Reproduction and larval development of *Polydora robi* (Polychaeta: Spionidae), an obligate commensal of hermit crabs from the Philippines

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Abstract. The reproduction of a recently described spionid polychaete, *Polydora robi*, is examined from the Philippines. Adults inhabit a burrow in the apex of gastropod shells occupied by hermit crabs. Females were found to deposit broods of 18–94 egg capsules in the summer (June–August) and winter months (January–March) sampled. Paired or single egg capsules are attached by stalks to the inside wall of the burrow and contain 40–106 eggs which average 97 μm in diameter. The total number of eggs per brood ranges from 941–8761 eggs and is positively correlated with the total number of segments and length of female worms. Adults of *P. robi* are polytelic, producing ≤9 successive broods over a 3-month period; a mean of 6.7 d was exhibited between broods in the laboratory. Females utilize sperm stored in the seminal receptacles during successive spawnings. Development occurs within egg capsules until the 3-segment stage, at which time the planktotrophic larvae are released. Juveniles of ~20 segments are competent to settle on gastropod shells inhabited by hermit crabs. Members of *P. robi* are relatively fecund, semicontinuous breeders; the life-cycle in this species is similar to the only other known obligate polydorid commensal of hermit crabs.

Additional key words: Annelida, fecundity, life-cycle, symbiosis

Polychaete worms exhibit a complex variety of life histories and reproductive modes. The reproductive biology of these worms has been reviewed with particular reference to endocrinology (Clark 1965), physiology (Bentley & Pacey 1992), and reproductive mode (Schroeder & Hermans 1975; Wilson 1991; Gian grande 1997). Recently, the reproduction and larval development of the Spionidae and related polychaete families have been reviewed (Blake & Arnofsky 1999). The life history, fecundity, and population ecology of soft-bottom spionids have been studied in significant detail (Levin 1984; Levin & Huggett 1990; Zajac 1991; Levin & Bridges 1994; Bridges & Heppel 1996). While larval development of shell-burrowing polydorids is well documented, studies of their fecundity are lacking due in part to the difficulty in extracting specimens from their burrows. Among the ~70 described species of *Dipolydora* and *Polydora*, the fecundity of individuals in 12 species has been investigated (Wilson 1928; Campbell 1955; Dorsett 1961a; Blake 1969; Radashevsky 1986, 1988, 1989, 1994; Sato-Okoshi et al. 1990; Lewis 1998; MacKay & Gibson 1999; Williams & Radashevsky 1999). Of these species, only one has been investigated from the tropics; the rest are restricted to temperate regions. The purpose of the present investigation is to provide data on the reproduction and larval development of *Polydora robi*, a recently described burrowing polydorid from the Indo-West Pacific (Williams 2000).

Members of *P. robi* are obligate commensals of hermit crabs, found only in the apex of gastropod shells occupied by host crabs (Williams 2000). These burrows extend from a hole in the apex to an opening on the columella along the upper body whorls. Adults are known to ingest the embryos attached to the pleopods of host hermit crabs and the worms are found in 2.5–35% of gastropod shells among sites in the Philippines. The morphology, ecology, and feeding behavior in *P. robi* have been documented (Williams 2000). This present report provides data on fecundity and larval development to the juvenile stage in *P. robi* adults isolated in glass capillary tubes in the laboratory and from field collected specimens. Life history in the species is discussed with reference to its association with hermit crabs.

Methods

Hermit crabs inhabiting gastropod shells were collected by hand, shallow subtidally (<5 m) in coral reef
areas from six provinces of the Philippines (Bataan, Batangas, Oriental Mindoro, Aklan, Palawan, and Cebu) from June to August 1997 and January to April 1999. Hermit crabs were either fixed *en masse* in the field (relaxation in 3% MgCl₂ followed by fixation in 10% formalin-seawater solution) or transported to the laboratory and isolated in divided plastic boxes until examination. Isolated hermit crabs were maintained in aerated unfiltered seawater. Hermit crabs were removed by cracking the gastropod shells in a mortar and pestle constructed of 60-mm galvanized-steel pipe.

After exposing the burrows of *P. robi* individuals by cracking the shells, the worms were forced from their tubes by pipetting a stream of seawater through the opening in the apex. The worms were then placed in glass capillary tubes (10–25 mm long, open at both ends; inside diameter between 0.9–1.1 mm). Total length, palp length, width at segment 7, and total number of segments of the worms were recorded after relaxation in 3% MgCl₂. The worms were maintained at room temperature (~27°C) and under ambient light conditions in 20 ml of artificial seawater (Tropic Marin®, salinity ~32‰) in plastic petri dishes for the duration of the observations. The seawater was completely refreshed every 24 h and worms were fed Tetra® fish food (crushed morsels or baby fish food) or hermit crab embryos, of the species *Calcinus gaimardi* (H. Milne Edwards 1848), in excess approximately every 48 h.

After isolation, the worms were monitored for the production of egg capsule strings between 4 February 1999 and 9 April 1999. For removal of egg capsule strings, the capillary tubes containing worms and capsules were immersed in 3% MgCl₂ for 1 min followed by a stream of seawater applied via pipette through the tube ending containing the posterior end of the worm. The number of capsules per string were recorded and the number of eggs per capsule were counted in a subset (n=3–18) of capsules. Total eggs per brood were determined by multiplying the mean number of eggs per capsule by the number of capsules per string. Worms were returned to the capillary tubes and successive broods were enumerated as described above; egg capsules typically were removed from the capillary tubes ≤12 h of deposition. Data on the brood size, number of ovigerous segments, and total number of segments were also recorded from specimens preserved in the field prior to examination. Gametogenic segments were counted using a compound microscope; developing eggs appeared yellow in the body coelom; sperm appeared white. Regression analysis was used to examine the relationship between measures of fecundity (mean number of capsules per string and total eggs per brood) and female size (total segments and length). All means are reported with standard deviations.

Larval development was documented through the release of 3-segment larvae from egg capsules. The timing of egg production, larval development, and larval release was followed by examination of the worms at ~12 h intervals. Sketches of egg capsules, larvae, and recently metamorphosed juveniles relaxed in 3% MgCl₂ in seawater were completed using a compound microscope with drawing tube attachment. These sketches were scanned into a Macintosh® computer and images were prepared using the programs Adobe Photoshop® and Adobe Illustrator®. Three-segment larvae relaxed in 3% MgCl₂ in seawater, fixed in 10% formalin-seawater solution and stored in 70% ethyl alcohol, were prepared for scanning electron microscopy (SEM). The specimens were dehydrated in an ascending ethyl-alcohol series followed by 4 changes of 100% ethanol. Dehydration was achieved with Peldri II (Ted Pella, Inc.) by placing the specimens into a 1:1 mixture of 100% ethyl alcohol and Peldri II for 1 h at 34°C. The specimens were transferred to 100% Peldri II for 3 h and then placed in a cool water bath and allowed to sublime overnight. Dried specimens were mounted on a copper stub, coated with gold-palladium mixture, and viewed in a JEOL 1200EX SEM.

**Results**

**Field collected specimens**

Individuals of *P. robi* ranged in size from 3.0–41.0 mm in length (mean=16.4 ± 9.5, n=40) for 24–171 segments (mean=75 ± 25, n=111). Developing gametes were found in a total of 70 individuals, of these 33 had ova in the coelom, 22 had sperm, and 15 individuals had both stored sperm and eggs visible. Ova were present from segments 17–38 to 28–127 with a mean number of 24.2 ± 17.5 (n=48) segments containing ova (Fig. 1A). Sperm were present in segments 15–26 to 21–110 with a mean number of 38.1 ± 20.9 (n=37) containing sperm (Fig. 1B). The total number of gametogenic segments was positively correlated with the total number of segments of the worms (Fig. 1A, B). Egg capsules were found in all months examined (June–August 1997 and January–March 1999); 8–80 capsules (mean=27.1 ± 19.2; n=14) were found joined in strings on the inside of shells from the field.

**Reproduction in the laboratory**

Isolated specimens of *P. robi* produced 1–9 broods during the observational period (Table 1). The number of egg capsules per string did not vary significantly from their mean over the spawnings, examined in 12
of the worms producing multiple broods ($\chi^2=0.11–7.08$, $p=0.35–0.83$, $df=1–8$). Two worms exhibited a significant difference ($\chi^2=13.78$, $p=0.03$, $df=6$; $\chi^2=8.84$, $p=0.03$, $df=3$); the initial broods of these worms were 27 and 14 egg capsules less than the mean; additional spawnings of these worms did not differ significantly. The mean number of capsules per string ranged from 13.8–93.6 (Table 1) and was positively correlated with both the number of segments and body length of the worms (Fig. 2A, B). The mean number of eggs per capsule ranged from 40–106 (Table 1) and was not significantly correlated with either the total segments or body length of the worms. The total number of eggs produced per brood ranged from 941–8761 and was positively correlated with both the total segments and body lengths of the worms (Fig. 2C, D). Worms containing sperm in seminal receptacles prior to isolation appeared to be depleted of sperm at the end of the experiment, however all broods contained fertilized eggs. In 7 cases the worms were found to have ingested all or part of the egg capsule strings after deposition; these instances may have represented premature egg capsule dislodgment from the capillary tube followed by female ingestion.

**Larval development**

Females deposited egg capsules in paired or single rows, attached to the burrow wall by 2 stalks (Fig. 3A). Females usually deposited egg capsules during the night (91%, $n=58$), although on 5 occasions the females deposited the egg capsules during the day. Females were able to deposit a complete egg capsule string within 1 h. No unfertilized or nurse eggs were found and all eggs developed into larvae, which were released at the 3-segment stage. These larvae were released in 4.6–7.5 d (mean=$5.8 \pm 0.6$, $n=19$) and time between spawnings ranged from 5.5–8.6 d (mean=$6.7 \pm 0.7$, $n=19$).

Eggs early in development were circular and had a mean diameter of 97.0 ± 6.0 µm ($n=50$), with a white to light yellow/orange color (Fig. 3A). The prototrochophore had a rounded anterior end, a small ciliated mouth vestibule, paired ventro-lateral ciliary patches, and the center was composed of a large yolk area (Fig. 3B). At $-3$ d the prototrochophores measured 128 ± 7.0 µm ($n=15$). Later in development, the larvae possessed the cilia of the telotroch and prototroch, two kidney-shaped eyespots anterior to the prototroch, and yolk center composed of large, irregularly shaped macroceres (Fig. 3C). In 4 d the early 3-segment larvae were 208 ± 18 ($n=8$) in length (Fig. 4A). The larvae had 2 kidney-shaped eyespots and 3 segments with developing larval spines; only the first set of spines protruded through the cuticle. Small ventro-lateral ciliary patches remained and the yolky macroceres were reduced in size and were approximately circular in shape (Fig. 4A).

In 5 d, the mid- to late-3-segment larvae were 279 ± 18 µm ($n=9$) and were competent to swim (Fig. 4B). Two sets of eyespots, a round median pair and a
kidney-shaped configuration of 2 pairs of lateral eye- spots were observed; 2 tactile cilia were present on the head (Fig. 4B). The prototroch extended from approximately the mouth vestibule to the lateral eye-spots. The telotroch contained a dorsal gap; nototrochs were present on segments 3 and the developing segment 4. Segment 3 projected laterally and each side contained a short curved seta (Fig. 4C), ~2 μm in length. The yolk had been partially depleted and the gut was beginning to form at this stage; no larval pigmentation was observed.

Fourteen juveniles of *P. robi* were observed in the apex of hermit crab shells; additional juveniles were found together with large females in the apex. Those shells inhabited by juveniles only did not contain a hole in the apex as observed for adult worms. Juveniles were composed of 24–27 segments and measured ~1660 μm in length and ~230 μm in width at segment 7. The palps were short, extending back to segments 5–6. Distinct eyespots were no longer present, but pigmentation was present on the prostomium between the palps; in some individuals, irregular patches were found on the dorsal side of the middle segments (Fig. 5A). The caruncle was short, the triangular ocipital tentacle found in adults had not yet developed. Nototrochs were found on segments 2–3, 7, and posterior segments.

Notogetae in the posterior 1/3rd of the body were found in small bundles of fine needle-like spines protruding through the cuticle. The bundles of notosetae contained ~7–10 spines in posterior segments, with two longer anterior notosetae. Segment 5 was almost twice as large as segments 4 and 6 and had a slightly curved row of 3–4 major spines. The major spines were falcate with a lateral obliquely curved flange. A tear-shaped gland on each side of the fifth segment was present (Fig. 5A, C). The gland was ventral to the major spines and emptied via a duct leading to each side of segment 5. The glands were composed of ~20–25 tear-shaped lobes (Fig. 5C, D) which contained a granular secretion. Later in development, the gland was reduced in size and the lobes of the glands were no longer visible (Fig. 5A). Three bidentate hooded hooks began on segments 7 or 8. Up to 6 hooded hooks were found in middle body segments and were not accompanied by capillaries. Branchiae began on segment 7 and extended to the middle body segments. Notopodia of posterior segments sometimes contained knob-like structures and non-motile cilia (Fig. 5B). The pygidium was cusp-shaped with irregular, rounded knobs surrounding the anus; knobs possessed non-motile cilia (Fig. 5B). Black pigmentation was present on the pygidium of some specimens. Glandular pouches were present in segments 7–9.

**Discussion**

Members of *P. robi* are polytelic, capable of producing as many as 9 broods during a 2-month period.
This Indo-Pacific worm produces large broods of eggs; the largest specimens can produce >8000 eggs in a single brood. Prior reports on the fecundity of polydorids, all confined to temperate waters, had shown that these species produce 200–5800 eggs per brood (see Sato-Okoshi et al. 1990). Dipolydora armata (LANGERHANS 1880), from the tropical waters of the West Indies, produces small broods (50–100 eggs) composed largely of nurse eggs (Lewis 1998) and shows no evidence of reproductive seasonality. As indicated by Blake & Arnofsky (1999), most spionids exhibit seasonality in reproduction, producing eggs during periods of highest water temperature. Seasonal variation in water temperature of the Philippines is limited (26–31°C; Cordero 1981; Yap & Gomez 1981) and broods of P. robi were found deposited in field samples during all months examined. The species is a rapid breeder producing successive broods in <7 d.

Egg capsule production has been documented in P. cornuta Bosc 1802 (Rice & Reish 1976). The eggs of P. cornuta exit the body via paired nephridiopores, and enter two thin mucous tubules attached to the burrow wall. As the eggs enter the tubules, the mucus expands and coalesces, creating a single capsule. These capsules become joined in a string, with additional egg capsules deposited along the length of the female dorsum. P. robi individuals often produce paired capsules, apparently because the mucous tubules from each nephridiopore do not coalesce. It is not known if this is due to environmental, behavioral, or morphological factors, but the paired capsules are produced in the field as well as in the laboratory.

Individuals of P. robi exhibit a short period of development in egg capsules (~6 d) until 3-segment larvae are released. The larvae develop in the plankton until settlement; this length of time is unknown but
Fig. 3. Larval development in *P. robi*. A. Partial egg capsule string, showing paired egg capsules and attachment stalks connected to a mucous string (arrow). B. Protrochophore larva in ventral view. C. Trochophore larvae in dorsal view. Scale: A = 300 μm; B = 25 μm; C = 50 μm.
Fig. 4. Larval development in *P. robi*. **A.** Early 3-segment larva in dorsal view. **B.** Late 3-segment larva in dorsal view. **C.** Curved seta of third segment (redrawn from SEM micrograph). Scale: A, B = 50 μm; C = 2 μm.

Larvae of other polydorids often remain in the plankton for ~1–2 months (e.g., Woodwick 1960; Dorsett 1961a; Hatfield 1965; Day & Blake 1979; Sato-Okoshi 1994). Larvae of *P. robi* metamorphose at ~20 segments, settling on gastropod shells inhabited by hermit crabs and completing their life cycle. The observation that some shells lacking a hole in the apex contained single juveniles, indicates that the juveniles bore from the inside of the shell. Juveniles may settle on the outer or inner lip of the aperture and crawl to the apex of shells occupied by hermit crabs. After movement to the apex the worms begin to produce a mucous burrow along the columella and create a hole in the apex. Fig. 6 shows a diagram of the life-cycle in *P. robi*. The life span of most polydorid species is ~1 yr, although in *P. brevipalpa* ZACHS 1933, a burrowing associate of scallops from Japan, individuals have a life span of 2.5 years (Sato-Okoshi et al. 1990).

Similarities are exhibited in reproduction, ecology, and behavior between members of *P. robi* and *D. commensalis* (ANDREWS 1891), the only other obligate commensal polydorid of hermit crabs. Individuals of *D. commensalis* produce large broods (~2400 eggs) and larvae of this species are released at the 3-segment stage (Hatfield 1965; Blake 1969; Radashevsky 1989). As documented in *P. robi* during the present study, Hatfield (1965) found males and females of *D. commensalis* closely associated in burrows; two specimens...
Fig. 5. Juvenile of *P. robi*. **A.** Anterior end of juvenile in dorsal view. **B.** Posterior end of juvenile in dorsal view. **C.** Fifth-segment spines and gland of juvenile; arrow indicates duct of gland. **D.** Tear-shaped lobe of fifth-segment gland. Scale: A, B=100 μm; C=25 μm; D not to scale.
she examined possessed eggs in the coelom and sperm in the seminal receptacles. Based on these observations, she concluded that copulation takes place in *D. commensalis* but did not suggest a mechanism for sperm transfer (Hatfield 1965). Rice (1978) indicated that sperm stored in the seminal receptacles of females may not have resulted from direct copulation. Instead, spermatophores produced by males could be picked up and broken by the palps of females; such sperm would subsequently be transported and stored in the seminal receptacles. He suggested that sperm transfer and fertilization in this manner would allow for continuous breeding and alleviate the need for synchronized spawning (Rice 1978). The present findings support this hypothesis.

Specimens isolated for reproductive studies at first exhibited stored sperm which became depleted during successive spawnings. The stored sperm were used to fertilize as many as 9 broods, and all eggs from each brood followed normal patterns of development. Sperm storage has also been reported in *P. herma- phroditica* Hannerz 1956 and more recently in *P. cornuta*, where specimens isolated in the laboratory were capable of storing sperm for at least a month (MacKay & Gibson 1999). However, females of *P. ciliata* (Johnston 1838), isolated in glass capillary tubes, were not observed to produce egg capsules unless paired with a male (Dorsett 1961a). Spermatophores were not produced by isolated worms or observed in any field collected specimens of *P. robi*, although they are documented in other members of the genus (Rice 1978). Sex determination appears to be similar to that in *D. commensalis*, in which the first worm to settle on hermit crab shells mature as females and subsequent worms develop as males (Radashevsky 1989). Transitory Hermaphroditism can occur when the female dies and the largest male becomes a female (Radash- evsky, pers. comm.).

The larval morphology in *P. robi* is similar to that of other members of the genus *Polydora* (see Blake & Arnofsky 1999). However, the 3-segment larvae possess a distinct curved seta on the third segment, which has not been previously documented. The seta may serve a similar function as the grasping cilia found in some polydorids (Wilson 1928; Blake 1969). The fifth-segment glands found in juveniles of *P. robi* have been described in other polydorids (Hannerz 1956; Hatfield 1965; Radashevsky 1994), but their function remains
poorly known. Hannerz (1956) revealed the duct of the glands terminate ventral to the major spines in *P. ciliata*. In all species noted, the glands become reduced or absent shortly after juvenile settlement. Hannerz (1956) considered the glands to be homologous to the glandular pouches found in segments 8–10 of some adult polydorids (e.g., Claparède 1870; Fauvel 1927; Dorsett 1961b). If these glands are homologous, then they may produce acidic mucopolysaccharides as found in *P. ciliata* (Dorsett 1961b). The distinct termination of the gland duct by the major spines (Fig. 5C) would allow the mucopolysaccharides to be utilized, in combination with mechanical abrasion, to burrow in calcareous substrata (Hannerz 1956). In spite of past research conducted on the burrowing behavior of polydorids, there is still considerable debate over the exact mechanism for substrate penetration (see Sato-Okoshi 1999). A detailed histochemical study of this gland as it develops and is subsequently lost may provide insight into its function.

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