

**A SCANNING ELECTRON MICROSCOPE
ANALYSIS OF MORPHOGENESIS OF
EMBRYOS AND JUVENILES OF THE
DIRECT DEVELOPING ISOPOD,
Cyathura polita (Stimpson, 1855)**

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Isopods are a species rich, morphologically diverse group characterised by direct development of young within a marsupium. Collectively, these traits make isopods excellent models for understanding the changes to morphogenesis that occur in the evolution of direct development, but the overall understanding of this process is limited by a lack of comparative data. We investigated morphogenesis in *Cyathura polita*, an isopod common in estuaries along the Atlantic coast of North America, from gastrulation to release of young as benthic juveniles, using Scanning Electron Microscopy. We found that early development of *C. polita* from gastrulation to hatching from the extraembryonic membranes was generally similar to that described by earlier work using sectioned material, but found that development through the manca stage to free living juveniles differed from previous studies. For instance, the embryonic dorsal organ differed from that described generally for isopods as the apical surface is covered by a sieve plate and the typically reported microvilli were not observed. Differences in timing were also noted: for example, segments appeared throughout the pereon and pleon well before the post-naupliar appendages, and not coincident with them as described previously for isopods. Limb development occurred simultaneously except that pereopod 7 did not form until the post-manca stages, as is typical for this group, suggesting there is non sequential (or suppression of) teloblast activity in postnaupliar development. Also, some broods contained young of different developmental stages (i.e., early embryos and late manca), indicating that spawning events are repeated and overlap, which is perhaps adaptive in the shortened reproductive season characteristic of our study population that is at the northern limit of the known range for this species. Mean brood size was 53.2 ± 18.9 young (range 4-108), the largest reported for Anthuridae (a family of isopod crustaceans).

Key words: scanning electron microscopy, marsupium, manca, embryonic dorsal organ, direct development, crustaceans.

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INTRODUCTION

Marine isopods are a large, diverse group (~4500 spp; Brusca 1997), which, as in other peracaridan crustaceans, are characterised by direct development of maternally brooded young (Anderson 1973). Reproductive biology is well known for a few species and presents some fascinating examples of mating behaviour, mate guarding, and both protandrous and protogynic sex change (reviewed by Johnson et al. 2001). Unfortunately, the understanding of isopod development is limited to a relatively few, but thorough, descriptions of embryonic development (McMurrich 1895, Strömberg 1965, 1967, 1971, 1972) and several descriptions of young following release from the marsupium (Elizalde and Sorbe 1993, Araujo et al. 2004). Detailed comparative data of morphogenesis throughout ontogeny is lacking despite the importance of these data to overall understanding of isopod diversity. Description of external morphogenesis in embryos and juveniles of *Cyathura polita* (Stimpson, 1855) (Anthuridae) will provide a more complete understanding of ontogeny for an isopod that is relatively well known in terms of early embryology (Strömberg 1972), physiology (Kelley and Burbanck 1976) and reproductive biology (Frankenberg and Burbanck 1963, Burbanck and Burbanck 1974, Burbanck et al. 1979).

Most isopods have large, centrolecithal eggs (>300 μ m) that are released as zygotes into a ventral marsupium composed of overlapping oostegites (Strömberg 1965, 1971, Johnson et al. 2001). Cleavage is superficial and results in a thin blastoderm and a thickened, ventral germ band. As the germ band elongates, head bands give rise to 3 naupliar segments and associated antennae and mandibles. The metanaupliar region forms as teloblasts sequentially divide to generate the maxillulary and maxillary segments, followed by the segments of the pereon (thorax) and pleon (abdomen) (Strömberg 1967). Embryos hatch from the extraembryonic membranes as manca that continue to develop within the marsupium. Manca become morphologically similar to free living juveniles but lack legs on pereonite 7 (Strömberg 1967, Johnson et al. 2001). After the manca are released from the marsupium, they develop into adults through a series of molts as pereopods 7 are formed. Descriptions of isopod reproductive biology are provided in the comprehensive reviews of Anderson (1973) and Johnson et al. (2001).

The single previous study of embryology in the Anthuridae is Strömberg's (1972) analysis of internal morphogenesis of *C. polita*, and was based on sectioned material to describe cleavage, cell arrangement

and organ formation in early embryos. *Cyathura polita* is a benthic isopod that occurs in estuaries from the Gulf of Mexico to the Bay of Fundy (Burbanck et al. 1979). Adults are interstitial in coarse sand of intertidal and shallowly subtidal areas and feed mainly on detritus and algae in the sediment (Burbanck 1959, Burbanck et al. 1979). Most *C. polita* are protogynic hermaphrodites, however, a few mature directly as males (Burbanck and Burbanck 1974). Life-history traits vary with latitude: northern populations, as described here, have longer life cycles, reproduce later in the summer, contain larger females and have higher individual fecundity than do southern populations (Mercer et al. 2007). Strömberg's (1972) description of embryos ended at hatching from the extraembryonic membranes, and later development of young within the maternal brood pouch was not described.

Our objective was to examine external morphogenesis in embryo and juvenile *C. polita* using Scanning Electron Microscopy (SEM) to build a more complete understanding of the development of this relatively well known species. Our study builds upon Strömberg's (1972) description of *C. polita* embryos by focusing on external structures and extending the period of ontogeny in question to include the entire brooded period until release and then growth as benthic juveniles.

METHODS

Collection and Preparation for Scanning Electron Microscopy

Cyathura polita were collected from the lower Saint John River Estuary, New Brunswick, Canada, the northern-most population known for this species (Mercer et al. 2007). Isopods were collected from four stations: Bell Point, Westfield Beach, Summerville and Millidgeville (approximately 45°20'N; 66°20'W). Samples were obtained bi-weekly from May 29 to August 27, 2004, by collecting surface sediments from shallow sub-tidal areas with water depths of 20 to 150 cm. Sediment was collected with a shovel and sieved on 1.0 mm or 0.50 mm mesh. Gravid females were fixed in formalin for 3 to 4 hours on the day of collection and stored in 70% ethanol until examination. During examination, young were removed from the marsupia, measured, and stage of development determined.

Most specimens examined with scanning electron microscopy were collected from Millidgeville on July 27, 2004. This included twelve females with gravid marsupia and eleven juveniles. The day following

collection, young were removed from 10 of the marsupia. Intact adults and isolated young were anaesthetized in 3.5% magnesium chloride, fixed in 2.5% glutaraldehyde (1 hour) and post-fixed in 1% osmium tetroxide (4 hours), both in 0.1M cacodylate in seawater. Additional young were removed from the marsupia of a few females that were fixed in formalin (above) and prepared for SEM by post fixing in 1% osmium tetroxide. Specimens were dehydrated in ethanol and critical-point dried using a Bio-Rad E3000 critical point drier in liquid CO₂. As embryos were placed on stubs, they were gently rolled on carbon tape to remove the chorion and vitelline membrane. Samples were coated with gold palladium using a Technics Hummer II sputter coater and viewed with a JEOL 5600LV Scanning Electron Microscope at 10kV. Digital images were adjusted for size and brightness in Corel Photo Paint 11, and arranged in plates using Corel Draw 11.

Identification of adults and classification of young

Females were identified by the presence of oostegites forming a ventral marsupium. Males were identified by the presence of an appendix masculina on pleopod 2. Post-manca individuals that did not demonstrate either of these sexual dimorphisms were considered to be juveniles or non-reproductive adults.

Young were categorized into six stages of development: Class 1) embryos that were oval in shape and housed within two extra embryonic membranes; Class 2) embryos that had naupliar appendages (antenna 1, antenna 2, mandible); Class 3) embryos with post-naupliar appendages (other mouthparts, pereopods, pleopods); Class 4) early manca that had hatched from the embryonic membranes but were still housed within the marsupium; Class 5) late manca, also housed within the marsupium but with an arrangement of appendages similar to adults although pereopods 7 were absent; and Class 6) free-living juveniles with pereopods 7 but lacking sexually dimorphic traits.

RESULTS

Female anatomy and brood structure

Mature females examined were 13.8 ± 2.14 mm long (mean \pm S.D., $n = 163$). The pereon consisted of seven elongate pereonites, each with a pair of pereopods (Fig 1A). In both sexes, pereopod 1 is modified into large gnathopods and is used for feeding and mating. Pereopods

2-7 are ambulatory and are arranged such that P2-P3 are anteriorly directed and P4-P7 are posteriorly directed. Pleopod 1 is operculate and covers pleopods 2-5. Gravid females had a well-developed marsupium that consisted of 4 pairs of oostegites arising from the coxa of pereonites 3-6 (Fig 1A, B). The leaf-like oostegites loosely overlapped on the ventral surface of the body to form a single pouch that housed the young (Fig 1C). Brood size was 53.2 ± 18.9 (mean \pm S.D.) with a range of 4-102 young per brood ($n=72$ females).

Development

Embryos

Class 1 embryos were slightly oval-shaped, flattened on the ventral side, and covered by two extraembryonic membranes including an outer chorion and an inner vitelline membrane (Fig 2A, B). Class 1 embryos observed were blastulae and had a single-layered cellular blastoderm that covered a large central yolk mass (Fig 2B). Class 1 embryos were $450 \pm 60 \mu\text{m}$ in length ($x \pm$ standard deviation; $n=956$). We were unable to measure the size of mature oocytes or zygotes (not collected) but the size of Class 1 embryos measured here were similar to the egg size given for the 8-cell stage of development ($0.40 \times 0.45\text{mm}$; Stromberg 1972).

Class 2 embryos were $1.05 \pm 0.15 \text{ mm}$ in length ($n=630$). The body regions, now well defined, were similar in size and the cephalon had two naupliar appendages forming the buds of antennae 1 and 2 (Fig 3A). The two dorsolateral embryonic organs, located on either side of the anterior pereon, were approximately $70 \mu\text{m}$ in length and consisted of an ectodermal rosette around a central pore (Fig 3A, B). Early Class 2 embryos lacked external evidence of segmentation while later Class 2 embryos showed initial differentiation of pereonites and pleonites as paired whorls along the dorsal midline (Fig 3A, C).

Class 3 embryos were on average similar in size to Class 2 embryos ($1.12 \pm 0.19 \text{ mm}$; $n=605$), but showed considerable variation in length among embryos (from 0.69 to 2.21 mm) with most growth occurring in the pereon (Fig 4A, B). After the chorion was removed, buds of the postnaupliar appendages were visible including the remaining mouth parts, pereopods 1-6, pleopods 1-5, and uropods. All appendages, held closely to the body, were positioned posteriorly and toward the ventral midline (Fig 4B). The antennae were segmented (antenna 1 had 4 segments, antenna 2 had 6 segments) but lacked setae or spines (Fig 4C). The buds of the mouthparts also lacked setae but the basic

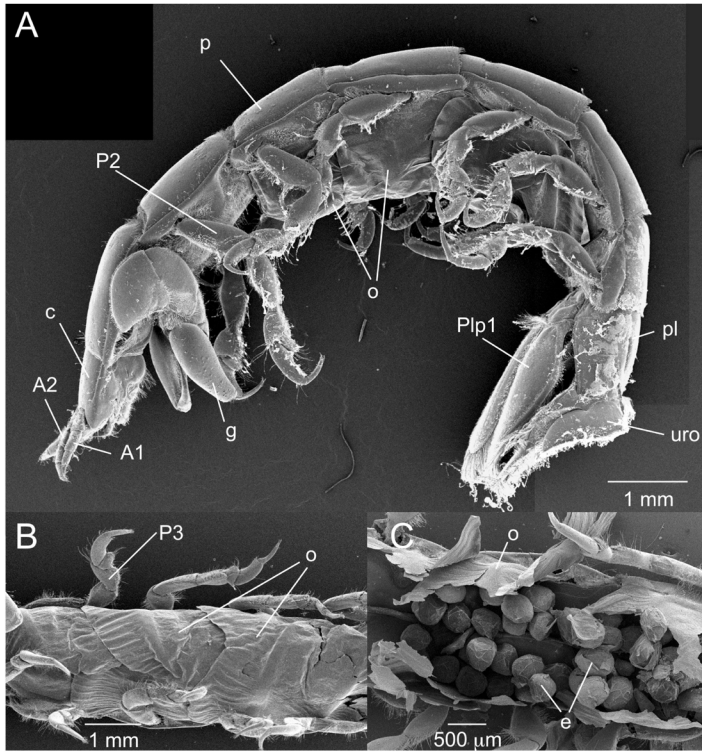


Fig 1 Scanning electron micrographs of female *Cyathura polita*. **A.** Composite image of a female with intact marsupium on ventral surface. **B.** Ventral view of marsupium, composed of overlapping oostegites. **C.** Marsupium with oostegites removed to show the brood of Class 1 embryos. A1=first antenna, A2= second antenna, c=cephalon, e=embryos, g=gnathopod, p=pereonite, P2=pereopod 2 (1st pair of walking legs), P3=pereopod 3, pl=pleon, Plp1=pleopod 1, o=oostegites, uro=uropod.

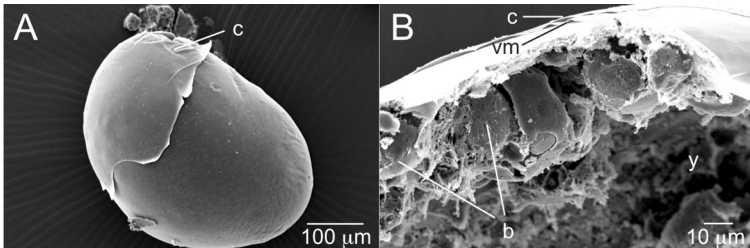


Fig 2 Scanning electron micrographs of Class 1 embryos of *Cyathura polita*. **A.** Ventral view of embryo with extra embryonic membranes partially removed. **B.** Fractured embryo showing the two extra embryonic membranes and the cellular blastoderm. Note that most of the central yolk mass is removed. b=blastoderm, c=chorion, vm=vitelline membrane, y=yolk.

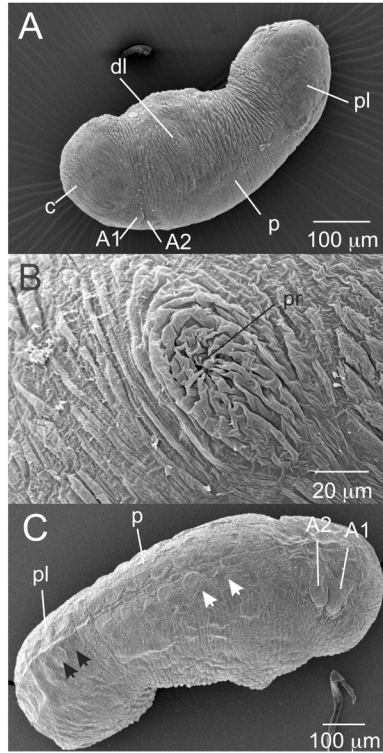


Fig 3 Scanning electron micrographs of Class 2 embryos of *Cyathura polita*. A. Lateral view of an early Class 2 embryo showing body regions but lacking defined segments. B. Dorsolateral organ. C. Dorsal view of a more advanced Class 2 embryo, showing early segmentation in the thorax (white arrows) and abdomen (black arrows). A1=antenna 1, A2=antenna 2, c=cephalon; dl=dorsolateral organ, p=pereon, pl=pleon, pr=pore.

segmentation pattern of their adult morphology was complete: the mandibles consisted of a stout peduncle, a palp with 2 articles and an incisor; maxilla 1 with single rudiments of both outer and inner lamella; maxilla 2 was uniaarticulate and maxilliped with 4 articles (Fig 4C).

The pereon of Class 3 embryos was composed of seven elongate segments. The six anterior pereonites were equal in size, and each had a pair of uniramus pereopods that were also similar in size (6 segments). All were oriented with the dactyl pointing posterior. Pereonite 7 was small and lacked legs (Fig 4D, E). The buds of all pleopods and uropods were biramus and approximately the same size (Fig 4E). The embryonic dorsal organ was visible medially on pereonite 1 as a

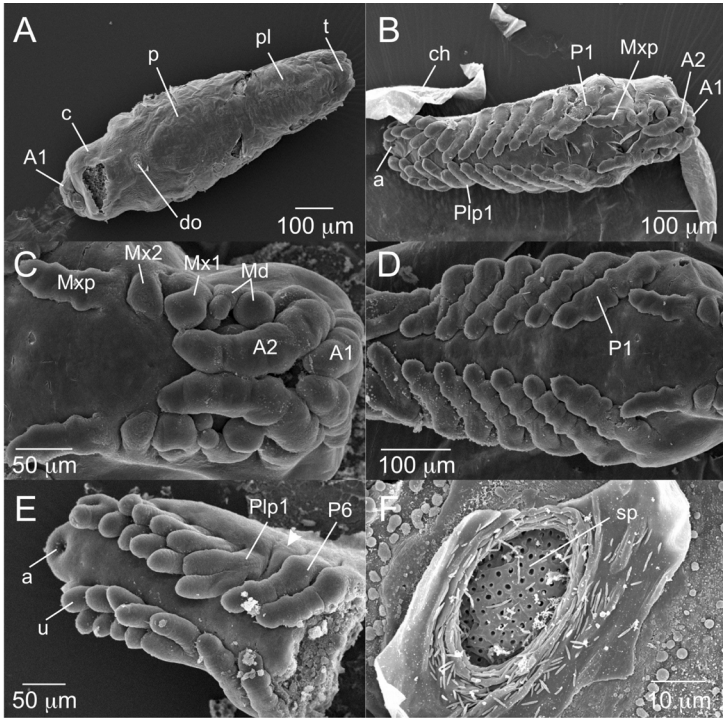


Fig 4 Scanning electron micrographs of Class 3 embryos of *Cyathura polita*. **A.** Dorsal view of embryo with the chorion intact. **B.** Ventral view of embryo partially removed from the extraembryonic membranes. **C-D.** Ventral view of the head and thorax, respectively. Note that small setae are visible at the tips of the pereopods. **E.** Ventral view of posterior thorax and abdomen. Note that pereonite 7 (indicated by the arrow) lacks legs at this stage of development. Exopods and endopods are present on the pleopods. **F.** Dorsal organ. a=anal pore, A1=antenna 1, A2= antenna 2, c=cephalon, ch=chorion, do=dorsal organ, Md=mandibles, Mx1=maxilla a, Mx2=maxilla 2, Mxp=maxilliped, p=pereon, P1= pereopod 1, P6= pereopod 6, pl=pleon, Plp1=pleopod 1, sp=sieve plate, t=telson.

central sieve-like plate, surrounded by the chorion (Fig 4A, F). The dorsolateral organs were no longer visible at this stage.

Manca

Manca were hatched from the extraembryonic membranes but were still sheltered within the female marsupium. Two size classes were observed. Class 4 young (early manca) had body proportions that were similar to the adults but still had rounded, well separated segments and a smooth exoskeleton with small tufts of setae (Fig 5A, B). Average

length was 1.8mm. The appendages had obtained the adult positions: the first antennae extended anteriorly; the mandible and maxilliped curved anteriorly over the mouth; pereopods 1-3 had rotated so that the dactyls were oriented anteriorly while pereopods 4-6 retained their original configuration; and pleopod 1 extended posteriorly to begin to enclose the other pleopods in the gill chamber (Fig 5B-G). The head appendages had the number of segments typical of adults: antenna 1 had 5 segments; antenna 2 had 6 segments and a broad peduncle; mandibles had a palp with 3 segments and apical setae; maxilla 1 and 2 were both smooth; and maxillipeds had 3 segments (Fig 5E). The gnathopods were much larger than the other pereopods. The pleonites

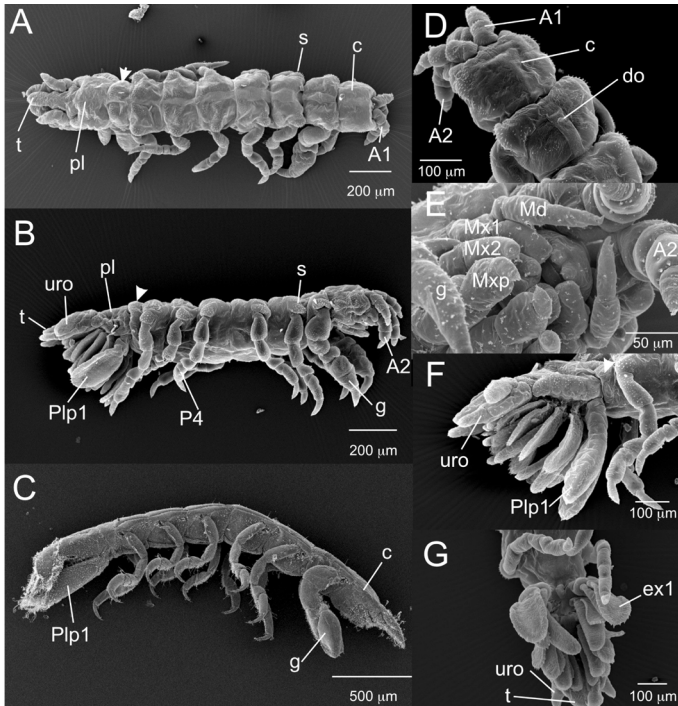


Fig 5 Scanning electron micrographs of Class 4 (early) and Class 5 (late) manca of *Cyathura polita* that were removed from the marsupium. A, B. Early manca, dorsal and lateral view. C. Late-stage manca. D, E. Dorsal and ventral aspects of the cephalon of an early manca. F, G. Lateral and ventral aspects of the pleon of an early manca. Arrow indicates pereonite 7 that lacks appendages at this stage. A1=antenna 1, A2=antenna 2, c=cephalon, do=dorsal organ, g=gnathopod, ex1=exopod of pleopod 1, Md=mandible, Mx1=maxilla 1, Mx2=maxilla 2, Mxp=maxilliped, P4=pereopod 4, pl=pleon, Plp1=pleopod 1, t=telson, uro=uropod.

were short and fused dorsally, but small lateral divisions were visible between pleonites 1-5 with a fairly deep groove between pleonites 5-6 (Fig 5A,F). The pleopods had an exopod that was shorter, broader and with more setae than the corresponding endopod (Fig 5F,G). The uropods had migrated to their adult position and appeared to be fused with the telson (Fig 5F). The dorsal embryonic organ was visible on the dorsal midline of pereonite 1 as a small rosette of ectoderm; the central sieve plate, found in earlier stages, was not observed (Fig 5D).

Class 5 mancas were much larger (approximately 2.5 mm in length) and exhibited considerable differentiation of the exoskeleton indicating that perhaps at least one molt occurred between classes 4 and 5 (Fig 5C). Class 5 manca had a pointed rostrum, anterolateral corners of the cephalon and smoothly articulated pereonites. The pereopods were considerably modified, with very large gnathopods with a large propodus, and extensive bands of setae on the posterior side of the anterior pereopods, and the anterior side of the posterior pereopods. Pereopod 7 was absent. The lateral divisions between the pleonites were no longer visible. The exopods of pleopod 1 were similar to those of adults in terms of relative size and development of the setae (Fig 5B,C).

Free-living juveniles

Class 6 or free-living juveniles, collected from the sediments, had the same overall morphology as adults but generally had fewer setae and lacked sexually dimorphic traits. Newly released juveniles (age 0) were first observed on July 31, 2004 and had a mean length of 2.70 ± 0.24 mm (Mercer et al. 2007) close to the length of Class 5 mancas and suggesting that Class 5 manca were released as juveniles without a molt occurring (i.e., no pereopod 7).

The juveniles collected for our SEM study were taken on July 24, 2004, had a mean length of 10.82 ± 2.50 mm, were age 1 juveniles, and pereopod 7 at this stage was similar in size and orientation to the other posterior walking legs (Fig 6A). The antennae were covered in setae, and the peduncle of antenna 2 had the broad, sculpted morphology typical of adults. The juvenile mouthparts consisted of: mandibles with 3 palp articles, broad peduncle and flat, sculpted incisor, and the maxillipeds with 3 elongate, bristly articles that curved flat over the mouth and covered maxilla 1 and 2 (Fig 6B, C). The gnathopod of the juvenile was much larger in comparison to the walking legs and had both propodal and carpal teeth (Fig 6A).

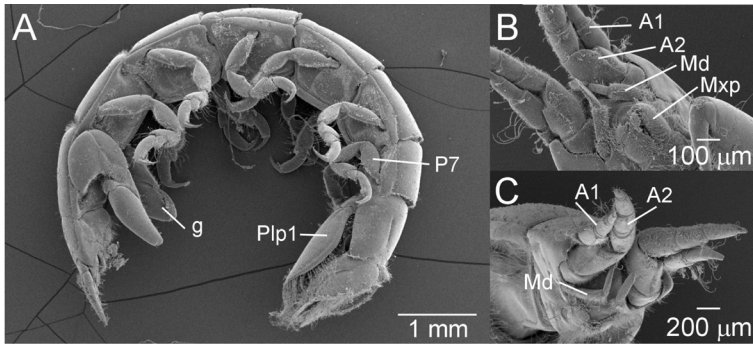


Fig 6 Scanning electron micrographs of free-living juveniles of *Cyathura polita*, collected from sediments. **A.** Lateral view of juvenile. **B, C.** Mouthparts of juvenile and adult, respectively. A1=antenna 1, A2=antenna 2, Plp1=exopodite of pleopod 1, g=gnathopod, Md=mandible, P7=pereopod 7.

Reproductive Activity and Intra-Brood Synchrony in Development

Gravid females were found from July 1- August 17 and females brooding early embryos (Class 1) were collected throughout that period (Fig. 7). All offspring per brood were at the same stage of development for most broods ($n=60$ females; Fig 7). The marsupia of a few females ($n=14$) contained young of different but sequential developmental classes (i.e., Class 2 and 3 embryos). Females with young at sequential stages of development were found slightly later in the summer (July 17- August 17) and were collected from all 4 study sites. A few females ($n=4$) contained broods with young at non-sequential stages of development. In all 4 broods, the majority of young per marsupium were at an advanced stage and a few (<10%) were at an earlier stage. In 3 broods, most young were manca while a few were Class 1 (2 broods) or Class 2 (1 brood), while in the fourth brood, most young were Class 3 and a few were Class 1. All 4 females were collected later in the reproductive season (July 31).

DISCUSSION

The marine peracaridan crustaceans are a large and diverse group, yet our understanding of their development rests on a few, well-studied species and incomplete descriptions of some additional taxa (Anderson 1973, Johnson et al. 2001). Our objective was to build upon a well

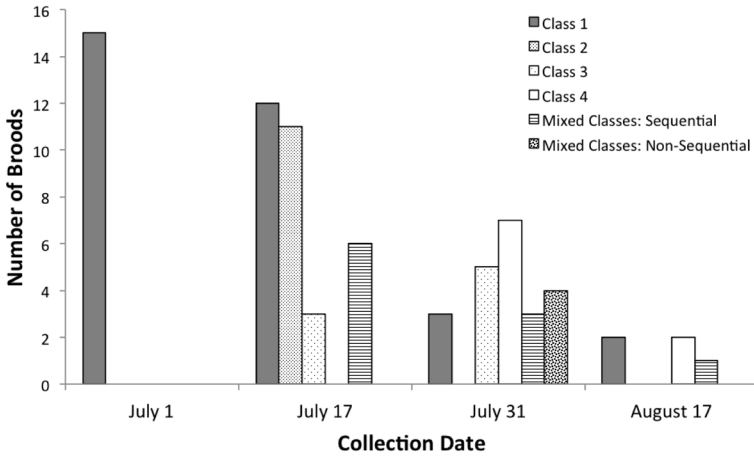


Fig 7 Stage of development of embryos brooded by female *Cyathura polita* collected throughout the study period (n=73 females).

established literature on *Cyathura polita* by investigating external morphogenesis, thus focusing on gaps in the existing knowledge that addresses their early embryology (Strömberg 1972), physiology (Kelley and Burbanck 1976) and life history (Frankenberg and Burbanck 1963, Burbanck and Burbanck 1974, Kruczynski and Subrahmanyam 1978, Burbanck et al. 1979, Mercer et al. 2007). Our study of morphogenesis throughout most of the life cycle is, to our knowledge, the first such description for the Isopoda using SEM. SEM revealed differences from earlier studies in timing of segmentation, new structural information on the embryonic dorsal organ, and suggests that several key modifications of adult morphology are acquired during the molt to the first manca stage.

Overall, our results support earlier descriptions of isopod embryology based largely on sectioned material (Strömberg 1965, 1967, 1971, 1972). Differences in timing of segmentation and appendage formation were noted. Strömberg (1965) describes the development of the naupliar appendages in isopods as arising almost simultaneously from the naupliar ecto-mesodermal headbands, with the mandibles arising slightly before the buds of the first and second antennae. We found the buds of the antennae appeared first, but the mandibular buds, once present, grew faster and soon outstripped the antennae in size. We also found external evidence that segmentation of the pereon and pleon began earlier in development than previously reported, as segments were observed at the same time as the naupliar appendages

were forming while Strömberg (1972) observed segmentation did not occur until the metanaupliar region was forming. In the present study, the segments developed before the limb buds were externally visible and not sequentially with them, as in previous reports (Strömberg 1972). These differences likely reflect differences in method of observation. For example, the small antennae buds are much easier to observe with SEM than in section, and differentiation of the surface of the ectoderm (SEM) may provide earlier indication of segmentation than may be observed by internal cell differentiation (section).

Our study also revealed new information on the structure of the embryonic dorsal organ. With few exceptions (epicarids, Charmantier and Charmantier-Daures 2001), peracaridan embryos typically have three embryonic dorsal organs: a pair of dorsolateral organs that extend from the yolk mass to the space between the chorion and the vitelline membrane; and, a single dorsal organ that develops later and extends through both extraembryonic membranes to the marsupial fluid (Strömberg 1971, Johnson et al. 2001). These dorsal organs are lost at hatching and are not equivalent to the dorsal organ typical of adults (Martin and Laverack 1992). Earlier reports of the dorsal organ (Strömberg 1965, 1967, 1972) illustrate an apical surface covered with microvilli (observed in section), while our observations (SEM) reveal a distinct sieve plate covering the organ and surrounded by a rosette of extraembryonic membrane. The sieve plate would likely appear as apical folds in section. The actual function of the dorsal organ, as yet undetermined, has been suggested to include yolk absorption, cell degradation, secretion of the larval cuticle, molting, and osmoregulation (Strömberg 1965, Johnson et al. 2001, Charmantier and Charmantier-Daures 2001). We also noted minor differences in timing including: the onset of the dorsolateral organ occurred earlier (here, before segmentation of the thorax) and the loss of the dorsal organ occurred much later (here, well after hatching), than was found in the analysis of the embryos observed by Strömberg (1972).

The morphological diversity of the telson has made it an important character in phylogenetic analysis (Brusca and Wilson 1991). The Anthuridae are characterised by uropods that extend dorsally over the pleotelson (a unique synapomorphy), while the presence of a true pleotelson (i.e., pleonite 6 fused to the telson) has been much discussed for this clade (Brusca and Wilson 1991). We found that the uropods originate as do pleopods 1-5 in size and early orientation and that differential growth to produce the adult arrangement occurs

in the molt to the first manca stage (Class 4 young). In early manca (Class 4), the division between pleonite 6 (with uropods) and pleonite 5 is distinct, while the articulation between pleonite 6 and the telson was not externally visible. This supports Brusca and Wilson's (1991) conclusion that pleonite 6 and the telson are fused in the Anthuridae, and also, that fusion may occur in the molt to the first manca stage. The first manca is also the stage of development where the walking legs acquire their adult orientation (except for leg 7), and differential growth of the gnathopods is most evident.

Lack of intra-brood synchrony in development was found in approximately 20% of the marsupia observed, although cyathurans were generally thought to have synchronous development (Strömberg 1972). Most of these females carried embryos of sequential developmental classes suggesting young were in the process of hatching or molting at the time of collection; similar observations are reported for *C. carinata* (Bamber 1985). We also found a few gravid females (4 of 78 in total) that were brooding young of non sequential developmental stages. All of these females were collected late in the reproductive season and the majority of young in these broods were manca. This suggests that perhaps a few females underwent a second, smaller reproductive event shortly before the offspring from the previous event had been released, or, that late season broods contained a few embryos that were less viable or at least slower to develop than their siblings. Both conditions may be a consequence of the short reproductive season associated with the northern location of the Saint John population. Cyathurans are known to spawn several times per reproductive season (Marques et al. 1994) in populations where the reproductive season is long (e.g., 5 months for *C. polita* populations in Florida; Kruczynski and Subrahmanyam 1978). *Cyathura polita* in the Saint John population have a short reproductive season (2 months) but early broods can be found throughout that time suggesting repeated spawning may occur (Mercer et al. 2007). The presence of several developmental stages within a single marsupium was also observed in a parasitic entoniscid isopod by Müller (1862; in Strömberg 1971).

Marine isopods are typically thought to undergo 3 molts inside the marsupium (Johnson et al. 2001). These molts include: the first, or hatching molt, that involves loss of the extraembryonic membranes by naupliar intermolts with three pairs of naupliar appendages and poorly developed thoracic appendages; the second molt in which the embryonic cuticle is shed by early manca with well developed pereopods

and pleopods (excepting pereopods 7); and the larval molt into a more advanced manca, occurring just prior to release from the marsupium (Johnson et al. 2001). We did not observe a typical hatching molt in *C. polita*. Instead, the loss of extraembryonic membranes (chorion and fertilization envelope) and embryonic cuticle appeared to occur at the same time and young hatched from the extraembryonic membranes after they had well developed abdominal and thoracic appendages (i.e., the first manca stage). The larval molt, from first to second manca stage, appeared to be more typical and was characterised by changes in size, shape and differentiation of the exoskeleton.

These, plus other, observations suggest that *C. polita* undergo 5 phases of development during their life cycle. The first 2 phases occur within the marsupium and include the development of embryos (organization of the body regions and appendages) and manca (young with a juvenile-like morphology but lacking the 7th pair of pereopods). The length of the brood period has been estimated as 30-45d (Mercer et al. 2007). Manca are released from the marsupium and as free living juveniles undergo at least one additional molt to become subreproductive adults; i.e., those with the 7th pair of pereopods. *Cyathura polita* are protogynic hermaphrodites (Burbanck and Burbanck 1974, Mercer et al. 2007) and as they mature, they undergo at least two additional molts: the first producing mature females with oostegites, and the second producing males with the appendix masculina. Mercer et al. (2007) estimated the life cycle takes 3 years in the Saint John River population.

This study of external morphogenesis supports earlier work on isopod embryology based on sectioned material and additionally describes the development of manca and free-living juveniles. Comparative analysis is difficult as so few developmental studies exist, but this preliminary work suggests that direct development in isopods includes shifts in the timing of the development of naupliar appendages relative to segmentation; the non-sequential development of appendages (i.e., the late appearance of the 7th pair of pereopods) suggesting temporary suppression of teloblast activity part way along the anterior-posterior axis; and the acquisition by most pericaridans of an additional juvenile stage (i.e., manca) in a life cycle that has lost a free-living larval stage. Our understanding of isopod development remains in its infancy as we lack sufficient comparative studies, undertaken within a phylogenetic framework, to both understand isopod diversity and also to more fully appreciate the origins of direct development in this group.

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