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Freshwater snail *Pomacea bridgesii* (Gastropoda: Ampullariidae), life history traits and aquaculture potential

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Abstract. Investigations on the reproductive biology, life cycle and feeding habits of *Pomacea bridgesii* have been undertaken to assess its potential as a cultured species for the ornamental trade. The species is dioecious and, under optimal culture conditions of temperature and food supply, it can breed all year round. The total developmental period at 23±1ºC varied from 15 to 24 days after oviposition. Hatching can last for up to 20 hours in the same egg cluster. Hatching success was very high (mean 94.56±0.62%) and no significant differences were observed in hatching rates between different clutch sizes. Development is direct and juveniles hatch at shell length (SL) = 2.4±0.25 mm. Maturity is reached 192±1.5 days after hatching and at SL = 32.80±2.03 mm. Two feeding experiments were undertaken to assess the impact of food type on juvenile survival during the first 8 days post-hatching and subsequent growth until 90 days post-hatching. Compatibility between other fish and plants fresh-water aquarium species were performed. A combination of environmental tolerance, moderately amphibious behavior, fast growth, short development and hatching at an advanced stage, compatibility with other aquarium species (fishes or other invertebrates), and simple low cost diet, make *P. bridgesii* highly suitable for intensive culture for the ornamental trade.
Key Words: *Pomacea bridgesii*, snail, freshwater, aquaculture, ornamental.

Introduction. Aquarium keeping is a popular hobby with millions of enthusiasts worldwide. Although exact figures on the value and trade of the ornamental fish industry are not available, the value of ornamental fish and invertebrates imported into different countries worldwide is approximately 278 million US dollars (FAO 1996-2005). An aquarium enthusiast can easily become overwhelmed by the enormous variety of fish, invertebrates (including mollusks), plants and live rock available, and ultimately forget to consider their source and method of collection. Although most freshwater fish species available in the trade have been raised in culture, it is important to remember that the majority of invertebrate species are still wild-caught and the ecological impact of their capture remains largely unknown.

From the early days of aquarium culture, the concept of a “balanced aquarium” included living plants and snails, as well as fish. Historically, native and non-native species from areas all over the world have been introduced in aquaria to control pests such as aquatic plants and periphyton (Levy & Miller 1979; Genthner et al 1993; Cowie 2001). Freshwater snails belonging to the family Ampullariidae, commonly called Apple Snails are well known and popular aquarium snails throughout the world. Due to their aquarium-cleaning feeding habits and their attractive appearance, shape and size, Apple Snails have become a common aquarium product traded all over the world (Cowie et al 2006).

Most Apple snails are voracious herbivores that eat a wide range of vegetation (Perera & Walls 1996). Held in captivity, they do well on common vegetables in combination with fish food. Unfortunately, although many species have a great appetite for aquatic vegetation, algae are not their preferred food. In such cases the snails can reduce the aquatic vegetation very quickly, and can ruin a beautiful aquarium within days. *Marisa cornuarietis* (Linnaeus, 1758) and *Pomacea canaliculata* (Lamarck, 1822) are a good example of this type of non-selective vegetal feeding behavior, which has turned them into pests.

This does not seem to be the case with *Pomacea bridgesii* (Reeve, 1856). This species prefers decomposed animal food, or dead and rotting plants rather than fresh green ones (Aditya & Raut 2001). These snails are thus a good choice for an aquarium with a nice collection of water plants. Also, their bright yellow color and large size (maximum length 57 mm), along with their ability to eat encrusting algae and dead animal matter, makes them attractive as both ornamental and cleaning species in aquaria.

Given the appropriate conditions, *P. bridgesii* will thrive in home aquaria almost as well as its "pest relative" *P. canaliculata*. Its moderately amphibious behavior, rapid growth, compatibility with other species, short embryonic development and advanced hatchling form are among the characteristic that makes it very promising for intensive culture (Lum-Kong & Kenny 1989). One of the most important considerations for viability in intensive culture is the feeding cost.

Compared to the many studies on its relative, *P. canaliculata*, considered a pest due to its herbivorous feeding habits (Estebenet 1995; Carlsson & Brönmark 2006), little is known about growth and longevity of *P. bridgesii*. To the best of our knowledge, information on this species is limited to works by Scholnick et al (1994), Jordan & Deaton (1999) concerning some aspects of its physiology, by Mendoza et al (1999, 2002), Aditya & Raut (2001) and Jarusiewicz et al (2004, 2005) on biochemical and ecological aspects of this species' natural and artificial diets, and by Perera & Walls (1996) in a general account of ampullariids, and nothing has been published on life history traits of *P. bridgesii*.

The objective of the present work is to describe *P. bridgesii* early development patterns, growth and lifespan under laboratory conditions. Also research on aquaculture techniques, husbandry and diets, were performed to assess its suitability to ornamental aquaculture trade.

Material and method

Broodstock husbandry and embryos incubation. Average-size (3.5cm shell length) broodstock (8 individuals) were obtained from a local aquarium store. Animals were maintained in one laboratory glass tank (70L) containing gravel substrate, filled with oxygenated, filtered, fresh water and maintained under a natural photoperiod. During the study (April 2007 to September 2010), water quality was monitored daily for temperature and pH, and weekly for nitrites, nitrates and phosphates. Water temperature was maintained approximately constant at 25±1^{o}C. Approximately two times per week, 15-20% water changes were performed to maintain water quality.

Due to the requirement of this species to deposit eggs above the water level, the tank was filled only to within 15 cm of the top.

Specimens were provided with abundant food, namely green peas, Goldfish and Koi pellets (Laguna ®) and tropical fish flakes (Sera Vipan ® and Tetra Dr. Wu®) as an *ad libitum* maintenance diet. The food chosen was based on the natural diet of *Pomacea* species (Cazzaniga 1981; Lum-Kong 1989; Estebenet & Cazzaniga 1992), on previous laboratory feeding studies (Mendoza et al 1999; Aditya & Raut 2001; Ruiz-Ramirez et al 2005; Selck et al 2006) and on commonly available aquarium/pond fish food. For feeding experiments three different types of food and their combinations were used.

Mating, spawning and egg-laying behaviour were photographed and filmed. After they finished spawning, females were measured (Figure 1) for mean shell length (SL; measured from the top of the spire to the base of the aperture) with callipers. Five to six

hours after oviposition, the hardened egg masses deposited on the glass walls of the tank were carefully removed with a scalpel, photographed and measured.

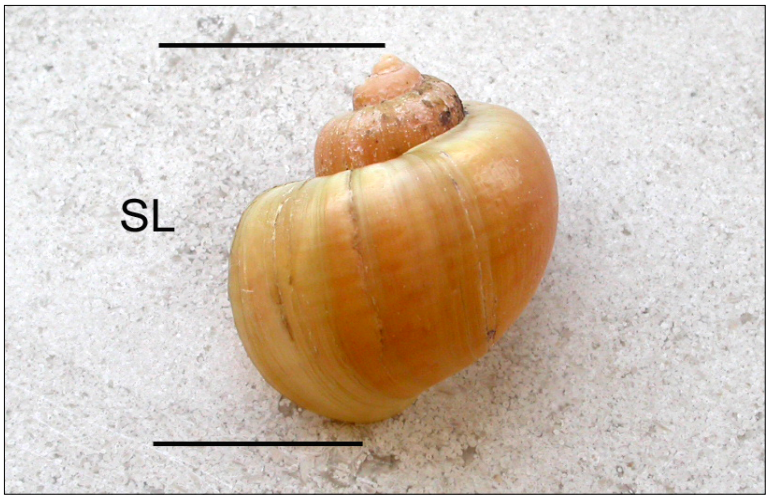

Figure 1. Measurement of *P. bridgesii* shells.

Afterwards, each egg mass was individually transferred to an incubation device (mesh on top of a floating fish-breeding container) which was placed inside the main tank, until hatching (Figure 2).

Figure 2. Front view of floating incubation device.

In order to calculate hatching rate, 15 egg masses were randomly selected, separated individually in floating incubation devices and grouped in three cluster-length class sizes: <4cm; 4.1-6cm; >6.1 cm. When hatching was finished, the numbers of hatchlings and of unhatched eggs were counted and the percentage hatched was calculated.

Transfer procedure. Due to demands of space and experimental design, juveniles and adults needed to be transferred from one tank to another several times during the culture procedure. Each time the following protocol was used.

A temporary container was prepared with 5 L of water from the original tank and a submersible heater used to ensure the smallest temperature variation between both tanks. Individuals were then carefully removed from their tank using a fish net and placed inside the temporary container. Every 15 minutes, 200 mL of water from the destination tank was introduced in the temporary container. This process was continued

for 3 hours, after which the animals were released in the destination aquarium, again using the fish net, and discarding the water from the temporary container.

Larval culture and grow-out. Immediately after hatching, ten early post-metamorphic individuals from each egg mass were photographed and measured with a calibrated micrometer under a binocular microscope. This procedure was repeated every two days until the individuals were one month old.

After this period, juveniles from each spawn were transferred to separate 20L tanks prepared with 50% conditioned water from the main aquarium and 50% new fresh water. The water quality in these aquariums was maintained with a Hang-On biofilter, and food leftovers were frequently cleaned from the bottom using a vacuum hose. No gravel was added to these aquaria in order to facilitate observation of juveniles and recording of deaths.

Three-month old juveniles were transferred to 40L tanks with gravel bottom. To estimate hatchling and juvenile growth rates, ten individuals from a group of juveniles from the same batch, hatched on the same day, were transferred to a plastic mini-box kept in the same tank, measured for SL every two days until one month old and then weekly until three months old. Initially, measurements were made with a calibrated micrometer under a binocular microscope until specimens reached approximately 10 mm SL, when they could be measured with callipers. Juveniles were fed *ad libitum* the same types of food offered to adults, but crushed in smaller particles. After 90 days, snails were observed and fed daily, but measured only monthly until first copulation was observed. The females were then measured (SL) and the life cycle was considered closed.

Feeding experiments. Two feeding experiments were conducted with *P. bridgesii* juveniles. The first experiment was designed to investigate the importance of diet type, during the first eight days of juvenile life, on survival rate. In the second experiment five different diets were used and the growth performance of the young snails assessed for 90 days.

Animals were placed in 5L tanks and held for 1 day prior to the experiments. During this period no food was provided to standardize the hunger levels.

During both experiments, the daily food ration was given in two equal portions (Mendoza et al 1999), in the morning and in the afternoon, and animals were allowed to feed *ad libitum*. A mixture of fish-food flakes and green peas was prepared by grinding the ingredients, and then divided in 15g cube portions, which were frozen. A daily ration consisted of one cube for each feeding session, giving a total of 30 g per day. A supply sufficient for two days (12 cubes, 15g each) was kept in a refrigerator, for easy handling. Every morning before the first feeding session, each tank was siphoned out to remove faeces and food leftovers and mortality was recorded. Water quality and characteristics were monitored for all the experiments as described in the general procedures, above.

In the first experiment, batches of 40 recently hatched juveniles (SL = 2.4±0.25 mm) were used per tank in each of the following treatments: (1) control: hatchlings were not fed and the glass walls were kept clean of algae, diet 1: no food cubes were provided but an abundant algal film was available on tank walls; diet 2: snails were fed with diet mixture and the tank walls were kept clean of algae; diet 3: algal film was available on the tank walls and snails were fed with diet mixture. At least three replicates were conducted for each treatment.

In the second experiment, batches of 20 uniformly sized, 8 days post-hatching juveniles, were selected per tank in each of the following treatments: (1) control: no food was provided but an abundant algal film was available on tank walls; diet 1: animals were fed fish-food flakes (5g per day); diet 2: animals were fed green peas (25g per day); diet 3: Koi pellets (25g per day) were provided; diet 4: animals were fed fish-food flakes and green peas (2.5g and 12.5g respectively, per day); diet 5: animals were fed fish-food flakes and Koi pellets (2.5g and 12.5g respectively, per day). At least three replicates were conducted for each treatment.

Interaction experiments. After maturity was reached, animals were transferred to a 250L tank, designed to reproduce a standard domestic freshwater aquarium, in order to assess possible negative interactions between our target species and common fish and plants in the aquarium trade. Fifteen species of plants and twenty species of fish were observed for interactions with mature *P. bridgesii*.

Behavior of fish towards snails was categorized as Ignored (I; snails are ignored); Noticed (N; snails are watched closely but no damage occurs); Harmed (H; damage is done to snails and death may occur). For the behavior of snails towards plants only I and H categories were used.

Data Analysis. One-way analysis of variance (ANOVA) was used to analyze the influence of cluster length on hatching rates. Differences among treatments for mortality rates of juveniles until day 8 were tested by a Chi-square goodness of fit. All results were considered statistically significant when *P*-values were lower than 0.05 (Sokal & Rohlf 1995). Shell growth rates (length gain per day; SGR) were calculated according to the equation

$$SGR = (Lf - Li) \times t^{-1},$$

where *Lf* and *Li* are the final and initial length measurements, and *t* is the time, in days, between measurements (Chapman, 1971).

To analyze growth differences within treatments of juveniles raised to day 90, a repeated-measures ANOVA was used. All statistic analyses were performed using Statistica 6.0 (Statsoft Inc.).

Results

Reproduction and grow-out. After the first 20 days of keeping the adults, courtship and copulation behavior began to be observed. The male usually approaches the female from behind, crawls over her shell and when at the last whorl searches for the genital aperture, grasping with his penial sheath, and inserts it (Figure 3).

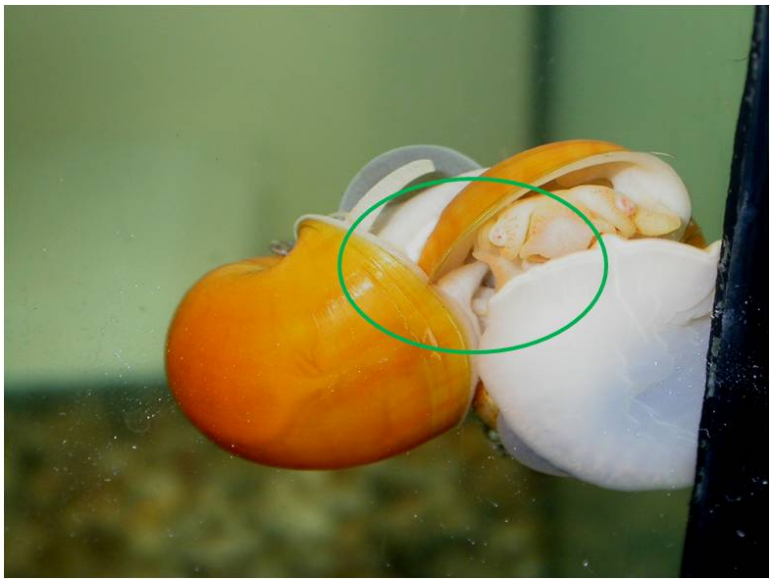

Figure 3. Copulation behavior. Male inserting penial sheath in female genital aperture.

Copulation can last for 1-5 hours and during this period the female usually crawls around feeding, while the male retracts inside his shell. The pair may be lifted out of water without interrupting copulation, showing that the male is firmly attached to the female during the entire copulation period.

Oviposition was difficult to observe since it usually occurs at night. It was observed only once. The female crawls from the water and deposits eggs on the tank wall. The eggs slide, one by one, from the mantle to the lower edge of the clutch. The

female descends the glass backwards while depositing the eggs and, when finished, simply lets herself fall down into the water. Freshly-deposited egg-masses are soft, milky pink and conspicuous (Figure 4).

Figure 4. Freshly-laid egg mass, showing milky appearance of soft eggs.

After 24 hours the eggs become white and harden (Figure 5). This is the appropriate time to scrap the cluster from the glass and transfer it to the incubation device. At the lower edge of the cluster eggs still show the soft, milky appearance.

Figure 5. Egg mass 24 hours after oviposition.

Data on oviposition and cluster morphology data are presented in Table 1. Hatching occurred 19.47±2.03 days after oviposition; the range was 15-24 days at 23.0ºC. Hatching can last up to 20 hours in the same cluster. Hatching success was very high,

ranging from 89.3 to 99.8% (mean 94.56±0.62%; N=15). No significant differences in successful hatching rate were found between clutches of different size classes ($P>0.05$).

Table 1

Oviposition and clusters data of 15 egg-masses laid in laboratory tanks in October-November 2006

Cluster Reference	*Cluster length (cm)*	*Cluster width (cm)*	*Distance from water surface (cm)*	*Incubation period at 23°C (days)*
1	6.2	1.3	5	18
2	6.8	1.5	7.5	18
3	7.6	1	5	15
4	4	1.5	8	18
5	6.4	1.2	8	21
6	7	1.2	5.5	24
7	4.3	1.2	7.5	22
8	4.5	1	7	19
9	5	1.5	7	20
10	3.5	1	5	19
11	5	1.5	7	18
12	3	1	8.5	20
13	2.3	1.5	7	20
14	3.5	1.2	15[a]	19
15	4.5	1	7.5	21
MED±SD	4.53±1.4	1.24±0.2	6.82±1.16	19.47±2.03

[a] Spawn deposited in aquarium glass cover.

Recently hatched juveniles of SL= 2.4±0.25 mm are similar to adults, with white body and yellow shell (Figure 6).

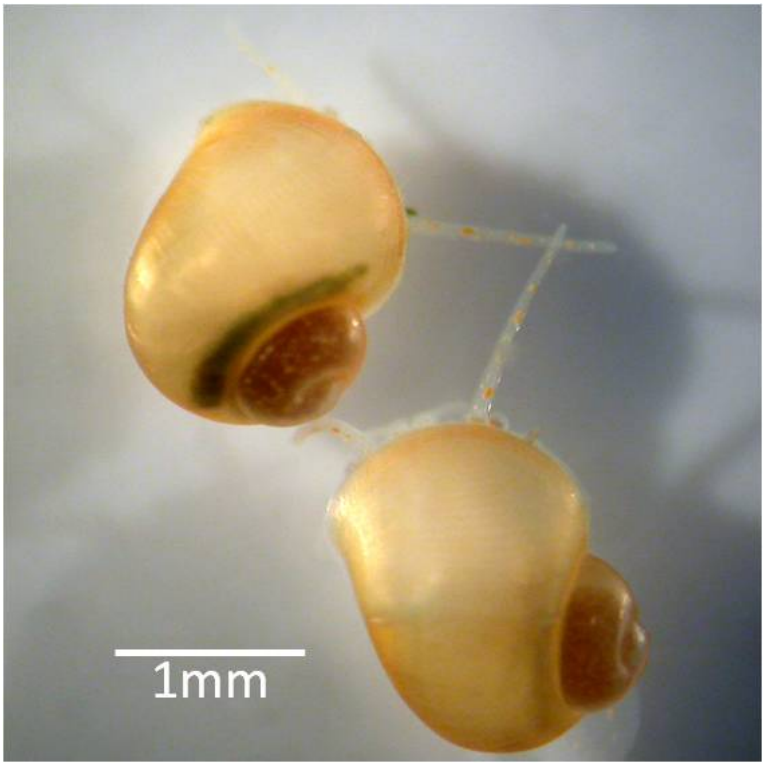

Figure 6. Recently-hatched juveniles.

The hatchlings fall or crawl into the water (Figure 7). Reproductive maturity was achieved 192±1.5 days after hatching in individuals of SL = 32.80±2.03 mm. These mature individuals showed the same reproductive behavior as the first breeding pairs. The growth curve is shown in Figure 8.

Figure 7. Juveniles hatching and crawling down to the water.

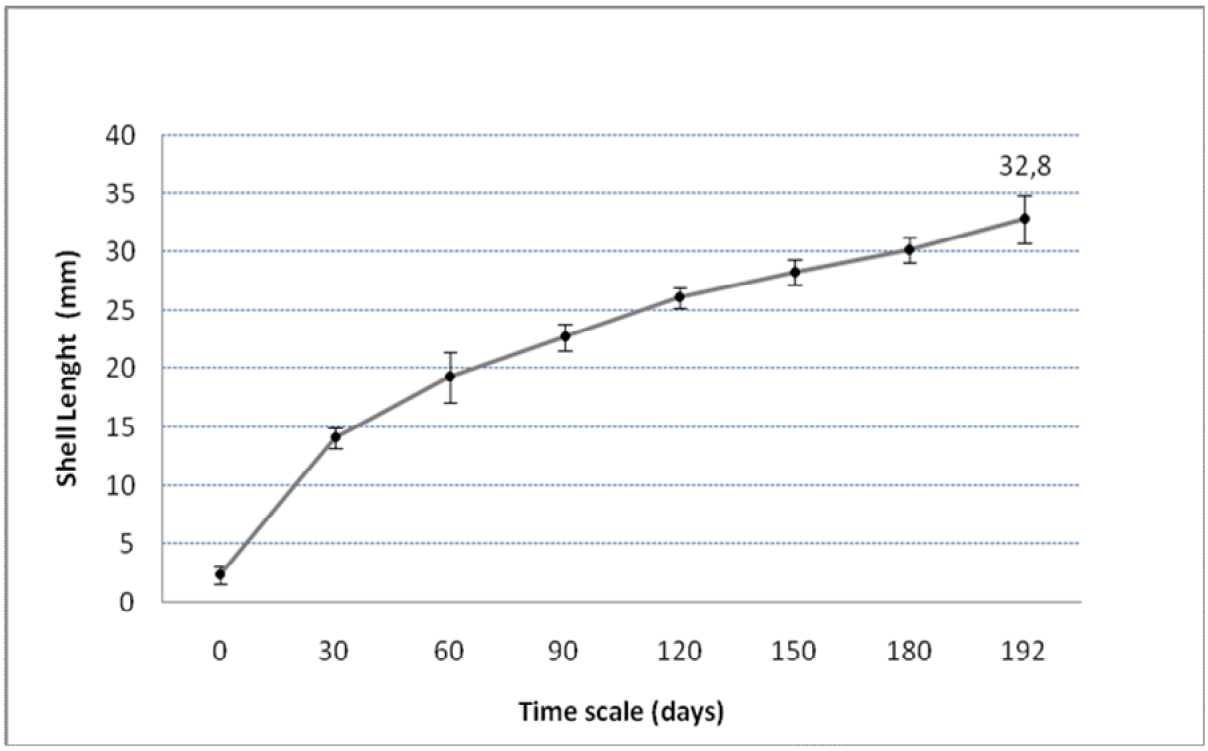

Figure 8. Growth curve of laboratory-reared *P. bridgesii*, from hatching to reproductive maturity.

Feeding experiments. From hatching day (day 0), juveniles seem to be able to ingest the same food as adults, but in smaller sizes. Despite this, hatchlings seem to depend more on their endogenous resources than on ingested food, as shown by the results of the first experiment. The survival during the first 8 days of juvenile life was 90.7% in the clean control tank, 90.8% if only algal film was available, 88.9% if algal film and crushed food were available and 64.4% if only crushed food was provided. The differences

between all four treatments were significant (Chi-Square = 13.34, df = 2 $P < 0.05$), with higher mortality values obtained for crushed-food diet, as seen in Figure 9. No significant differences in mortality were found between clean control tank and exclusively algae film treatment ($P>0.05$).

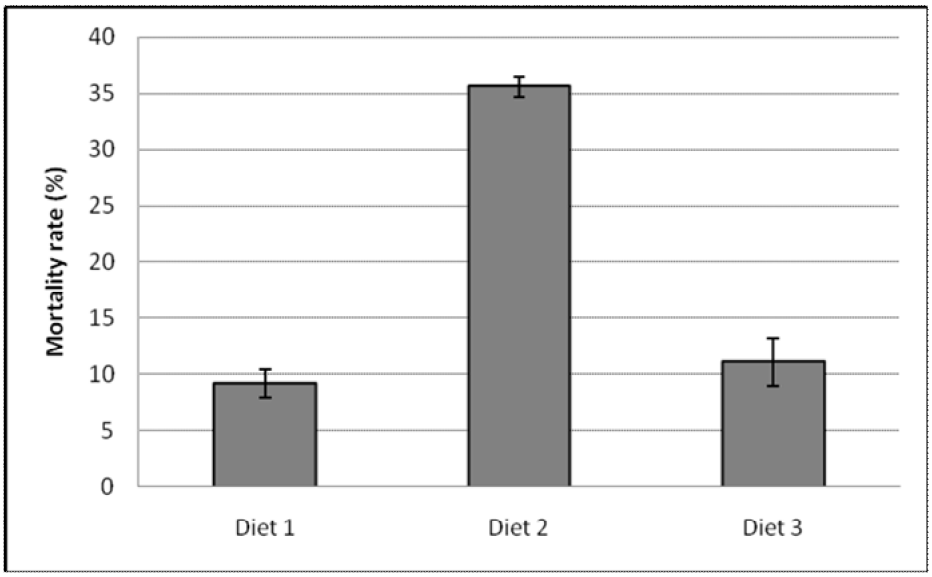

Figure 9. Mortality rate of *P. bridgesii* juveniles during the first 8 days of life, reared on 3 different diets.

A week after hatching, regardless of the diet regime used, juveniles in all treatments had doubled their shell length. Overall, juvenile SGR was influenced by diet (repeated measures ANOVA; F=11505.73; df=5.15; $P<0.01$). Snails fed exclusively on Koi pellets (diet 3) showed the lowest SGR, and those on diet 4 (fish food flakes and green peas) the highest SGR, although difference with diet 5 were not statistically significant ($P>0.05$). Data for all five diets tested are presented in Figure 10.

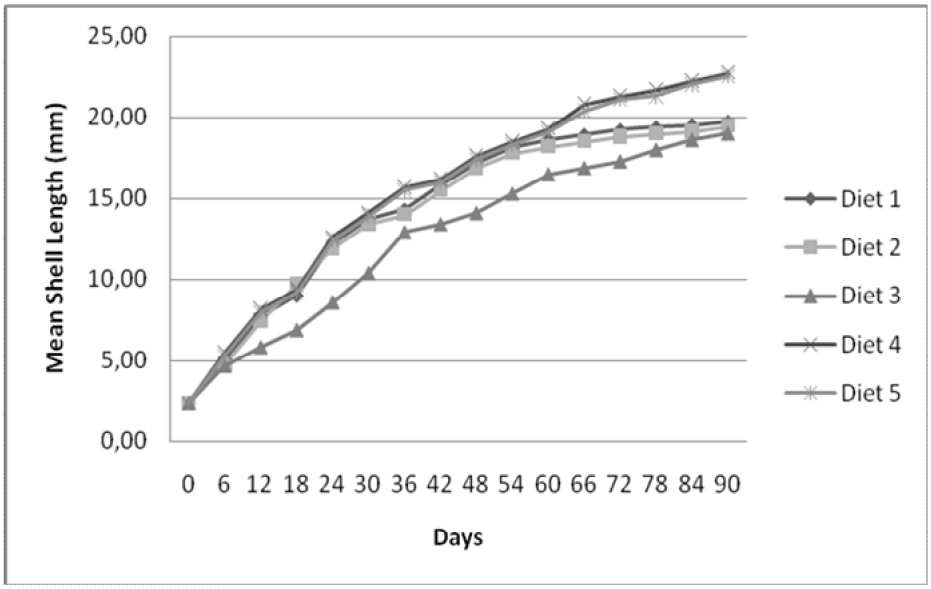

Figure 10. Growth curves of juvenile *P. bridgesii* reared on five different diets.

Juvenile growth rate calculated in mm per month showed that the highest SGR was achieved with diet 4, fish-food flakes and peas (5.17 mm/month) and that SGR was highest during the first month, gradually decreasing through the second and third months. This growth pattern was similar for all five diets tested.

When SGR was calculated in mm per day over 90 days, the combined diet of fish-food flakes and peas (diet 4) showed the highest value (5.17 mm/month and the diet of Koi pellets alone (diet 3) the lowest (4.3 mm/month).

Interaction experiment. The mature snails in a regular aquarium, with fish and plants, showed no interest in any of the 15 submersed aquatic plants as a food source and could even starve if no other food was provided. Of the 20 fish species tested only two harmed the snail, *Botia lohachata* Chaudhuri, 1912 and *Chromobotia macracanthus* Bleeker, 1852. The list of all species tested and recorded behaviours are presented in Table 2.

Table 2

List of fish and plant species tested for negative interactions with *P. bridgesii* in aquarium conditions

Fish species	*Behavior (I, N, H)*	*Plant species*	*Behavior (I, H)*
Ameca splendens Miller & Fitzsimons, 1971	N	*Anubias barteri*	I
Betta splendens Regan, 1910	I	*Aponogeton boivinianus*	I
Botia lohacata Chaudhuri, 1912	H	*Aponogeton crispus*	I
Botia macracanthus (Bleeker, 1852)	H	*Bacopa australis*	I
Brachydanio rerio (Hamilton, 1822)	I	*Bacopa caroliniana*	I
Corydoras aeneus (Gill, 1858)	I	*Cabomba caroliniana*	I
Corydoras panda Nijssen & Isbrücker, 1971	I	*Ceratophyllum demersum*	I
Crossocheilus oblongus Kuhl & Van Hasselt, 1823	I	*Cladophora aegagrophila*	I
Hyphessobrycon axelrodi (Travassos, 1959)	I	*Cryptocoryne wendtii*	I
Hyphessobrycon eques (Steindachner, 1882)	I	*Cyperus helferi*	I
Paracheirodon axelrodi (Schultz, 1956)	I	*Echinodorus bleheri*	I
Poecilia reticulata Peters, 1859	I	*Egeria densa*	I
Poecilia sphenops Valenciennes, 1846	I	*Heteranthera zosterifolia*	I
Pterophyllum scalare (Schultze, 1823)	N	*Hygrophila polysperma*	I
Puntius conchonius (Hamilton, 1822)	I	*Limnophila sessiliflora*	I
Puntius tetrazona (Bleeker, 1855)	I		
Puntius titteya Deraniyagala, 1929	I		
Trichogaster trichopterus (Pallas, 1770)	I		
Xiphophorus hellerii Heckel, 1848	I		
Xiphophorus maculatus (Günther, 1866)	I		

Discussion. Like the other members of the family Ampullariidae, *P. bridgesii* is gonochoristic and fertilization is internal. There is no visible sexual dimorphism, but in all copulations observed, males were smaller in size than females, as also reported for *P. canaliculata* (Estebenet & Cazzaniga 1998; Tanaka et al 1999; Estebenet & Martin 2002). Breeding in many *Pomacea* species is seasonal and related to latitude, temperature and rainfall (Andrews 1964). In equatorial regions, many species aestivate during the dry season as their habitat dries up, and breed in the rainy season; in subtropical regions, they may only breed during summer, once temperatures reach a certain level (Scott 1957; Andrews 1964). As shown here, if provided with suitable conditions in aquaria (constant 26ºC temperature and *ad libitum* food) copulation and spawning occur all year round. As in *P. canaliculata*, these activities are time-consuming (Estebenet & Martin 2002), particularly during mating, where the male is firmly attached to the female

throughout the copulation period of up to 5 hours. Like other species of the genus, *P. bridgesii* lays eggs above the waterline (6.82±1.16 cm), which imposes an additional cost on females, perhaps in order to avoid aquatic predators or in response to low oxygen tension in their often near-stagnant aquatic habitats (Snyder & Snyder 1971). The pink and conspicuous eggs are each enclosed in a calcium carbonate capsule, which might be used as a source of calcium for the developing embryo (Andrews 1964; Tompa 1980; Turner & McCabe 1990). The egg color changes as the capsule surface dries following oviposition, and subsequently as the embryo inside develops. The oviposition of a single egg clutch occurs predominantly at night or in the evening. This nocturnal oviposition behavior (Schnorbach 1995; Albrecht et al 1996) probably reduces risks of predation and desiccation (Estebenet & Martin 2002). Each egg clutch can contain between 50-200 eggs, depending mainly on female size. As Lacanilao (1990) pointed out for *P. canaliculata*, it is probable that clutch size of *P. bridgesii* is also affected by diet. However, our results demonstrate that hatching success is not dependent on clutch size, as also found by Teo (2004) for wild *P. canaliculata.*

Our results showed that laboratory growth of *P. bridgesii* is influenced by diet. During the first week of juvenile life, although able to ingest particulate food, survival seems to depend more on endogenous sources (Heras et al 1998; Heras et al 2007). The high mortality figures recorded when hatchlings were offered a food mixture were probably related to some toxic effect of the food, a bacterial problem or to oxygen depletion in the tank, and not necessarily related to ingestion habits. During the entire growth process until maturity is reached, dietary regime plays an important role, with the more complete and balanced diet (fish-food flakes and peas) promoting the fastest growing. This is consistent with reports for other invertebrate species by Alava & Lim (1983), that diets with containing a mixture of two or more proteins are better utilized by the animals. The poor results shown by juveniles fed on Koi pellets seem to be explained mainly by the feeding difficulties presented by this type of round floating food. Although *P. bridgesii* can feed effectively at the water surface, its technique seems to be more suited to capture of flake food or crushed particles (pers. obs.). *P. bridgesii* reared in this project reached maturity in 192 days at constant temperature, under natural photoperiod and with food *ad libitum,* at an average shell length of 32.8 mm.

The last part of the project consisted of testing the behavior and interactions of *P. bridgesii* in a standard ornamental aquarium. This was done in order to assess its potential as an ornamental species. The results were encouraging because, in contrast to *P. canaliculata*, *P. bridgesii* does not show any interest in the ornamental plants available as a food source (Andrews 1965; Neck 1986; Schnorbach 1995), which makes it suitable for planted aquariums. Concerning the fish species, *P. bridgesii* does not harm any of the species with which it shared the aquarium, but is attacked by *B. lohacata* and *B. macracanthus.* Both these species are anatomically adapted to remove snails from their shells and eat them. This hardly happens with *P. bridgesii,* due to the presence of the operculum, which allows it to completely withdraw inside the shell and close the aperture when attacked by the fish. Nevertheless, if under constant attack by the fish, the snails spend most of their time withdrawn, are unable to feed, and eventually perish.

Conclusions. At the end of the study we were able to conclude that *P. bridgesii* exhibits characteristics that make it suitable for intensive ornamental aquaculture. The young begin to feed as soon as they hatch and accept a wide range of food, if of suitable particle size. They hatch at an advanced stage of development and hatching success is high (89 to 100%). They require minimal care in culture since they exhibit similar behavior patterns to adults. Juveniles and adults use both aerial and aquatic respiration, allowing them to inhabit waters low in dissolved oxygen and to withstand some crowding, which can be important during shipping and wholesale stocking. Our feeding and diet experiments showed that it is possible to breed *P. bridgesii* on a low cost and widely available fish-food diet. All of these characteristics make *P. bridgesii* highly suitable for intensive culture for the freshwater aquarium trade.

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