Development and movement of the opisthobranch, *Hydatina physis*, in the Solomon Islands

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Abstract

This study investigated several aspects of the life history of the opisthobranch, *Hydatina physis*, including the circadian foraging cycle, monthly breeding activity, development, settlement preferences and growth, using laboratory trials and field observations. We have recently published results on the nocturnal circadian rhythm mediated by photic intensity and modulated by food availability (Hamel and Mercier 2006). The present study further showed that the average absolute distance covered daily was around 471 cm. All individuals exhibited apparently random movements, changing direction after each surfacing, although a degree of homing behaviour was apparent. The courtship, copulation, egg-laying and hatching of *H. physis* was primarily influenced by the lunar cycle (Hamel and Mercier 2006). Each capsule contained between 0 and 14 eggs and/or embryos. In almost all capsule masses, abnormal development and high mortality rates were observed in the first third of the capsules released. In part of the mass that developed normally, veligers developed around 3 days after capsule-laying. They emerged from the capsules and began to feed on phytoplankton about 2 days later, settled after 7.5–9 days, and reached a size of around 4 mm after 5.5 months.

Introduction

The green-lined paperbubble, *Hydatina physis* Linnaeus, 1758, is an opisthobranch gastropod that is found circum-globally in shallow tropical waters of the Atlantic and Indo-Pacific Oceans (Rudman 1972; Kilburn and Rippey 1982; Wirtz 1999; Abbott and Dance 2000). Even though *H. physis* is widely distributed and is attractive to aquarists and shell collectors (Kilburn and Rippey 1982), data on its biology remain scarce and mostly anecdotal. According to Beeman (1977), opisthobranch populations tend to be sporadically explosive, which can partially account for the rarity of published data on their reproductive habits.

We have recently found that adult *H. physis* express a well-defined activity cycle (Hamel and Mercier 2006). The majority of individuals remain burrowed in sand for ca. 12 h each day, surfacing at dusk to forage during the night with only short periods of re-burrowing. Because individuals surface at sunset and burrow at sunrise, a photically entrained circadian rhythm is the most probable underlying factor. Apart from their nocturnal burrowing habits, adult *H. physis* display a well-marked mobility pattern with a maximum distance recorded between 20:00 and 22:00, followed by a progressive decrease in the distance travelled until the next morning (Hamel and Mercier 2006).

Our earlier study also revealed that the reproduction of *H. physis* follows a lunar periodicity and that larval settlement preferably occurs on substrata that are rich in food items sought by the adults (Hamel and Mercier 2006). For four consecutive months, hermaphroditic reciprocal copulation, preceded by pre-copulatory courtship behaviour, occurred at night, 5–7 days (d) before the full moon. Spawning occurred 3–5 d later for up to five consecutive nights, the egg mass gradually decreasing in size with each spawning. An overcast sky or rain prevented or delayed both copulation and egg release. Settlement of veligers was largely influenced by the nature of the substrate. In multiple-choice experiments, settlement occurred predominantly on sand containing cirratulid polychaetes. Juveniles reached around 3.9 mm in shell length after 5.5 months (mo) of growth (Hamel and Mercier 2006).

The present paper provides complementary data that further elucidates the movement patterns of *H. physis* and provides details on the larval development until metamorphosis into juvenile.

Methods

Numerous *H. physis* specimens (Fig. 1) were commonly found in the intertidal zone off Aruligo, Solomon Islands (9°25.59′ S and 159°56.58′ E). For laboratory trials, individuals were collected on the

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Figure 1. The green-lined paper bubble, *Hydatina physis*, in its natural environment at night. Shell length of this specimen is approximately 4 cm.

sand at low tide, or in tide pools, and immediately transferred to holding tanks. Whenever possible, all observations were concurrently carried out in the field and in the laboratory. For details on the collection, maintenance and experimental procedures, see Hamel and Mercier (2006).

Movements of adult *H. physis* were monitored by making a slight mark on the shell of 11 specimens prior to the trial to allow identification. Every 2 h, the position of each specimen was marked (using a small numbered plastic flag) and its general behaviour (i.e. moving, burrowing, surfacing) was noted. The absolute distance traveled by an individual over the 2-h interval was measured as the distance between two consecutive flags. All individuals were followed for three clear days, as well as during rainy or overcast days. Movement and orientation of *H. physis* were tested for randomness with the Rayleigh test. The directionality of movement by individuals was examined with a second-order analysis, which allowed inferences to be made about the populations of individuals examined. In this case, the cumulative frequency distribution of the length of the mean vector "r" (the Rayleigh statistic) of each individual's direction of movement was compared with the theoretical distribution of "r" for the same sample size by the Kolmogorov-Smirnov goodness of fit test (Batschelet 1981).

Whenever egg-laying was observed, groups of capsules were collected at regular intervals from at least three different masses. Samples were collected every 2–5 minutes (min) during the first 2 h, then every hour for the next 12 h, twice daily for 7 d and approximately once a day for the remainder of the trial. Some of the capsules were observed live and others were preserved in 4% formaldehyde/sea water for subsequent morphological examination and measurements. Capsules were routinely collected in two different sections of the mass to compare their development and mortality rates: close to the anchor (i.e. among the first released) and at the distal tip (i.e. among those released last). Between 20 and 25 embryos were sampled from capsules in both locations within each mass. Sizes were measured under a light microscope equipped with a graduated ocular. A new stage was considered attained when 50–60% of the embryos and/or larvae reached it. The egg masses under observation were not moved for the duration of development.

Results

Movements

Globally, all *H. physis* exhibited apparently random displacements (Rayleigh test of directionality, $p>0.05$), changing direction after each surfacing and every day. The only recurrent pattern was observed in the 22:00 records, which systematically corresponded to the outside most positions (Fig. 2) at the end of the peak locomotor activity period.

Embryonic and larval development

Embryonic development was not entirely synchronous between various parts of the capsule mass (Table 1). In the central section of the rosette, which harbours the capsules that were spawned first (i.e. close to the anchor), up to 85% of the embryos developed abnormally, compared with 5–10% in the capsules located farther from the anchor. The time course of normal development is shown in Table 1. Although the approximately 10,000–30,000 capsules

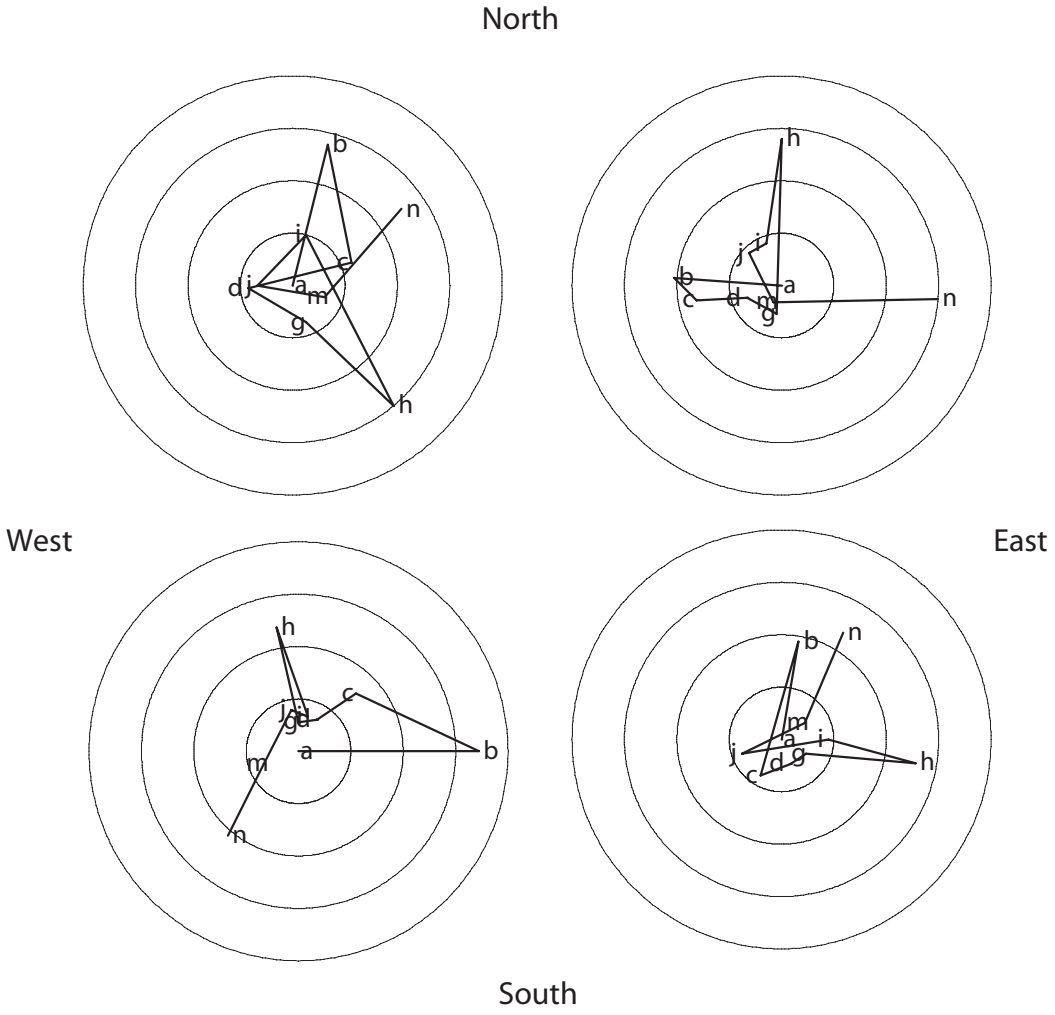

Figure 2. Absolute displacement of four *Hydatina physis* individuals under clear conditions (<30% cloud cover). Positions were recorded every 2 h for 3 d, but for the sake of clarity, data are shown at 4-h intervals for the first 52 h. The letter "a" corresponds to initial time (18:00 on day 1) and the letter "n" to the last value recorded (22:00 on day 2). Note that "b" and "h" are the two other 22:00 values.

near the anchor were spawned first, the development of their embryos was slower, and the asynchrony in development increased with time. Moreover, the first capsules contained embryos that did not develop into veligers and consequently never hatched. In normally developing capsules throughout the mass, most of the embryos developed synchronously (Table 1).

H. physis had telocithal eggs with holoblastic (or total) cleavage, the first two divisions being equal and the third unequal. The first cleavage was vertical through the animal and vegetal poles of the embryo, dividing it into two blastomeres of equal size. The fertilized oocytes measured about 50 µm in diameter. At the 2-cell stage, the embryos reached a diameter of 67 µm. The second cleavage was also vertical, perpendicular to the first and began about 30 min after fertilization, forming four symmetrically arranged blastomeres of equal size. Another cleavage took place 10 min later in the horizontal plane and at right angles to the two earlier cleavages, forming four micromeres and four macromeres. A short time thereafter, the embryos underwent an additional division of the macromeres, thus producing four more micromeres.

As development proceeded, the micromeres piled up and formed a pointed dome at the animal pole. About 9 h after fertilization, the numerous micromeres extended over almost the entire surface of the embryo, leaving only a small area of the macromeres uncovered. This was the blastula stage. After 34 h, the larva emerged from the fertilization envelop as a late gastrula (Table 1). After 42 h of development, the larva was slightly tapered

Table 1. Development of *Hydatina physis*. A new stage was considered attained when around 50–60% of the embryos/larvae reached it. Temperature varied with the daily cycle between 25°C (night) and 29°C (day). Data pooled from seven different spawning events are expressed as mean ± S.D.

Time	Typical development throughout the egg mass		
	Stage	Capsule diameter (µm)	Embryo/larva diameter (µm)
0	Fertilization	230 ± 30	49 ± 4
15 min	1-cell	230 ± 35	58 ± 5
30 min	2 cell	284 ± 45	67 ± 5
60 min	4-cell	250 ± 50	66 ± 6
70 min	8-cell	255 ± 55	66 ± 5
85 min	16-cell	250 ± 40	63 ± 5
95 min	Division stage	245 ± 50	83 ± 7
5 h	Division stage	245 ± 35	81 ± 4
6 h	Division stage	250 ± 40	84 ± 5
9 h	Early blastula	240 ± 35	75 ± 5
16 h	Late blastula	260 ± 50	76 ± 6
31 h	Gastrula	255 ± 45	78 ± 5
34 h	Hatching from fertilization envelope	250 ± 40	69 ± 4
42 h	Trochophore	275 ± 40	89 ± 4
50 h	Late trochophore (early shell development)	280 ± 35	93 ± 3
3 d	Early veliger	285 ± 50	100 ± 7
3.8 d	Veliger spinning in capsule	390 ± 65	110 ± 8
4.9 d	Veliger hatching from capsule	410 ± 32	111 ± 9
7 d	Veliger (extrusion of muscular foot, searching behaviour)	-	112 ± 8
7.5–9 d	Metamorphosis and settlement	-	109 ± 4
7.9–9.5 d	Juvenile	-	112 ± 3
2 wk	Juvenile	-	145 ± 7
1 mo	Juvenile	-	211 ± 9
2 mo	Juvenile	-	485 ± 42
3 mo	Juvenile	-	1543 ± 140
5.5 mo	Juvenile	-	3900 ± 310

at the anterior end, which displayed a small tuft of short cilia that enabled the larva to rotate and move backwards and forwards. This was the trochophore stage. About 24 h later two large, ciliated lateral lobes and a smaller median lobe with shorter cilia developed. The foot also developed posterior to the ciliated lobes. Beneath it, near the base, was a short, pointed operculum. Furthermore, the shell began to form around the posterior part of the body. About 3 d after fertilization, the larva had a well developed shell with an operculum and a bilobate ciliary tuft, typical of the veliger stage (Table 1).

Upon reaching the veliger stage, the larvae began to spin within the capsule at a rate of 1 revolution s^{-1}, thus disintegrating the residual underdeveloped embryos (representing <5% of all eggs/embryos in a capsule). The resulting small fragments and lipid droplets were ingested by the developing veligers, filling their digestive tracts. At the same time, the capsules grew from 285 to 390 µm in diameter. At the veliger stage, the ciliary rings of the velum oscillated without interruption. Four days after fertilization, the veliger displayed circular movement and the capsules reached 410 µm in diameter. The swimming-veliger hatched from the capsule after 4.9 d of development to enter the pelagic stage (Table 1). Immediately after hatching, the free-swimming veliger began to swim in the water column, near the surface. Approximately 2 d later, the veliger started to exhibit a searching behaviour by repetitively touching the bottom. At this time, its swimming capacity decreased considerably; the two well-developed ciliary lobes atrophied to form a tuft between the shell and the closing operculum. This searching period lasted around 1–2 d before settlement occurred after 7.5–9 d of development. Juveniles reached a size of 4 mm after 5.5 months.

Discussion

Movements

Adults foraged between 19:00 and 05:30, with an activity peak between 20:00 and 22:00, and remained inactive and burrowed in the sand during the day. This pattern was clearly influenced by cloud cover, rain and laboratory manipulated light:dark cycle (Hamel and Mercier 2006). Circular plotting of the data over three consecutive days hinted at the occurrence of a homing behaviour, the individuals showing a strong tendency to forage on the periphery of the tanks and to rest and/or bury closer to the central area where they were initially placed. As field and laboratory observations were carried out in the presence of large amounts of potential food items (i.e. cirratulids; Rudman 1972), the first intense foraging phase after sunset may have fulfilled most of the nutritional requirements of *H. physis*, thus lessening the need for further activity. However, intestinal contents were not examined to confirm this hypothesis.

Development

In opisthobranchs, development from egg-laying to hatching takes an average of 11 d (Hadfield and Switzer-Dunlap 1984). The short period in *H. physis* (5 d) may be partly attributable to the warm water in which the embryos and/or larvae develop. However, growth and mortality rates in *H. physis* differed considerably according to the location of the capsule in the rosette. Deformities and abnormal development were predominant in the first third of the spawned capsules, generally those located close to the anchor. This was observed in all the egg masses examined, and could be due to polyspermic fertilization. Such abnormality was not evident in the last two-thirds of capsules released. The spermatozoa:oocytes ratio may be controlled less efficiently at the beginning of the laying period than later in the process.

There may be nutritional benefits to encapsulation, particularly in marine gastropods, in which encapsulating structures enclose extra-embryonic yolk or nurse eggs in addition to developing embryos (Thorson 1950; Spight 1976; Rivest 1983; Pechenik 1986). Veligers of *H. physis* were observed to ingest fragments of dying embryos, which represented <5% of the total in nearly all capsules observed, although this does not seem to be a common trait in opisthobranchs. The presence of these dying or fragmented embryos did not seem to impede the growth and hatching of normal larvae but their possible role as food for surviving embryos remains to be clarified.

The release of offspring from encapsulating structures has been described for a number of molluscs (Vaughn 1953; Davis 1967; Gamulin 1973; West 1973; Pechenik 1975; Webber 1977), although Pechenik (1986) mentioned that the hatching process remains poorly understood. While chemical mediation of hatching has been documented in the marine gastropod *Ilyanassa obsoleta* (Sullivan and Bonar 1984; Sullivan and Maugel 1984), the rotation of the growing larvae, the enlargement of the capsules and their non-uniform rupture support a mechanical process in the case of *H. physis*.

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