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Short communication

Use of Amphipods as alternative prey to culture cuttlefish (*Sepia officinalis*) hatchlings

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ABSTRACT

The effects of feeding two alternative live prey (exclusively caprellids (*Caprella equilibra*) or several species of gammarids, mainly *Ericthonius brasiliensis*, *Jassa marmorata* and *Elasmopus* sp.), to cuttlefish hatchlings were compared to feeding mysids (*Mesopodopsis slabberi*), which are normally used during the first weeks of the life cycle. Weight (g) and growth rates (GR, % BW d^{-1}) were determined. Cuttlefish hatchlings fed with mysids and gammarids grew faster (6.7 ± 0.4 and 5.7 ± 0.9% BW d^{-1}, respectively) compared to caprellids (1.6 ± 0.2% BW d^{-1}). Survival was higher (96.7 ± 5.8%) for hatchlings fed mysids, compared to 83.3 ± 15.3% and 76.7 ± 5.8%, for those fed gammarids and caprellids, respectively. According to the results obtained, gammarids could be used as an alternative prey to mysids, while *Caprella equilibra* did not deliver appropriate growth rates and should be disregarded as alternative prey for rearing early stages (hatchlings) of *Sepia officinalis*. This is the first study revealing a successful use of amphipods, mainly gammarids, as alternative prey for cuttlefish hatchlings.

1. Introduction

Cephalopods can be cultured with both natural live or dead prey (Boletzky and Hanlon, 1983; Toll and Strain, 1988; DeRusha et al., 1989; Castro, 1991; Castro et al., 1993; DiMarco et al., 1993; Castro and Lee, 1994; Domingues et al., 2002, 2005, 2006). Nevertheless, during the first part of their life, live prey have to be provided, with mysids being the live prey that promotes better growth (Domingues et al., 1998, 1999, 2001a, 2003a). The lack of alternative prey that can be successfully used to culture early stages is an important bottleneck for cephalopod large-scale culture (Lee, 1994; Domingues et al., 2003a, 2004). Adult *Artemia* has been used to culture cuttlefish hatchlings with poor results on growth and survival (Domingues et al., 2001b).

Sepia officinalis is one of the most easily cultured cephalopods, and has been cultured in aquaria since the late 1960s (Richard, 1971; Pascual, 1978; Boletzky and Hanlon, 1983; Forsythe et al., 1994; Lee et al., 1998; Domingues et al., 2001a, 2001b, 2002, 2003b).

According to Pinczon du Sel et al. (2000), amphipods are the main prey for *S. officinalis* during the first 3 months. Among these, caprellids and gammarids could be possible alternative prey to mysids, since they are easier to collect and also less expensive to culture, as they can feed on suspended organic matter (Caine, 1974) and can be cultured at high densities.

Caprellid amphipods are small crustaceans that inhabit littoral zones on erect hydrozoans, bryozoans, macroalgae and seagrass (Guerra-García and Tierno de Figueroa, 2009). They are relatively sedentary and important components of epibiotic communities, colonizing also artificial structures. Under appropriate conditions, caprellids can attain high biomass, particularly in environments with high organic content, such as around fish farms (Guerra-García et al., 2004; Woods, 2009). They are opportunistic feeders which have fast growth, quick time to reproductive maturity and short interbrood periods (Woods, 2009), and also graze on epibiotic fauna and flora (Caine, 1974). Their importance as prey for many coastal species is high (Caine, 1989, 1991; Woods, 2009). The nutritional value of caprellids has been studied; Woods (2009) reported high concentrations of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in these animals, making them good candidates for larval stage culture of several species, but differences in body composition should be taken in consideration, as they reflect different feeding habits among species (Guerra-García et al., 2004; Guerra-García and Tierno de Figueroa, 2009). Some species, such as *Caprella mutica*, have been cultured in the laboratory for over 5 years and several generations (Nakajima and Takeuchi, 2008).

The life-history traits of 214 gammaridean species were reviewed by Sainte-Marie (1991), who stated that life-history patterns of gammarid amphipods are influenced by latitude, depth and salinity. Morino (1978) classified breeding activity and life history of amphipods into four categories and concluded that low-latitude species tend to breed throughout the year and have short life spans. According to Cunha et al. (2000), low-latitude, warm-water

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amphipods show iteroparous, multivoltine life-history patterns. Gammarids, similar to caprellids, show short life cycles. They are an important natural dietary component in a variety of coastal marine finfish, and also can be cultured in controlled conditions (Prato et al., 2006; Aravind et al., 2007). In fact, amphipods in general are potentially very useful as aquaculture resource.

In this sense the present research was designed to evaluate the effect of alternative live prey on growth and survival of cuttlefish hatchlings during the first 3 weeks of their life. Two alternative live prey were used: 1) caprellids, *C. equilibra* and 2) several species of gammarids, mainly *Ericthonius brasiliensis*, *Jassa marmorata* and *Elasmopus* sp., and compared to mysids (*Mesopodopsis slabberi*), which are normally used during the first weeks of the life cycle (Domingues et al., 2001b).

2. Material and methods

2.1. Organisms

Sepia officinalis were obtained from eggs collected in Portil Beach, Huelva (south Spain) during July 2009, and hatched in the "Centro IFAPA – Agua del Pino", in Cartaya, Spain.

The experiment lasted 21 days, corresponding to the hatchling stage where hatchings are usually fed live prey. All cuttlefish were born on the same day and from the same egg cluster. The embryonic development of eggs was assured using the technology described in Sykes et al. (2006).

A flow-through system composed of nine rectangular tanks was used. Each tank had a total volume of 10 L and a bottom area of 1410 cm^2. Water flow was of 10 L/h. Each diet had three replicates with 10 hatchlings each. The 90 hatchlings weighed 0.102 ± 0.018 g at the start of the experiment, with no significant differences ($p>0.05$) among replicates. Water was filtered through an industrial sand filter, decanted, and before entering the tanks, passed through a 25-μm mesh filter and a UV bacteriological filter. Water temperature, salinity and dissolved oxygen were measured every morning. Temperature and dissolved oxygen were measured with a CRISON OXI45 probe; salinity was measured with a Kikuchi optical salinity meter. Mean culture temperature was 20.5 ± 1.5 °C. Mean salinity was 36.7 ± 0.9 ‰ and dissolved oxygen values were 7.5 ± 0.1 mg/L and always near saturation (98.8 ± 0.7%). Low light intensity was used to maintain low stress levels (Koueta and Boucaud-Camou, 2003).

2.2. Diets

Three groups of natural live prey were used: 1) caprellids belonging to the species *C. equilibra*, 2) a mixture of several species of gammarids, mainly *E. brasiliensis*, *J. marmorata* and *Elasmopus* sp., and 3) the control mysids (*M. slabberi*), which are normally used during the first weeks of the life cycle. Live prey was collected from the wild. Mysids were collected in saltwater ponds close to the bottom in very shallow water, using small aquarium nets, while caprellids and gammarids were collected from algae and bryozoans attached to buoys and ropes used to anchor boats in the harbour. Prey was provided *ad libitum*, every day, such that there was always abundant live prey in each tank, to assure that this was not a limiting factor. Average total size of prey was of 8 mm, 7 mm and 7 mm, for caprellids, gammarids and mysids, respectively.

Every cuttlefish was weighed individually on a weekly basis, and data was used to calculate mean instantaneous growth rate (MIGR). Mortality was accounted in all diets tested.

2.3. Statistical analysis

The experiment was run simultaneously for the three diets. After every weighing period, statistical analysis was performed to determine differences in weight among groups. ANOVA (Zar, 1999) was performed on the three replicates of each group, and if no significant differences were found among the three replicates, all cuttlefish in those groups fed the same diet were gathered, and ANOVA was performed to compare differences in weight. ANOVA was also performed to compare growth rates. Homogeneity of variances was verified with the Cochran test (Zar, 1999).

3. Results

Average cuttlefish wet weight is showed in Fig. 1. Significant differences ($p<0.05$) were found among cuttlefish fed the three different prey at the end of the experiment. Final weight was 0.412 ± 0.057 g, 0.324 ± 0.043 g and 0.149 ± 0.009 g for hatchlings fed mysids, gammarids and caprellids, respectively. Hatchlings fed mysids were larger ($p<0.05$) that those fed gammarids, and these were also larger ($p<0.05$) compared to those fed caprellids.

Highest overall growth rates were obtained with the control (mysids) and gammarids (6.7 ± 0.4 and 5.7 ± 0.9% BW d^{-1}, respectively), and they were not different ($p>0.05$). Caprellids promoted very low growth rates (1.6 ± 0.2% BW d^{-1}), which were significantly lower ($p<0.05$) compared to the other two prey.

Survival was of 96.7 ± 5.8%, 83.3 ± 15.3% and 76.7 ± 5.8% for hatchlings fed mysids, gammarids and caprellids, respectively, and was not significantly different ($p>0.05$) among diets.

4. Discussion

The dependence on adequate natural prey such as mysids, which are expensive to culture, has been one of the bottlenecks for the large-scale culture of cephalopods such as cuttlefish. Less expensive live prey could reduce considerably production costs and enable large-scale culture (O'Dor et al., 1983; DeRusha et al., 1989; Lee et al., 1991; DiMarco et al., 1993; Domingues et al., 2000, 2006). Amphipods (gammarids and caprellids) are among the most adaptable species in the world (Woods, 2009). Due to their opportunistic feeding, fast growth and reproductive cycles, their culture would be considerably less expensive compared to mysid culture, which requires a constant supply of *Artemia* nauplii for their culture (Domingues et al., 1998). This would make them good candidates to be used as first live prey for cuttlefish hatchlings, and greatly reduce production costs.

Results obtained from the present research indicate that caprellids (*C. equilibra*) is not a good alternative prey for cuttlefish hatchlings during this delicate phase of the life cycle. Growth rates obtained (1.6 ± 0.2% BW d^{-1}), were considerably lower compared to when using mysids and gammarids (>5% BW d^{-1}), or those reported for similar water temperatures (Domingues et al., 2002, 2003a, 2003b, 2004). Previous to this study, another caprellid species (*C. dilatata*) was used in

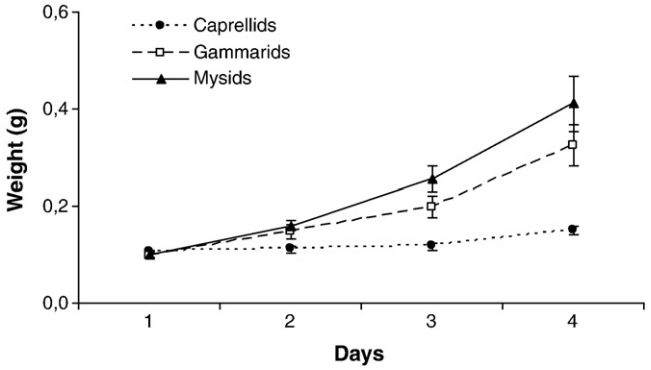

Fig. 1. Growth (g) of *Sepia officinalis* hatchlings cultured for 21 days, fed with three live prey (mysids, caprellids and gammarids). Hatchlings were weighed every 7 days. Bars indicate standard deviations.