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Direct intracapsular development: implications for feeding mechanisms in early juveniles of the gastropod *Crepidula dilatata*

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Embryos of *Crepidula dilatata* develop within egg capsules where they feed on nutritive eggs also deposited within the capsules. Nutritive eggs permit the embryos to develop to a juvenile stage at hatching without passing through a pelagic larval phase. The juveniles enter the local population in posession of a well developed radula and gill filaments covered by lateral, ventral, and dorsal cilia. Initial feeding by the juveniles, employing both the radula and the gill, occurs from the first day of release from the capsule. The radular band of recently hatched snails is functional from the first day in the benthos, and is used for grazing the substrate, driving the mucus cord from the neck-canal to the mouth, and also for extracting mucus balls produced in the food pouch. The gill is active in particle collection from the first day of the free living juvenile, and is noted by the presence of well developed dorsal and ventral ciliature, active in moving mucus and particles over the gill lamellae to form the mucus string at the extreme ends of the gill filaments. The food pouch becomes active two to three days after initiation of production of mucus cords. All the functions associated with feeding, such as the numbers of mucus cords, radular strokes, and mucus balls increase in activity with progressive development of the juveniles.

INTRODUCTION

Mixed development in gastropods, including benthic and pelagic stages, is typified by an initial embryonic stage contained within egg capsules followed by release of a pelagic, free swimming larval stage (Pechenik, 1979; Gallardo, 1989). Some gastropods have direct development, where all development is completed within the egg capsule, and juvenile snails are released directly to the parental population. In the first case, after a determinate planktonic period the pelagic larva settles on a suitable benthic substrate and passes metamorphosis, which includes, among other structural changes, those associated with the feeding process. In the second case, the larvae undergo metamophosis while contained in the capsule, the structures associated with feeding may become well developed prior to hatching of the juveniles from the capsules. Both forms of development have been found in Calyptraeids of the genus *Crepidula*, as reviewed by Collin (2003).

Information is available on feeding mechanisms related to the radula and gill in the genus *Crepidula*, and their comparative importance has been related to ontogenetic changes (Hoagland, 1979; Chaparro et al., 2002; Navarro & Chaparro, 2002). These studies have shown the use of the radula and gill to occur sequentially in recently settled snails, with the simultaneous use of both systems in more advanced juveniles and in motile individuals. Priority in use of the radula was reported for juveniles and males (Hoagland, 1979; Chaparro et al., 1998; Navarro & Chaparro, 2002), while in feeding by sessile females more importance was assigned to filtering than to grazing (Werner, 1951, 1953; Purchon, 1977; Declerk, 1995). The latter observation has probably generated the broadly held opinion that *Crepidula* spp. are, in general, suspension feeders (Orton, 1913–1915; Newell & Kofoed, 1977; Pechenik & Eyster, 1989; Pechenik et al., 1996).

Some information suggests that the radula of *Crepidula* was used as the initial mechanism by which young snails of this genus collect food from benthic surfaces upon which they hatch. For example, recently hatched *C. convexa* actively used the radula for gathering microorganisms and detritus from the substrate (Hoagland, 1979). Recently juveniles of *C. adunca* used radular action as a feeding mechanism (Putnam, 1964), as did similar-stage juveniles of *C. fecunda* (Montiel et al., personal communication). The morphology of the gill filaments of recently hatched *C. convexa* juveniles did not appear to Hoagland (1979) to be adequate for particle capture, although the gill lamellae of recently settled *C. fecunda* do have an active role in respiration beginning on the first day of settlement (Montiel et al., personal communication) and later develop the capacity to filter particulate food. The latter condition requires a few weeks to develop, since adequate ciliation for filter feeding is required (Montiel et al., personal communication). Contrary to the preceding, Eyster & Pechenik (1988) observed filter feeding in recently settled *C. fornicata*.

Contrasting information concerning the feeding mechanisms of early juveniles of *Crepidula* spp. may reflect differences in the degree of development undergone by larvae or juveniles prior to their settlement or capsular hatching and initiation of feeding in the benthos. With species having direct development, juveniles hatched from

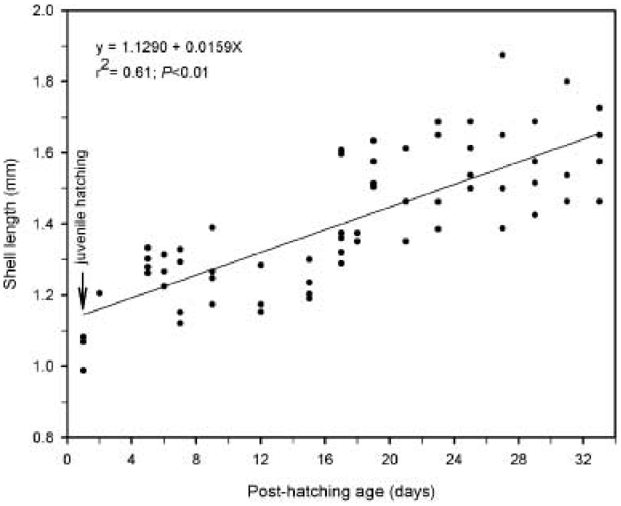

Figure 1. *Crepidula dilatata*. Growth rates of juvenile *C. dilatata* held at 18°C (N=67).

capsules have already passed metamorphosis within the capsule, and in the case of *C. dilatata*, measure between 804 and 1862 μm in shell length upon recruitment into local populations (Chaparro & Paschke, 1990; Chaparro et al., 1999). This high comparative degree of development could allow new juveniles of species with direct development recruit to local populations in a higher state of development than larvae of species with pelagic development (e.g. larvae of *C. fecunda* settle at a shell length of about 650 μm). Recruiting juveniles would have optimally developed structures for feeding, which allow complementary activity between grazing and a filter-feeding process. The preceding would contrast with species with mixed development where the two types of feeding develop out of phase with each other. The present study examines the capacity and activity of feeding by newly settled juveniles of *C. dilatata* to clarify the impacts of direct intracapsular development on early feeding behaviour of these juvenile snails.

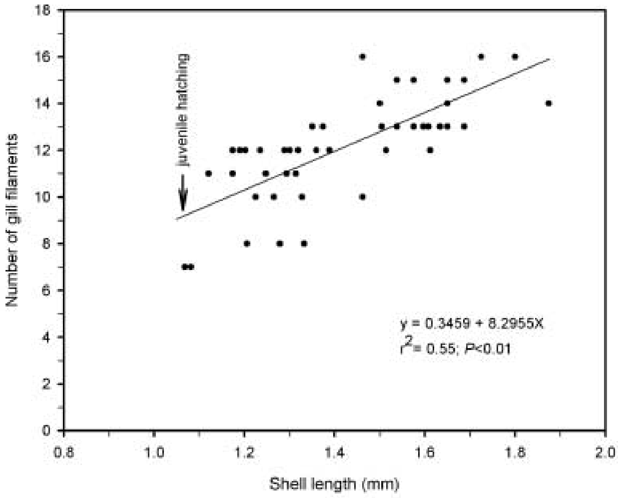

Figure 2. *Crepidula dilatata*. Relation between shell length and number of gill filaments in juvenile snails of different ages post-hatching.

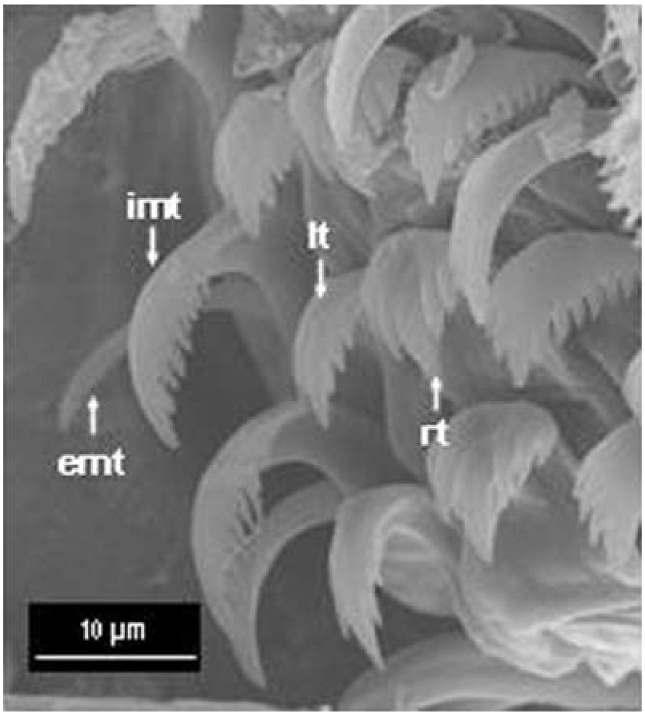

Figure 3. *Crepidula dilatata*. Photomicrograph of radular band (taenioglossa) in a recently hatched juvenile snail, one day in age. rt, rachidian or central tooth; lt, lateral tooth; imt, internal marginal tooth; emt, external marginal tooth.

MATERIALS AND METHODS

Adult *Crepidula dilatata* were obtained from the subtidal zone (0.5 to 1 m depth) of the Quempillen estuary (41°51′S 73°46′W), Chiloe Island, southern Chile, removing females from the substrate in order to obtain egg capsules. Capsule spawning masses adhered to the substrate and showed an advanced stage of development were collected and maintained in 10-l seawater aquaria in the laboratory. Seawater in the aquaria had a salinity of 30 ppt, was filtered to 0.47 μm, and maintained at 18°C. The water received constant aeration, and was changed every two days.

The capsules were observed daily until liberation of juvenile individuals began to occur. Sixteen groups of recently liberated juveniles were collected daily, and deposited in 5-l aquaria in order to separately maintain progressive age groupings.

The recently hatched *C. dilatata* juveniles were maintained in 10-l aquaria as described above and placed on glass slides covered with biofilm as a potential food on the surface. Biofilms were obtained on the slides by immersing them directly in the estuary for one week. About 3×10^4 cells per ml of artificially cultured *Isochrysis galbana* were also administered as a potential source of suspended food. An inverted microscope fitted with a Pulnix model TMC-7 camera was used to film feeding behaviour of the juvenile snails from the ventral aspect. Individuals of the different ages studied were filmed for periods of one hour under the same water conditions as maintained in the holding aquaria. During filming, the snails had access to both the biofilmed substrate and the suspended microalgae, and were observed for both grazing and filter feeding

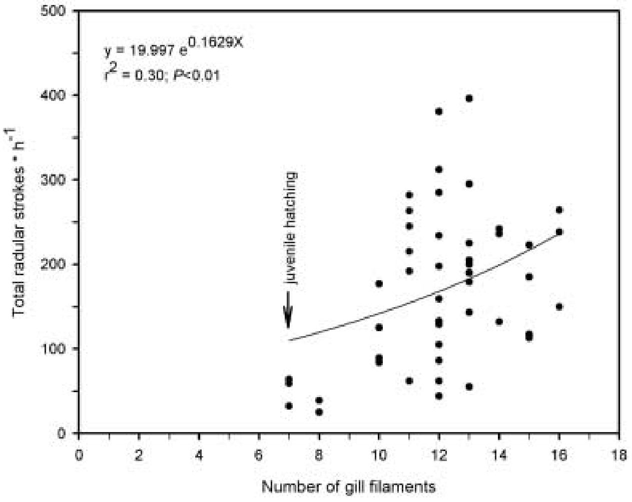

Figure 4. *Crepidula dilatata*. Grazing activity on the substrate biofilm in the collection of food by juvenile snails of different ages (expressed as number of gill filaments).

behaviour. Water containing microalgae was continually circulated through the observation chamber to maintain a constant concentration of the suspended food for the individuals under observation. Biofilms were maintained by replacing grazed substrates with newly fouled substrates as necessary.

Filming of feeding activity was done using 4–6 replicates for each of the post-hatching ages of the young juveniles observed every two days, to about one month post-hatching. The shells of the juveniles (longest axis) were measured during each filming session.

Film records of the grazing and filter-feeding by the *C. dilatata* juveniles were digitalized in a BMP format using Ati all in Wonder® image capture cards, and processed using Scion Image 3.0b software.

Figure 5. *Crepidula dilatata*. Photomicrograph of a mucus cord (arrow) produced in the gill of a recently hatched (one-day) juvenile.

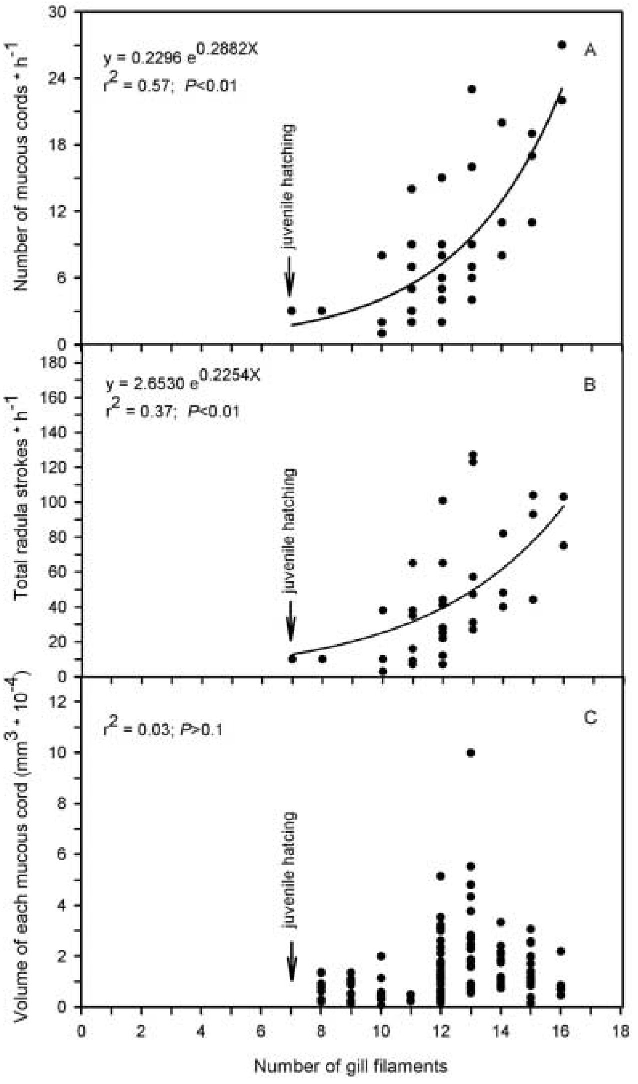

Figure 6. *Crepidula dilatata*. Production and consumption of mucus cords in juveniles between one and 33 days post hatching. (A) Rate of production of mucus cords; (B) grazing effected per ingestion of mucus cords produced per hour; (C) range of volume of mucus cords produced by post-hatching juveniles of different ages (expressed as number of gill filaments).

Analysis of the film records centred on the structure or functionality of the radula, the mucus cords, mucus balls, osphradia and the gill filaments.

The mucus cord (MC) is the term for a cylinder of food and mucus found within the neck canal of the snail (Chaparro et al., 2002). We counted the number of cords produced per hour, as well as the number of radular strokes required to effect their ingestion by each snail. The volume of each mucus cord was estimated by $\pi * r^2 *$ length, with direct measurement of the diameter and length of each cord just prior to its ingestion.

The mucus ball (MB) is a spherical body formed of mucus and food particles, located within the food pouch of the snail (Chaparro et al., 2002). The production of mucus ball per hour was determined for each specimen observed. The number of balls ingested were also counted and the number of radular strokes required to effect the ingestion of each bolus, as related to the age of each snail observed.

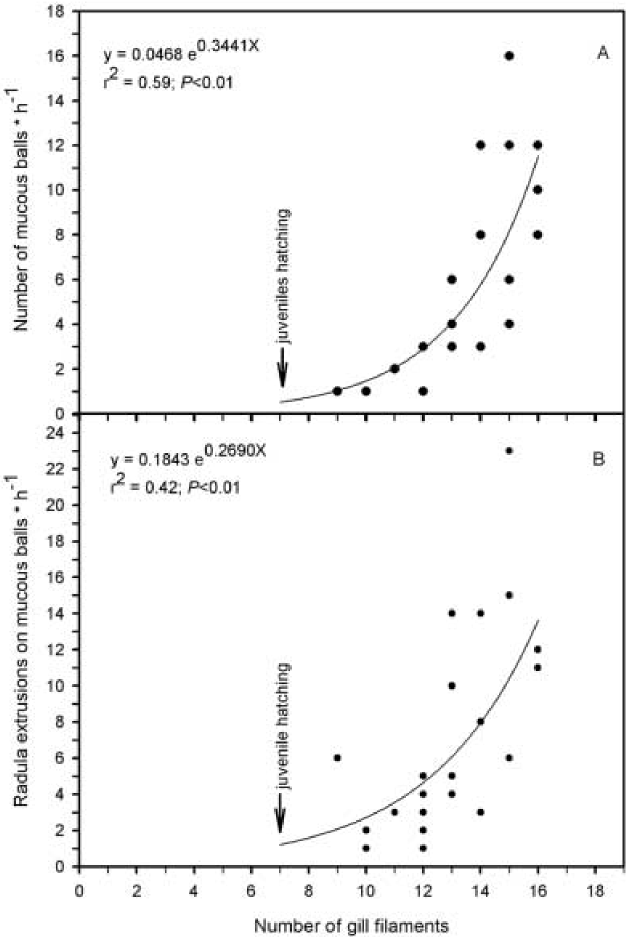

Figure 7. *Crepidula dilatata*. Production and ingestion of mucus balls from one to 33 days post hatching by juvenile snails. (A) Rate of production of mucus balls in the food pouch; (B) grazing strokes effected for the ingestion of all the mucus balls produced per hour by post hatching snails of different ages (expressed as number of gill filaments).

The condition of the radula, as well as the radular activity, was noted for each age-group of juvenile snail observed. Use of the radula was noted as strokes of the radula per hour by the snails as they obtained food from the substrate biofilm.

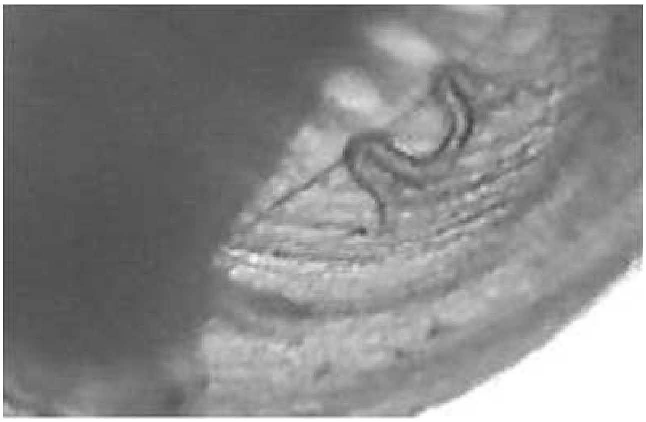

Figure 8. *Crepidula dilatata*. Photomicrograph of the osphradium of a juvenile snail at two days post hatching.

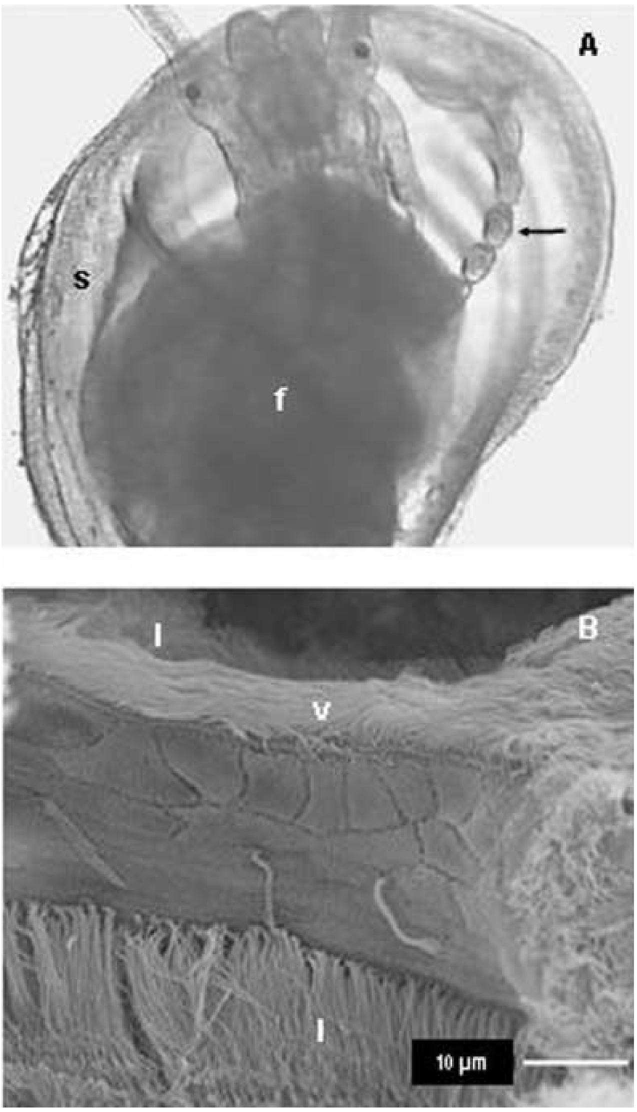

Figure 9. *Crepidula dilatata*. Photomicrographs of gill filaments in juvenile snails at one day post-hatching. (A) General view of gill filaments (arrow) in the juvenile, s: shell, f: foot; (B) SEM image of a gill filament (lateral view) to show the presence of lateral (l), and ventral (v) ciliature.

Groups of 15–20 juvenile snails for examination by scanning electron microscopy (SEM), were fixed every 2–3 days in 2% glutaraldehyde for 2–4 h at 4°C, washed in 0.1 M phosphate buffer, and dehydrated in a graded series of alcohols for 30 min periods until reaching 100% ethanol, followed by a 100% acetone wash. Samples were critical point dried in a Hitachi drier, using CO_2 as a transitional fluid. Portions of the shell of each specimen were removed with the aid of a stereoscopic microscope in order to expose the gill, and then were mounted on SEM stubs and shadowed with gold for observation in a LEO 420 SEM. Observations were concentrated on the radular band and the micro-structure of the branchial filaments, with special emphasis on presence and development of the ciliature.

RESULTS

Recently hatched juveniles of *Crepidula dilatata* held at 18°C, showed a significant relation between age and length of the shell (Figure 1: y=1.1290 + 0.0159 X;

$N=67$). The length of the shell was similarly related to the number of gill filaments (Figure 2: $y=0.3459+8.2955\ X$, $N=46$). The number of osphradia present showed no significant relation to the age of the juvenile snails ($P>0.01$).

Radula

A taenioglossan radula (dental formula 2-1-1-1-2; Figure 3) was present in the juveniles from the first day post-hatching, and was actively employed in the collection of food from the first day of release from the capsule. The radular activity (strokes h^{-1}) demonstrated a weak relationship to the development of the individuals. This species showed a maximum activity of 396 strokes h^{-1} (Figure 4: $y=19.997\ e^{0.1629X}$; $N=46$) in a specimen at about 20 days post-hatching (13 gill filaments).

Mucus cords

The mucus cords of *C. dilatata* were formed beginning on the first day of liberation from the capsule, when specimens had seven gill filaments (Figure 5). An increase in the number of mucus cords was observed as the juveniles grew, with three cords produced per hour in 1-day specimens and 27 cords per hour in 33-day post-release specimens (Figure 6A: $y=0.2296\ e^{0.2882X}$; $N=36$). The increase in production of mucus cords was paralleled by an increase in radular activity required to ingest the cords produced per unit time. The earliest juveniles employed 10 radular strokes per hour, while the most advanced specimens employed 127 strokes per hour (Figure 6B: $y=2.6530\ e^{0.2254X}$; $N=36$).

There was no significant relation ($P>0.01$) in this species between the number of gill filaments or size of the juveniles and the volume of mucus cords produced (Figure 6C, $N=130$). This was due to the fact that the volumes of the mucus cords produced by each juvenile snail were highly variable, althougth some tendency was observed toward production of larger-volume mucus cords by larger individuals (having the greater numbers of filaments).

Mucus balls

Recently hatched juveniles of *C. dilatata* were able to produce mucus balls in the food pouch at 2–3 days post hatching. These increased in number with development of the juvenile snails (Figure 7A: $y=0.0468\ e^{0.3441X}$; $N=25$), with extremes of one ball h^{-1} in the youngest juveniles to maxima of 16 balls h^{-1} in specimens at 30 days post hatching. The radular activity for ingestion of all mucus balls produced per hour also showed an increase with development of the snails (Figure 7B: $y=0.1843\ e^{0.2690X}$; $N=25$). In young specimens, an average of one radular extrusion was carried out per hour, while in the most advanced snails observed, 23 strokes were required to ingest all the mucus balls produced per hour.

Osphradia

The presence of an osphradium was noted in the anterio-lateral region of the mantle beginning at the first day post-hatching. Increase in number of these structures was almost nil. A second osphradial filament was observed in specimens at 2–4 days post hatching, and persisted without variation to the oldest (33 day) juveniles observed in the study (Figure 8).

Gill filaments

Observation of the ventral area of recently hatched *C. dilatata* juveniles showed the presence of seven gill filaments. These filaments increased in number with progressing development of the snails. The maximum number of filaments observed was 16, occurring in a juvenile snail at 33 days post hatching (Figure 1).

The gill filaments of *C. dilatata* were elongated in form, with a bulbous distal section. The ciliature was composed of lateral, dorsal, and ventral cilia from the first day post hatching (Figure 9A,B). The density of the cilia increased with advancement in development of the specimens, and no additional ciliary types appeared.

DISCUSSION

Existing information suggests that the feeding in post-hatching *Crepidula* is based on use of the radula beginning on the first day after hatching (e.g. *C. convexa*, Hoagland, 1979; *C. adunca*, Putnam, 1964). In some cases, the use of the gill was also identified as a mechanism for collecting food in very early juveniles (e.g. *C. fornicata*, Eyster & Pechenik, 1988). In recent studies it was shown that use of the radula and gill in *C. fecunda* juveniles occurred out of phase, with the radula employed first, followed by the use of the gill in particle capture beginning two weeks later (Montiel et al., in press).

Snails of the genus *Crepidula* bear a taenioglossan-type radula (Steneck & Walting, 1982; Black et al., 1988; Hawkins et al., 1989; Chaparro et al., 2002). In juveniles of *Crepidula*, this structure appears to be present and active for the collection of food from surfaces, beginning on their first day of life in the benthos (e.g. *C. fecunda*, Montiel et al., personal communication). This condition is now reported in recently hatched juveniles of *C. dilatata*. In the case of *C. fecunda* it was reliably shown that the radula was the only operational feeding mechanism during the first few weeks of benthic life of these snails. In contrast, the present study has shown that both the radula and the gill are used simultaneously in initial feeding by *C. dilatata*.

Thus, in juveniles from this species, the gill is able to capture particles from the plankton, whereas the radula is active in the food collection from the surface. Radula is also able to take the mucus cords from the neck channel to be moved to the mouth for ingestion, as it happens in adult specimens of *Crepidula* (Chaparro et al., 2002).

In the case of *C. convexa*, inefficiency of filtration as a feeding mechanism in its early juvenile phase is due to the scarcity of gill filaments as well as their low degree of development, particularly with regard to the ciliature (Hoagland, 1979). In juveniles of *C. fecunda* (<9 days) the gill filaments bear active lateral cilia which are capable of generating ventilatory water currents, and dorsal and ventral ciliature of more advanced juveniles is not yet present (Montiel et al., personal communication). The latter two ciliary types are responsible for moving mucus and food particles towards the distal region of the gill filaments (Chaparro et al., 2002), and appears in juveniles of *C. fecunda* after about nine days of benthic life. In contrast, the observation in the present study of initial filter feeding in *C. dilatata* can be attributed to the presence at settlement of dorsal and ventral ciliature, in addition to the lateral ciliature of the gill filaments.

The production of mucus cords, which reflects filter feeding activity, showed a strong increase with increase in age of the *C. dilatata* juveniles. However, the size of the mucus cords (based on volume) at ingestion showed no correlation with the stage of development of the juveniles observed. In spite of the preceding, there was evidence that as the individuals grew, they were able to generate mucus cords of increasing volume. The consumption of mucus cords of differing volumes suggested that their ingestion occurred independently of size. A similar situation was observed in early juveniles of *C. fecunda* (Montiel et al., personal communication). The ingestion of mucus cords with different sizes by juveniles of *C. fecunda*, could represent a partial development of the filter feeding mechanism, which in complement with the radula as another independent food collector mechanism, could meet the energetic requirement of the juveniles.

However, in sessile females of *C. fecunda*, a strong relationship was registered between food available and increment in the volume of the mucus cords ingested. This leads to the supposition that early juveniles, as observed in the present study (in the presence of a constant concentration of particulate food), did not appear to have developed the capacity to regulate the ingestion of mucus cords except for those which had reached a 'maximum size'. In spite of the preceding, the total number of grazing strokes on the mucus cords ingested per unit time increased with the age in early juveniles of *C. dilatata*, suggesting that their ingestive capacity increased with their age.

The occurrence of production of mucus balls in the food pouch about two days after the production of mucus cords by the gill as noted above for *C. dilatata* has also been observed in *C. fecunda* (Montiel et al., personal communication). This occurs because the filtration system must be functional before the food pouch is able to become active. The food pouch receives at least a portion of the particles obtained by the shorter branchial filaments, which are in contact with this structure. The tendency toward increase in the number of mucus balls produced per hour with development of the juveniles is evident, and similar to that observed for *C. fecunda* (Montiel et al., personal communication).

In conclusion, early juveniles of *C. dilatata* demonstrate simultaneous use of the radula and the gill as feeding mechanisms beginning on the first day of hatching from the egg capsule. The gill filaments are covered with ventral and dorsal cilia which are active in filter feeding. Immediate initiation of feeding using the radula and gill, as well as the degree of development of the gill, radula, and osphradia in the first day of benthic life in *C. dilatata* appears to be a consequence of its direct development within the egg capsule, supported by the fact that embryos of this species consume nutritive eggs within the capsules as they develop into pre-hatching juveniles. Future research may allow further corroboration that direct development in the genus *Crepidula* is associated with the capacity for feeding using the radula and gill by the juvenile snails immediately post-hatching.

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