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Evidence of external digestion of crustaceans in *Octopus vulgaris* paralarvae

Vicente Hernández-García, Ana Y. Martín & José J. Castro

Departamento de Biología, Universidad de Las Palmas de Gran Canaria, Apartado 550, Las Palmas de Gran Canaria, Canary Islands, Spain. E-mail: ana.martin@biologia.ulpgc.es

This paper reports, for the first time, the existence of external digestion of decapod larvae by the common octopus, *Octopus vulgaris* (Mollusca: Cephalopoda), paralarvae. Zoeae of three crab species were externally digested, leaving a whole and empty exoskeleton. The attack sequence on these prey is also described, and divided into the same three phases (attention, positioning and seizure) already known for *Sepia* hatchlings.

The common octopus, *Octopus vulgaris* (Cuvier, 1797), is probably the best known cephalopod, but knowledge of this species is still very limited (Mangold, 1983; Vecchione, 1994). Sampling of planktonic individuals in the sea is infrequent, and their trophic relationships have not been described, merely hypothesized (Mangold & Boletzky, 1973; Nixon, 1985; Boucher-Rodoni et al., 1987). Currently, only two successful rearing experiments have been reported up to the benthic phase (Itami et al., 1963; Villanueva, 1995) so information on the type of prey which supports survival is particularly limited.

Juveniles and adults demonstrate external digestion when feeding on crabs (Nixon, 1984; Nixon & Mangold, 1996). Feeding starts with the administration of a paralysing agent, cephalotoxin (Nixon, 1987). Immediately after capture of the prey, the octopus squirts enzyme(s) onto it in order to break the muscle–skeletal attachments, thus allowing for easy extraction of the edible contents from the crustacean exoskeleton. This process is well documented in adult *O. vulgaris*, but has not been described in the paralarvae, although it has been reported in Vecchione (1991) for *Loligo vulgaris* hatchlings preying on small shrimps. The aim of this paper is to describe the first evidence of external digestion of crab larvae by octopus paralarvae reared in the laboratory.

Male and female *Octopus vulgaris* were captured along the east coast of Gran Canaria Island (28°57′N 15°22′W) during autumn of 1996 and spring of 1997. Animals were housed in a 12,000 l tank with open seawater flow. Spawning females deposited and incubated the egg clusters in plastic burrows located inside the tank. The embryonic development took between 25 and 30 d at 19–22°C temperature range. Once hatched, the paralarvae were transferred to 12-l transparent containers with open seawater flow. Paralarvae density was variable between 8 and 100 paralarvae l^{-1}. The bottom was siphoned daily in order to clean, remove and count the dead individuals. The illumination was a natural photoperiod throughout the whole experiment.

Octopus paralarvae were fed with recently hatched zoeae obtained from ovigerous females of several crab species collected in rocky shores. The species utilized were *Pachygrapsus marmoratus* (Fabricius, 1787), *P. transversus* (Gibbes, 1850) and *Eriphia verrucosa* (Forsskal, 1775). The maximal length of the zoeae was 2 mm, approximately the mantle length of a recently hatched paralarva.

The paralarvae frequently swam near the surface next to the best illuminated walls of the tank, where the zoeae were also concentrated. In general, paralarvae did not usually attack the first zoea they found, but persisted swimming around several before attacking one. The imminence of an attack can be predicted, because the paralarva modifies its swimming behaviour. When a prey is selected, the hatchling swims with shorter movements than usual, and keeps near to the target prey. Then, the whole body is directed straight towards the zoea, in an arrow-like position, with arms joined and pointing at the prey. All this time, the paralarva is almost immobile, sometimes rotating around the prey, probably looking for a better position from which to attack. The attack occurs at distances of from 1–2 cm, and is very fast. The zoea is handled with all the arms, and the paralarva continues swimming while feeding. Sometimes, the first attack fails, and then the paralarva moves away from its prey and repeats the attack 0.5–2 s later. We have observed up to three successive attacks on a single zoea without any signs of escape behaviour on the part of the prey.

A change in the chromatophore pattern usually happens between the second and third phase of the attack sequence. During the attention phase the paralarvae maintains the chromatophores contracted, so that a nearly transparent appearance is achieved. However, during the positioning phase and/or in the attack movement towards the prey, the dorsal chromatophores covering the central zone of the mantle, the arms chromatophores and those situated between and above the eyes are expanded, leading to an arrow like appearance on the paralarva body. After seizure of the zoea, the chromatophores are contracted again, and the transparent appearance is recovered.

The zoeae of the crab species used were dark coloured, so a recently captured zoea could easily be seen as a dark spot among the arms of the paralarva. However, the prey becomes less visible with time, until it is totally invisible. When the paralarvae finish feeding, the hardly visible remains fall towards the bottom of the tanks. Observation of these remains under the stereomicroscope indicates that they are completely empty transparent exoeskeletons. The extraction of the zoea edible content leaves a molt like carapace, with attached appendages, as the only remains. This is the reason for the progressive invisibility of the zoea among the arms. Unlike the adult octopus, the paralarvae did not dismember the zoeae, but even so, all the appendages were empty of flesh.

The attack on a prey can be separated into the same three phases already reported for *Sepia officinalis* hatchlings (Boucher-Rodoni et al., 1987; Nixon & Mangold, 1996). In the attention phase, the paralarva selects the prey. This phase can be recognized because the hatchling reduces its speed, as reported by Villanueva et al. (1996). In the next phase (positioning), the

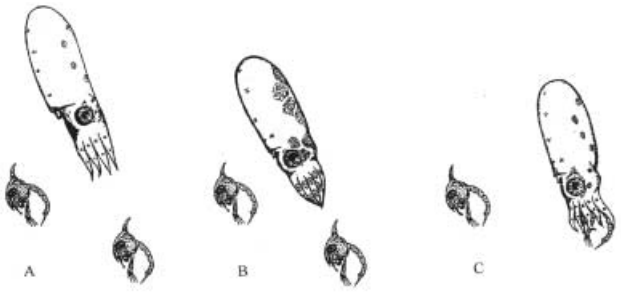

Figure 1. (A) In the attention phase, the paralarva selects its prey. (B) Positioning phase: an arrow like position and a change in chromatophore pattern indicate the imminence of the attack; (C) after seizure of the prey, the paralarva returns to its usual transparent coloration.

body is directed at the zoea, with the arm tips pointing towards the prey. In this position, the stretched body of the hatchling and the zoea form a straight line. So, the observation of the prey by the paralarva during the positioning phase probably implies a binocular fixation in this early stage, as suggested previously (see Nixon & Mangold, 1996). Seizure of the prey always takes place via a forward reaching movement (Villanueva et al., 1996), and the attack is directed towards the zoeae cephalothorax. The failure of an attack may be due to various reasons: the crab zoeae have long dorsal spines, that may damage the paralarva on contact, and also the adherence of the suckers on the prey may not be sufficiently efficient in a first attack, leading the hatchling to repeat the seizure.

At hatching, the paralarvae arms bear three large suckers, and their beaks have tooth like serration (Nixon & Mangold, 1996). Both facts may help them to keep the prey firmly attached to the oral zone, allowing the administration of the digestive enzyme(s) and, possibly, the paralysing toxin. Boucaud-Camou & Roper (1995) found a medium activity of N-acetylglycosaminidase in the posterior salivary glands of *Octopus* paralarvae. They suggested a crinophagic role for this enzyme in these glands. However, another possible function for the N-acetylglycosaminidase could be related to the external digestion of crab zoeae, since this enzyme can act consecutively to chitinase, hydrolysing the dimers and trimers of N-acetylglycosamine left by the action of this late enzyme on chitin (Grisley & Boyle, 1990). In adult octopuses *O. vulgaris* and *Eledone cirrhosa*, hole-boring of exoskeletons has been reported by various authors (see for example Nixon, 1985 and Boyle, 1990) when feeding on crabs. The mechanisms of hole-boring are still unknown, but Grisley & Boyle (1990) found a high chitinase activity in *Eledone* saliva, although no N-acetylglycosaminidase activity was detected.

Moreover, at this stage, the beaks have not reached the necessary degree of development to break fairly hard structures (e.g. crustacean exoskeletons). The beak lacks a pointed tip and the degree of darkening of the chitin implies that there is no great hardening. All of these factors have an important impact on the choice of the diet and on the related feeding behaviour (Hernández-García & Piatkowski, in press).

The external digestion of prey will make the study of trophic relationships of *O. vulgaris* paralarvae even more difficult in the sea. Visual identification of the prey which constitute the natural diet may be impossible. Thus, crustaceans would be underestimated in the diet. Therefore, it is necessary to determine how other prey are ingested via laboratory experiments, in order to get information on the state of potential prey in the digestive tracts of sampled paralarvae.

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