

Effect of Diet and Stocking Densities on Life History Traits of *Clea helena* (Philippi 1847) Reared in Captivity

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Abstract

The freshwater gastropod *Clea helena* has recently been targeted by the freshwater ornamental industry due to its predation abilities on other snail species. This work describes for the first time some life history traits of this species, including reproduction, growth and development under laboratory conditions. Additionally, appropriate stocking densities were studied in order to assess the production viability of healthy snails in close-circuits systems. The species is dioecious and breeds under laboratory conditions but fertility rates presented were low. Hatching occur 52 ± 6 days after oviposition, at 25.0°C . Development is direct and juveniles hatch at shell length $\text{SL}=3.1 \pm 0.3\text{mm}$ ($n=20$) and resemble the adults in shell shape and color. Growth rates during a maximum period of 60 days were compared between snails fed on three different diets and results showed they were influenced by the different diets treatments ($P<0.05$). Growth performance experiments at three stocking densities (5, 10, 20 snails/L) with three replicates per treatment were also performed. During the first month, individuals stocked at 5 snails/L had significantly faster growth rates than those stocked at 20 snails/L. No significant growth responses were found during the second month among all densities and overall growth rates for this experiment also revealed no density-dependent growth responses.

Keywords: Gastropoda; Freshwater; Carnivore; Diet; Stocking density; Ornamental; aquaculture

Introduction

The family Buccinidae (Gastropoda: Neogastropoda), commonly known as true whelks, presents a worldwide distribution, with the larger species occurring at the subtidal zones of both northern and southern temperate and cold seas. *Clea helena* (Philippi, 1847) is one of very few whelks that lives its entire life in freshwater habitats and can be natively found in Tropical Indo-West Pacific regions like China, Thailand and Indonesia [1].

Most *Clea* species live in clean, fast-flowing streams where they can be found in sandy or muddy substrates. *C. helena* is exceptional in being found in ponds and ditches too, and its tolerance for a wider range of water conditions is probably why this one particular species does so well in captivity.

Adult individuals present a typical ovate-conical thick shell (Mean Shell Length= 24.3 ± 3.6 mm; $n=35$) with brown and yellow lateral bands. The shell presents five to six whorls spiral with lateral ridges and an operculum. The body is yellowish with grey speckles all over. The mantle forms a foldable tube, while the siphon is equipped with chemoreceptors, that like in other predators or scavenger species, *Clea* uses to perceive water and locate food. There is no apparent sexual dimorphism in the shell structure. Virtually nothing is known about this species' natural life traits. However this poorly studied freshwater snail has recently been targeted by the freshwater ornamental industry, due to its predation abilities on other snail species that frequently became aquarium pests. For this reason it is commonly called "Assassin Snail" in the trade.

Despite its growing popularity, studies concerning this species' biology and developmental growth patterns are absent. This basic information is essential in order to develop an optimal rearing protocol and improve production systems. From an aquaculture production point of view, the culture system is one of the most influential factors

for growth and survival of various commercial species, but such information does not exist for *C. helena*.

The objective of this work was to describe for the first time *C. helena* life history traits, including collecting breeding, growth and development data under laboratory conditions as well as to determine appropriate stocking densities in order to assess the production viability of healthy snails in close-circuits systems.

Material and Methods

This study was conducted over a period of 17 months.

Broodstock and experimental setup

The broodstock (Mean Shell Length= 25 ± 2.3 mm; $n=20$) was obtained from an online Portuguese pet store. The animals were maintained in one aquarium (100 L) containing a substratum composed of a 4 cm layer of sterilized beach sand, and equipped with a filter (Rena® Filstar XP2) and aeration system, maintained under a natural daylight with no artificial illumination. Tropical freshwater large leaf plants, PVC pipes and plastic net pieces were added to the tank to provide spawning surfaces for the snails. The snail density was 0.2 individuals L^{-1} .

During the study, water quality was monitored daily for temperature and pH, and weekly for nitrites, nitrates and phosphates. Water

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temperature was maintained approximately constant at $25 \pm 1^\circ\text{C}$ using an aquarium heater (RenaCal Basic 150W). Two times per week, 15-20% water changes were performed to improve water quality.

Specimens were fed with a combined maintenance diet of dry food supply like catfish pellets (Nutrafin® Sinking tablets) and live prey such as *Physella acuta* (Draparnaud 1805) and *Melanooides tuberculata* (Müller 1774), *ad libitum*.

Reproductive behavior

Clea helena is dioecious and sexual dimorphism is absent, therefore total broodstock acquired (20 individuals) were stocked together in order to assure that the group could be sexually heterogenic. Notes on reproductive behavior (presence/absence copulation; copulation position and duration) were taken twice a day (10:00 am and 4:00 pm). After spawning was finished, females were measured with callipers (Figure 1) for mean shell length (SL; measured from the top of the spire to the base of the aperture). Egg sacs' length was measured on the attached edge. Objects with recently laid eggs were carefully transferred to fish floating incubation devices inside the broodstock tank till hatching occurred.

Juveniles-diet and growth

This experiment was conducted in a recirculating 250 L glass aquarium system, with no substratum and filled with oxygenated, filtered, freshwater, maintained under a natural photoperiod. *C. helena* juveniles (Mean Shell Length= 9.5 ± 31.5 mm; $n=20$) selected from the stock population, were held individually in floating plastic fish-breeding boxes (Hagen® Marina multibreeder: length 20.32 cm, width 9.16 cm, height 10.79 cm), after removing the bottom compartment.

Ten replicates for each of 3 different diet treatments were placed in the tank. The experiment tank contained thus a total of 30 semi-submerged floating devices, each containing one juvenile snail. The snail density was 0.12 individuals L^{-1} .

The fact that all cage devices were housed in the same tank ensured homogeneous conditions for all experiments. Water conditions were kept similar to those described in Broodstock and breeding setup section.

Prior to any feeding experiment, juveniles were fed *ad libitum* for five days and then fast (no food provided) for two days to standardize the hunger levels. Growth rates during a maximum period of 60 days were compared between snails fed on three different diets: A-Catfish pellets, B-live prey, C-combined dry and live food (broodstock maintenance diet). Juvenile snails were fed to excess every two days. Treatments were randomly allocated within the system.

Growth performance was measured as an increase in shell length



Figure 1: Measurement of *C. helena* shell length.

(from apex to the base of the spire) using a calibrated micrometer under a stereomicroscope (Nikon SMZ800) or with callipers to the nearest 0.05 mm, according to individual size. Shell length, as opposed to shell weight, was monitored because it is thought to be a more important factor for reaching sexual maturity (Hanning, 1979). Individuals were measured in regular periods (15, 30, 45 and 60 days). Average weekly growth increment (G) was calculated using the following equation:

$$G = \frac{W_1 - W_0}{t_1 - t_0}$$

Where W_0 and W_1 =shell length at times t_0 and t_1 , respectively [2].

Eventual casualties were recorded and removed from each tank as soon as detected to prevent water fouling and cannibalism.

Effect of stocking density

Laboratory raised *C. helena* juveniles (standard lengths=8-11mm) were randomly selected and stocked in similar plastic floating cages used for growth performance experiments at one of three stocking densities (5, 10, 20 snails/L) with three replicates per treatment. Treatments were randomly allocated to the location within the tank. To perform this experiment, snails were fed combined maintenance diet of dry food and live prey described in Broodstock and rearing setup section.

Shell length was determined using the same methodology described in Growth performance experiment with individuals being measured in regular periods (15, 30, 45 and 60 days). This experiment was conducted for a period of eight weeks.

Statistical analysis

The statistical significance of the effects of different diet treatments on growth rates of the juvenile snails was evaluated by one-way ANOVA [3] for data obtained at four different times (15, 30, 45, and 60 days).

Significance of differences among treatments was tested with a Tukey's post-hoc test. Differences among treatments were considered significant for $P < 0.05$. Data were tested for homogeneity of variances using a Shapiro-Wilk test and were found to be normally distributed. All statistic analyses were performed using Statistica 6.0 (Statsoft Inc.).

Results

Breeding and early development

Twelve days after broodstock accommodation started, mating behaviors were recorded, with males climbing over females shell. For a period of 20-30 minutes male stayed firmly attached to the female shell but no copulatory attempt could be seen. Afterwards, male slightly slides towards female right side and starts grasping with his penial sheath searching for genital aperture.

Copulation would usually last for $3 \text{ h } 40 \text{ min} \pm 45 \text{ min}$ ($n=7$). Spawning process was difficult to observe because this is mainly a nocturnal behavior. Eggs were laid individually in transparent sac deposits in solid smooth surfaces, like PVC pipes or plant leaves, but were frequently seen in aquarium walls too.

Eggs were 1.3 ± 0.4 mm in length and egg sacs 3.7 ± 0.6 mm in length on the attached edge (Figure 2). Each female deposits between 1 to 4 eggs per clutch in a straight line, separated from each other by 5 mm approximately. Eggs were frequently seen in aquarium walls too.

Hatching occurred 52 ± 6 ($n=25$) days after oviposition; the range

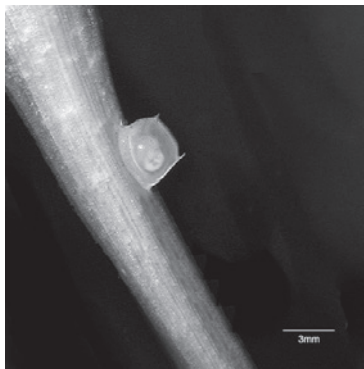


Figure 2: *Clea helena* fertilized egg sac attached to a plant leaf.

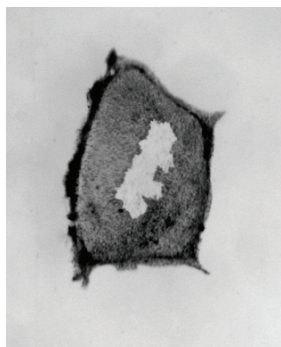


Figure 3: *Clea helena* recently hatched egg.

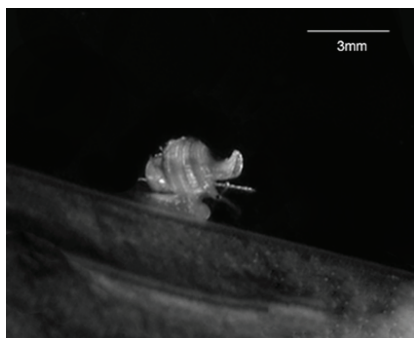


Figure 4: *Clea helena* newly hatched juvenile.

was 46-58 days at 25.0°C (n=25). Egg cases were found empty with open holes that provide evidence of successful hatches (Figure 3).

Newly hatched juveniles (Figure 4), measured SL=3.1 ± 0.3 mm (n=20) and resembled the adults in shell shape and color although typical shell stripes were not clearly marked.

Diets and growth

Growth rates of *C. helena* juveniles, estimated by shell length, were influenced by the different diets treatments (P<0.05) (Figure 5).

Juveniles reared with Diet 3 (dry food and live prey combined diet) presented significantly higher growth rates than those presented by both other diets (ANOVA; F=4.30; P<0.05; post-hoc Tukey, p<0.05). No significant differences were found between the use of Diet 1 and Diet

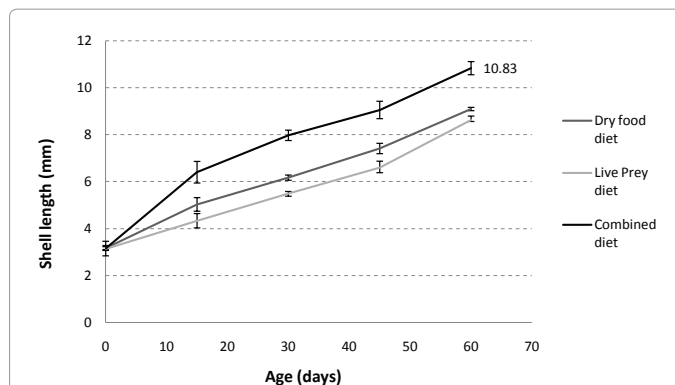


Figure 5: Mean (mm, ± Standard deviation, N=5) of *Clea helena* juveniles shell length reared at three different diets.

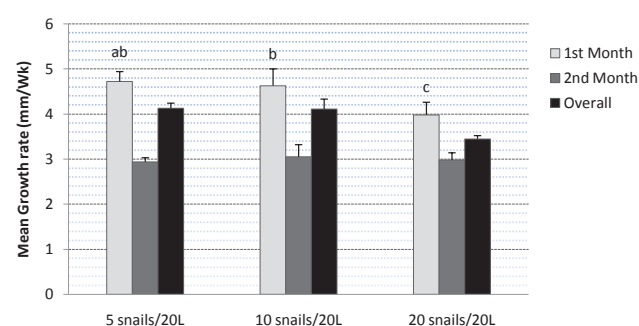


Figure 6: Mean Weekly growth rate (mm/WK ± <SD) of juvenile *Clea Helena* stocked at three different densities over an 8 weeks period. Significant pairwise differences between densities are shown by letter and were found only during the first month.

2 (P>0.05). Growth pattern was similar to all three diets and juveniles fed with combined diet (dry food and live prey) presenting the highest growth rate during the entire test duration.

Stocking density experiment

The mean shell length (SL) at stocking was 9.6 ± 1.5 mm (n=105) and there were no significant differences in initial SL among densities (P>0.05).

Average weekly growth rates of *C. helena* juveniles, tended to slightly decline with increased stocking density during the first month and were indistinguishable among densities during the second month (Figure 6).

During the first month, individuals stocked at 5 snails/L had significantly faster growth rates than those stocked at 20 snails/L (p=0.031). No significant growth responses were found during the second month among all densities and overall growth rates for this experiment also revealed no density-dependent growth responses. Survival ranged from 97-100% among individual replicates with no significant differences among treatments (p=0.691).

Discussion

Like the other members of the family Buccinidae, *C. helena* is gonochoristic and fertilization is internal. There is no visible sexual dimorphism and in all copulations observed, no size differences were recorded between males and females. This morphological pattern differs from those described for other Buccinidae species [4] were

sexual dimorphism was clearly registered, although no data is available on other *Clea* species.

To our best knowledge, no data on wild specimens' spawning season is available either, but results from the present study indicate that, under laboratory environmental controlled conditions, *C. helena* is able to breed all the year through.

Unlike other Buccinidae family members female, *C. helena* individuals lay only 3-4 eggs per clutch. Even under controlled conditions and *ad libitum* food availability, this species presented a very low fertility rate and a very high survival rate through the entire study [5].

Research on diet and growth clearly showed that a combined diet (dry food and live prey) produces significantly better results than those presented by other simpler diets used. That comes in line with the knowledge that this species is a very effective and non-specific snail predator, which results in a naturally diverse and enriched protein food intake. This is also consistent with reports for other invertebrate species [6], that diets which contain a mixture of two or more proteins are better utilized by the animals. We hypothesize that during the feeding experiments using a combination diet, the energy consumed by the snails while preying could easily be compensated by the dry food simultaneously available. The absence of significant differences among both other diets might also be related to the ratio between food intake Vs energy spent acquiring it.

Choosing an appropriate diet is crucial for maintaining successful snail stock cultures in laboratory. In order to fulfill the optimum growth rate possible for this species, further studies would have to be undertaken in order to find an artificial protein source that fulfills the dietary needs with inexpensive cost and ease of use that might suit an intensive production management.

Natural densities of *C. helena* are unknown, so comparisons with other freshwater snails (*Pomacea* and *Marisa* genera), marine snails (*Babylonia* genus) and land snails (*Helix* genus) stocking densities studies were considered. Average weekly growth pattern during the first month, with increased stocking density tended to decline slightly during the first month which is concordant with similar studies performed on *Pomacea* genus [7,8], *Marisa cornuarietis* [9], *Babylonia areolata* [10] and various land snail species [11-13].

In the present study, individuals stocked at 5 snails/L had significantly faster growth rates than those stocked at the upper limit tested (20 snails/L). This is also consistent with experiments performed with other previously referred genera and is likely to be related with competition for space and prey among individuals. No significant growth responses were found during the second month among all densities, which could possibly indicate that the amount of metabolites wastes accumulated in the water, that are usually considered detrimental to juvenile's growth, doesn't seem to influence our test responses.

Survival rates obtained allow us to consider *C. helena* a robust specie, which is an important feature when considering ornamental market issues, like shipping and wholesale stocking.

Conclusions

Besides the overall contribution to a systematic knowledge of *C. helena* life history important traits like breeding, growth and

development data under laboratory conditions this study also demonstrates that it is feasible to culture healthy marketable size snails in a re-circulating system. Nevertheless, further studies are encouraged namely focusing in finding a dietary artificial protein source substitute and trying to raise the extremely low fertility rate presented which can be impairments to a prospectus commercial farming of this species.

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