity. (A) Hatchlings with 0 arms (4×). (B) Hatchlings with 2 arms (4×). Scale bars correspond to 1 mm.

3. Results

The overall number of hatched paralarvae was 76,580 and there was no statistical difference in ventral mantle length ($p > 0.05$) between the initial and the final groups of octopus hatching paralarvae (1.48 ± 0.08 mm in both samples). Two examples of deformed paralarvae (displaying an absence of arms or only 2 arms) are shown in Fig. 1. The percentage of total deformed hatchlings decreased throughout the hatching period (Table 1). The number and percentage of deformations of paralarvae arms is presented in Table 1, ranging from 0 to 8 arms. The first paralarvae to hatch showed the most striking deformations and the highest percentage of paralarvae with no arms (43.3%) or only displaying 2 arms (33.3%). The percentage of deformed paralarvae showed a reduction tendency, with the number of normal developed paralarvae being of 75% in individuals hatching on the 5th day and 100% on the 7th day.

The histological sections of paralarvae tissues revealed no outstanding evidence of malformations between normal or possibly deformed paralarvae apart from the lack of arms in the latter (Fig. 2). Moreover, no evidence of malformations was observed in

Table 1
Percentage of hatchlings with mal-formations (number of arms) during the hatching period of *Octopus vulgaris*.

	No. arms	2nd day	5th day	7th day
Deformed	0	43.3	13.0	0.0
	1	0.0	0.0	0.0
	2	33.3	9.0	0.0
	3	6.7	0.0	0.0
	4	6.7	3.0	0.0
	5	0.0	0.0	0.0
	6	10.0	0.0	0.0
	7	0.0	0.0	0.0
Normal	8	0.0	75.0	100.0

Values represent percentage of total hatchlings for a given day.