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Effects of husbandry parameters on the life-history traits of the apple snail, *Marisa cornuarietis:* effects of temperature, photoperiod, and population density

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Abstract. These experiments are part of a larger study designed to investigate the influence of husbandry parameters on the life history of the apple snail, *Marisa cornuarietis*. The overall objective of the program is to identify suitable husbandry conditions for maintaining multi-generation populations of this species in the laboratory for use in ecotoxicological testing. In this article, we focus on the effects of photoperiod, temperature, and population density on adult fecundity and juvenile growth. Increasing photoperiod from 12 to 16 h of light per day had no effect on adult fecundity or egg hatching and relatively minor effects on juvenile growth and development. Rearing snails at temperatures between 22°C and 28°C did not influence the rates of egg production or egg clutch size. However, the rates of growth and development (of eggs and juveniles) increased with increasing temperature in this range, and when temperatures were reduced to 22°C egg-hatching success was impaired. Juvenile growth and development were more sensitive to rearing density than adult fecundity traits. On the basis of the present results, we conclude that rearing individuals of *M. cornuarietis* at a temperature of 25°C, a photoperiod of 12L:12D, and a density of <0.8 snails L^{-1} (with lower densities for juvenile snails) should provide favorable husbandry conditions for maintaining multi-generation populations of this species.

Additional key words: Ampullaridae, ecotoxicology, gastropod, growth, reproduction

The freshwater prosobranch ampullarid *Marisa cornuarietis* LINNAEUS 1758, also known as the apple snail, is used in many tropical countries as a biological control agent for molluscs hosting the trematode known to cause intestinal schistosomiasis (Pointier & Augustin 1999; Pointier & Jourdane 2000). Although feeding mainly on living and decaying aquatic plants, it also consumes eggs, newly hatched young, and possibly even the adults of other snail species (Demian & Lutfy 1965, 1966; WHO 1982). More recently, this snail has been the subject of ecotoxicological studies and has been reported to be particularly sensitive to certain hormone-disrupting chemicals (Oehlmann et al. 2000). Although not native to European freshwater habitats, it is presently being considered as a test species in the environmental risk assessment of certain suspected endocrine-disrupting chemicals within the European Union.

Members of *M. cornuarietis* have a widespread distribution in the Caribbean, Central America, and South America, and its natural distribution has been expanded by introductions for parasite control. It has also been investigated as a potential weed control agent in both Puerto Rico and Florida (Ferguson & Palmer 1958; Ortiz-Torres 1961; Radke et al. 1961). Although the species seems to be highly adaptable to varying environmental conditions in the field, there are relatively few controlled laboratory studies examining how its life-history traits respond to environmental changes, e.g., photoperiod, temperature, food quality and quantity, population density, etc. A knowledge of responses such as juvenile and adult

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survival, growth, and reproduction is essential for developing suitable husbandry conditions for maintaining populations of the species in the laboratory. Likewise, designing effective ecotoxicological test protocols relies critically on an understanding of how husbandry conditions influence snail performance as well as on having baseline information on the means and variances of life-history traits under standard laboratory conditions.

The present experiments are part of a larger study designed to investigate the influence of husbandry parameters on the life history in *M. cornuarietis*. The overall objective of the program is to identify suitable husbandry conditions for maintaining multi-generation populations of this species in the laboratory for use in ecotoxicological testing. In this first paper, we focus on the effects of photoperiod, temperature, and population density on adult fecundity and juvenile growth. Later papers in this series will focus on the effects of water quality, food quality, and feeding frequency, at different temperatures, on various juvenile and adult life-history traits.

Methods

Collection and maintenance

Individuals of *Marisa cornuarietis* were collected from the shoreline of Lake Guajataca, Puerto Rico by Sharon File-Emperador. The species was introduced into the lake in 1960 as part of a bilharzias (schistosomiasis) control project. The lake is a man-made reservoir located high in the Karst region of western Puerto Rico. Water and sediment quality at the collection areas were assessed by the Terran Corporation (Terran 2002). A variety of heavy metals, organochlorine pesticides, PCBs, and PAHs were measured, and all were determined not to be elevated as a result of human activities.

Snails were maintained at the University of Puerto Rico at a temperature of 25°C, pH 7.2, a density of ~ 0.7 snails L^{-1}, and fed washed Romaine lettuce (*Lactuca sativa* var. Romaine). Each aquarium was outfitted with an AquaTec filter pump (Aquaria Inc. Regent Pet Products, Moorpark, CA, USA), and water was changed weekly. Aquaria received indirect natural light for ~ 12 h d^{-1} supplemented by fluorescent light for ~ 8 h d^{-1}. Snails were wrapped individually in cheesecloth, packed in a styrofoam box, and shipped by overnight mail to ABC Laboratories (Columbia, MO). The snails were collected in April 2003 and shipped, in two batches, on April 23 and July 9, 2003.

Common husbandry conditions

Snails for all experiments were reared in 10-L glass aquaria under flow-through conditions. The renewal rates were 5.8 tank volumes per day, with each tank receiving ~ 500 mL of water/cycle and 115 cycles d^{-1} during the test period. The freshwater used in all experiments consisted of natural well water blended with well water that was demineralized by reverse osmosis to achieve a final water hardness of ~ 130–160 mg L^{-1} (as $CaCO_3$). Temperature and pH were measured with a Denver Instruments temperature/pH meter (Fisher Scientific, Pittsburgh, PA, USA). Target temperatures were maintained within ± 1°C and pH was maintained within the range 7.7–8.5. Dissolved oxygen was measured with a WTW Oxi 330 or WTW Oxi 330i dissolved-oxygen meter (Fisher Scientific) and kept above 4.0 mg L^{-1}. Total hardness (measured by titration) was kept within the range 134–154 mg $CaCO_3$ L^{-1}. Photoperiod was controlled by a timer connected to overhead fluorescent lighting, and light intensity in the aquaria during the light period was ~ 400–800 lx.

Snails in all experiments were fed an alginate snail diet consisting of an $\sim 1{:}4$ ratio of snail mix (20% salmon starter, 20% flake food, 45% dried algae, 15% lyophilized lettuce, and 3% vitamin mix) to gel fish food (56% crude protein, 16% crude fat, 0.6% crude fiber, 16% ash, and 11% amino acids, minerals and vitamins), administered as cubes. The exact amounts of food provided depended on the number and size of snails/aquarium, but was such that snails always had access to food. The experimental aquaria were cleaned and siphoned free of debris as needed.

E1: temperature and photoperiod versus fecundity

In this experiment, adult snails originally collected from Puerto Rico (F_0 generation) were reared for a total of 32 d under one of the following treatments: 25°C and 12L:12D, 25°C and 16L:8D, 28°C and 12L:12D, or 28°C and 16L:8D. There were four replicate aquaria/treatment, each containing four females and three males, giving a snail density of 0.7 snails L^{-1}. Snails were checked daily and eggs were removed, counted, and transferred to separate containers for hatching. The hatching containers consisted of 19-cm-tall nitex mesh columns (355-μm mesh size) attached with silicone to 100-mm, glass petri dish, bottoms. The hatching containers were maintained in identical aquaria as the adults. The following fecundity parameters were measured: eggs/clutch, egg clutches/female/week, eggs/female/week, egg-hatching success (% hatch), and the number of

days until the first egg in a clutch hatched (time to first hatch).

Temperature was monitored continuously, whereas dissolved oxygen, pH, and total hardness were measured weekly. All other husbandry parameters were as described for common husbandry conditions above.

E2: temperature and photoperiod versus juvenile growth

In this experiment, juvenile snails (F_1 generation) produced by field-collected adults were reared for a total of 103 d under one of the following treatments: 25°C and 12L:12D, 25°C and 16L:8D, 28°C and 12L:12D, or 28°C and 16L:8D. There were eight replicate aquaria/treatment, each containing ten juveniles, giving a snail density of 1.0 snail L^{-1}. Snails were 23 d of age at the start of the experiment. Body sizes were estimated by gently blotting snail dry and weighing them to the nearest milligram. The shell diameter of each snail was also measured to the nearest 0.1 mm using calipers. A representative sample of ten snails was used to estimate average starting wet weight (WW) (with shell; 16.4 ± 4.4 mg) and shell diameter (DIAM) (3.5 ± 0.5 mm). All remaining snails were randomly allocated to treatment aquaria. Snail WW and DIAM were measured by harvesting one of the replicate aquaria after 4, 6, 8, and 10 weeks, with the remaining four replicates harvested at test termination (~15 weeks). Additional DIAM measurements were collected from a single replicate aquarium after 12 weeks of the trial.

Plots of DIAM versus age (defined as days post-hatch) were linear in all cases, whereas WW versus age relationships were linearized by $\log_{10}$ transformations of both axes. Time to morphological maturity (TM), defined as the age at which males and females can be distinguished on the basis of head/foot coloration (occurs at a size of ~20 mm), was estimated by solving the best-fit regression of age and DIAM for a size of 20 mm. Likewise, time to first reproduction (TR), defined as the age at which females begin to lay egg clutches (occurs at a size of ~30 mm), was estimated by solving the same regression equation for a DIAM of 30 mm.

Temperature was monitored continuously, whereas dissolved oxygen, pH, and total hardness were measured biweekly. All other husbandry parameters were as described for common husbandry conditions above.

E3: temperature versus adult fecundity

In this experiment, adult snails that had hatched in the laboratory (F_1 generation) were reared for a total of 42 d under a 12L:12D photoperiod at either 25°C or 22°C. There were four replicate aquaria/treatment, each containing four females and three males, giving a snail density of 0.7 snails L^{-1}. Snails were checked daily and eggs were removed, counted, and transferred to separate containers for hatching as in Experiment 1. Fecundity measurements were performed as in Experiment 1.

Temperature was monitored continuously, whereas dissolved oxygen, pH, and total hardness were measured weekly. All other husbandry parameters were as described for common husbandry conditions above.

E4: temperature versus juvenile growth

In this experiment, juvenile snails (F_1 generation) produced by field-collected adults were reared for a total of 42 d under a 12L:12D photoperiod at either 25°C or 22°C. There were eight replicate aquaria/treatment, each containing ten juveniles, giving a snail density of 1.0 snail L^{-1}. Snails were 44 d of age at the start of the experiment. A representative sample of ten snails was used to estimate average starting weight (189 ± 178 mg) and DIAM (8.1 ± 2.8 mm). All remaining snails were randomly allocated to treatment aquaria. Snail WW and DIAM were measured by harvesting one of the replicate aquaria after 1, 2, 4, and 5 weeks. The remaining four aquaria/treatment were harvested at the end of the experiment (6 weeks). Treatment effects on juvenile growth rates were assessed as in Experiment 2.

Temperature was monitored continuously, whereas dissolved oxygen, pH, and total hardness were measured biweekly. All other husbandry parameters were as described for common husbandry conditions above.

E5: density versus adult fecundity

In this experiment, adult snails (F_1 generation) that had hatched in the laboratory were reared for a total of 26 d under a 12L:12D photoperiod at 25°C and at a density of 0.2, 0.8, or 2.0 snails L^{-1}. There were two replicate aquaria/density treatment each containing two (0.2 snails L^{-1}), eight (0.8 snails L^{-1}), or 20 (2.0 snails L^{-1}) adults in a 50:50 sex ratio. Snails were checked daily and eggs were removed, counted, and transferred to separate containers for hatching as in Experiment 1. Fecundity measurements were performed as in Experiment 1.

Temperature was monitored continuously, whereas dissolved oxygen, pH, and total hardness were measured weekly. Because of low dissolved oxygen levels in the 0.8 and 2.0 snails L^{-1} treatments, it was

necessary to provide gentle aeration to the aquaria. All other husbandry parameters were as described for common husbandry conditions above.

E6: density versus juvenile growth

In this experiment, juvenile snails (F_2 generation) produced by laboratory-reared adults were grown for a total of 73 d under a 12L:12D photoperiod at 25°C and at a density of 0.2, 0.8, or 2.0 snails L^{-1}. There were eight replicate aquaria/density treatment, each containing two (0.2 snails L^{-1}), eight (0.8 snails L^{-1}), or 20 (2.0 snails L^{-1}) juveniles. Snails were 11 d of age at the start of the experiment. A representative sample of ten snails was used to estimate average starting DIAM (3.3 ± 0.4 mm). All remaining snails were randomly allocated to treatment aquaria. WW was measured for the first time in week 4 (together with DIAM) and then in weeks 6, 8, and 9 by harvesting a single replicate aquarium. The remaining four replicate aquaria were harvested at the end of the experiment (approximately week 10). Treatment effects on growth rates, time to maturity, and time to first reproduction were assessed as in Experiments 2 and 4.

Temperature was monitored continuously, whereas dissolved oxygen, pH, and total hardness were measured biweekly. All other husbandry parameters were as described for common husbandry conditions above.

Statistics

The effects of temperature, photoperiod, and their interaction on adult fecundity traits (Experiment 1) were analyzed by two-way analysis of variance (ANOVA). In cases in which interaction effects were significant, Student's t-tests were performed for each factor separately. The effects of temperature and density on adult fecundity (Experiments 3 and 5, respectively) were analyzed by one-way ANOVA. If >2 treatments were included, pairwise differences were tested with Tukey's HSD test in the case of significant main effects. Data were checked graphically for normality and homogeneity of variances before ANOVA. Treatment effects on juvenile growth were tested by analysis of covariance (ANCOVA), with diameter or weight as the dependent variable, age as the covariate, and temperature, photoperiod, or density as the treatment. ANCOVAs were followed by Tukey's HSD tests of significant main effects for cases in which there were >2 treatment groups.

All statistical analyses were performed with SYSTAT (ver. 10) (Richmond, CA, USA). A significance level of $p \leq 0.05$ was used throughout; comparisons in which $0.1 \leq p \leq 0.05$ are defined as marginally significant.

Results

E1: temperature and photoperiod versus fecundity

There was 100% survival of adults in all treatments in this experiment. There was no difference in the number of egg clutches/female/week in response to temperature ($p=0.45$), photoperiod ($p=0.99$), or their interaction ($p=0.35$). Likewise there were no differences in the numbers of eggs/female/week in response to temperature ($p=0.72$), photoperiod ($p=0.80$), or their interaction ($p=0.33$). The number of eggs/clutch was also unaffected by temperature ($p=0.38$), photoperiod ($p=0.31$), or their interaction ($p=0.79$). Whereas percent hatch was unaffected by temperature ($p=0.16$), photoperiod ($p=1.00$), or their interaction ($p=0.18$), time to first hatch was significantly longer at 25°C compared with 28°C ($p<0.001$). The effects of photoperiod ($p=0.18$) and the interaction between photoperiod and temperature ($p=0.84$) on time to first hatch were not significant. Average values ($\pm$SEM) for fecundity traits in each treatment are given in Fig. 1.

Measured temperatures during the course of the experiment averaged ($\pm$SD) 25.1 (± 0.1)°C and 28.4 (± 0.3)°C under the 12L:12D photoperiod, and 25.3 (± 0.1)°C and 28.5 (± 0.3)°C under the 16L:8D photoperiod.

E2: temperature and photoperiod versus juvenile growth

Survival of juveniles in all treatments was $>93\%$. In the 12L:12D photoperiod, snails grew significantly faster at 28°C than at 25°C (DIAM: $p=0.001$; WW: $p=0.01$). However, in the 16L:8D photoperiod, temperature had no significant effect on juvenile growth (DIAM: $p=0.50$; WW: $p=0.45$). Although at 28°C photoperiod did not have a significant effect on juvenile growth (DIAM: $p=0.15$; WW: $p=0.35$), at 25°C the effects of photoperiod were marginally significant (DIAM: $p=0.05$; WW: $p=0.09$). Average values ($\pm$SEM) for juvenile WW and shell DIAM at the end of the experiment are shown in Fig. 2. Using the individual regressions of age versus diameter for each treatment group and the sizes at morphological maturity and first reproduction, we estimated that it would take snails between 89 and 101 d to reach morphological maturity and between 132 and 145 d to reach age of first reproduction under these conditions.

Measured temperatures during the course of the experiment averaged ($\pm$SD) 25.1 (± 0.1)°C and 28.5 (± 0.6)°C under the 12L:12D photoperiod and 25.1

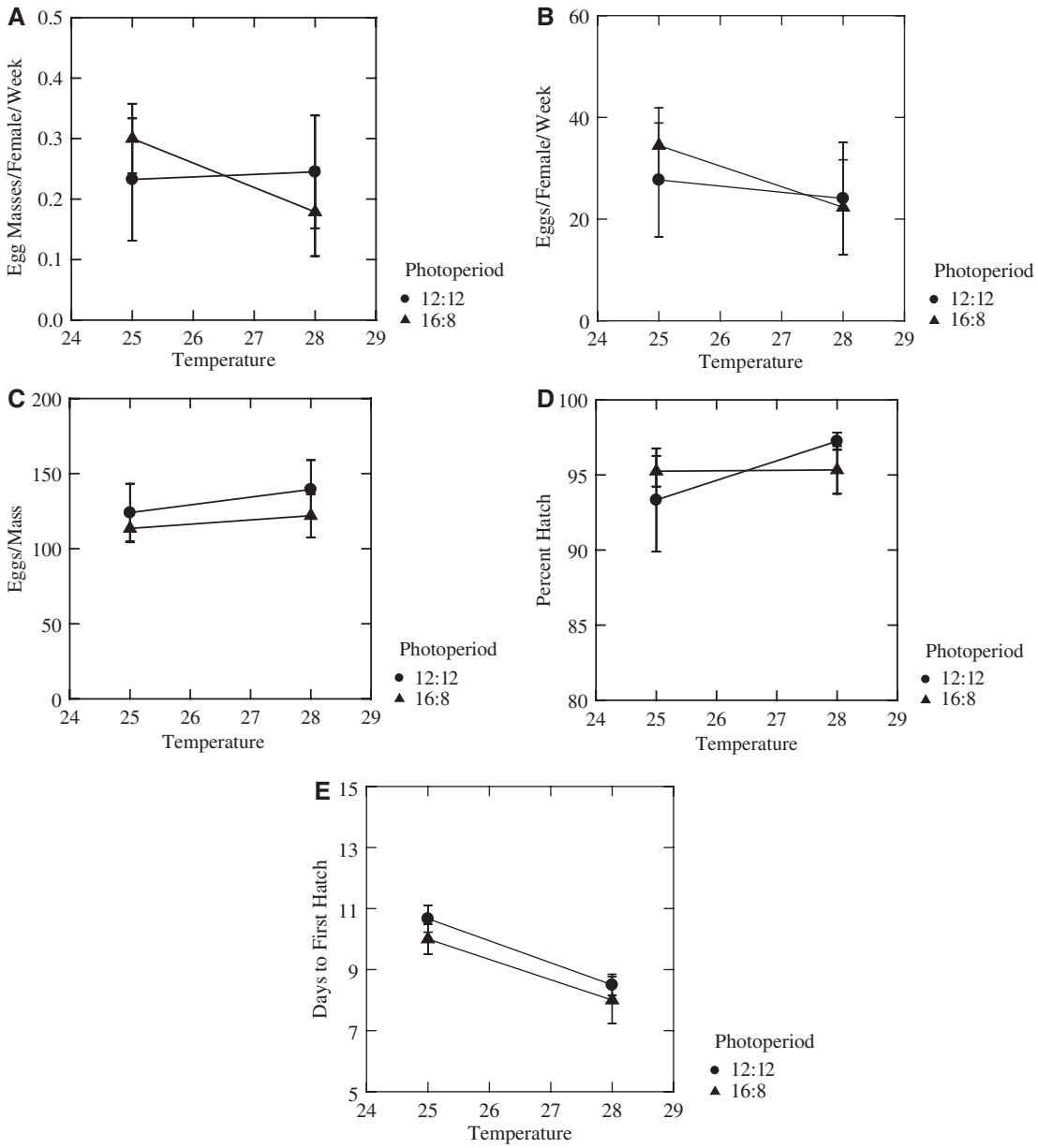

Fig. 1. Experiment 1. Effect of temperature (25°C vs. 28°C) and photoperiod (12L:12D vs. 16L:8D) on adult reproductive traits. Symbols are means; error bars are SEM. **A.** Egg masses/female/week. **B.** Eggs/female/week. **C.** Eggs/mass. **D.** Egg-hatching success. **E.** Number of days for first eggs in a clutch to hatch.

(±0.4)°C and 28.7 (±0.1)°C under the 16L:8D photoperiod.

E3: temperature versus adult fecundity

There was 100% survival of adults in all treatments. There was no difference between 22°C and 25°C in the number of egg clutches/female/week ($p=0.99$), the number of eggs/female/week ($p=0.30$), or the number of eggs/clutch ($p=0.18$). However, hatching success was higher at 25°C than at 22°C ($p=0.002$), and time to first hatch was significantly shorter at 25°C that at 22°C ($p<0.001$) (Fig. 3).

Measured temperatures during the course of the experiment averaged (±SD) 24.4 (±0.1)°C and 22.3 (±0.2)°C.

E4: temperature versus juvenile growth

Survival of juveniles in both treatments was 95%. Juveniles grew significantly faster at 25°C than at 22°C (DIAM: $p=0.02$; WW: $p=0.02$) (Fig. 4). We

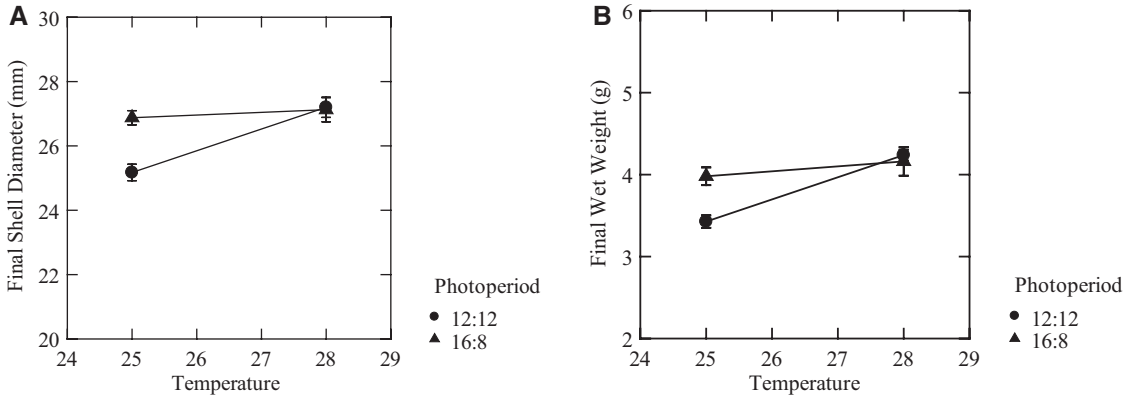

Fig. 2. Experiment 2. Effect of temperature (25°C vs. 28°C) and photoperiod (12L:12D vs. 16L:8D) on juvenile growth rate measured in terms of wet weight (**A**) and shell diameter (**B**). Symbols are means; error bars are SEM.

estimated that snails would reach morphological maturity at 89 d at 25°C and at 102 d at 22°C. Age at first reproduction was estimated to be 127 d at 25°C and 148 d at 22°C.

Measured temperatures during the course of the experiment averaged (±SD) 24.7 (±0.1)°C and 22.3 (±0.2)°C.

E5: density versus adult fecundity

There was 100% survival of adults in all treatments. Density had a significant effect on the number of egg clutches/female/week ($p=0.02$), with snails grown under the highest density producing significantly fewer egg clutches (Tukey HSD: 0.2 vs. 0.8, $p=0.86$; 0.2 vs. 2.0, $p=0.03$; 0.8 vs. 2.0, $p=0.03$). The number of eggs/female/week was also reduced with increasing density ($p<0.001$). Whereas egg production did not differ between 0.2 and 0.8 snails L^{-1} (Tukey HSD: $p=0.36$), it was significantly reduced at 2.0 snails L^{-1} (Tukey HSD: 0.2 vs. 2.0, $p=0.001$; 0.8 vs. 2.0, $p=0.002$). There was no significant effect of density on the number of eggs/clutch ($p=0.47$). However, percent hatch was reduced with increasing adult density (ANOVA: $p=0.03$; Tukey: 0.2 vs. 0.8, $p=0.64$; 0.2 vs. 2.0, $p=0.03$; 0.8 vs. 2.0, $p=0.06$). Time to first hatch was 11 d in all replicates at all adult densities. Average values (±SEM) for fecundity variables as a function of density are shown in Fig. 5.

Measured temperatures during the course of the experiment averaged (±SD) 25.2 (±0.1)°C.

E6: density versus juvenile growth

Survival of juveniles in all treatments was >99%. Density had a significant effect on juvenile growth measured as WW ($p<0.001$). Tukey pairwise comparisons of WW growth indicated that juveniles maintained at a density of 0.2 snails L^{-1} grew significantly faster than snails grown at 0.8 snails L^{-1} ($p<0.001$) or 2.0 snails L^{-1} ($p<0.001$). For DIAM, the slopes of the regressions between age and size differed significantly among density treatments (age × density interaction: $p=0.005$), and the slope of the regressions decreased with increasing density. Using the best-fit line for each density, we estimated that it would take snails 67, 83, and 98 d to reach morphological maturity at 0.2, 0.8, and 2.0 snails L^{-1}, respectively. The corresponding values for age at first reproduction were estimated to be 98, 123, and 148 d. Average values (±SEM) for juvenile WW and DIAM at the end of the experiment are shown in Fig. 6.

Measured temperatures during the course of the experiment averaged (±SD) 25.2 (±0.1)°C.

Discussion

Effects of temperature

There were no differences in adult fecundity between snails reared at 25°C and 28°C. In Experiment 1, females produced an average of 0.24 egg clutches/week, with an average clutch size of 125 eggs, giving an average of 30 eggs/week. Although it took eggs 2–3 d longer to hatch at 25°C (10.5 vs. 8 d at 28°C), there were no differences in hatching success between the two temperatures, which was >93% in all treatments. Reducing temperature from 25°C to 22°C had no effect on adult egg production or egg clutch size. However, egg-hatchability was reduced by 72% and eggs took 5 d (i.e., 42%) longer to hatch at the lower temperature. Demian & Yousif (1973) reported an

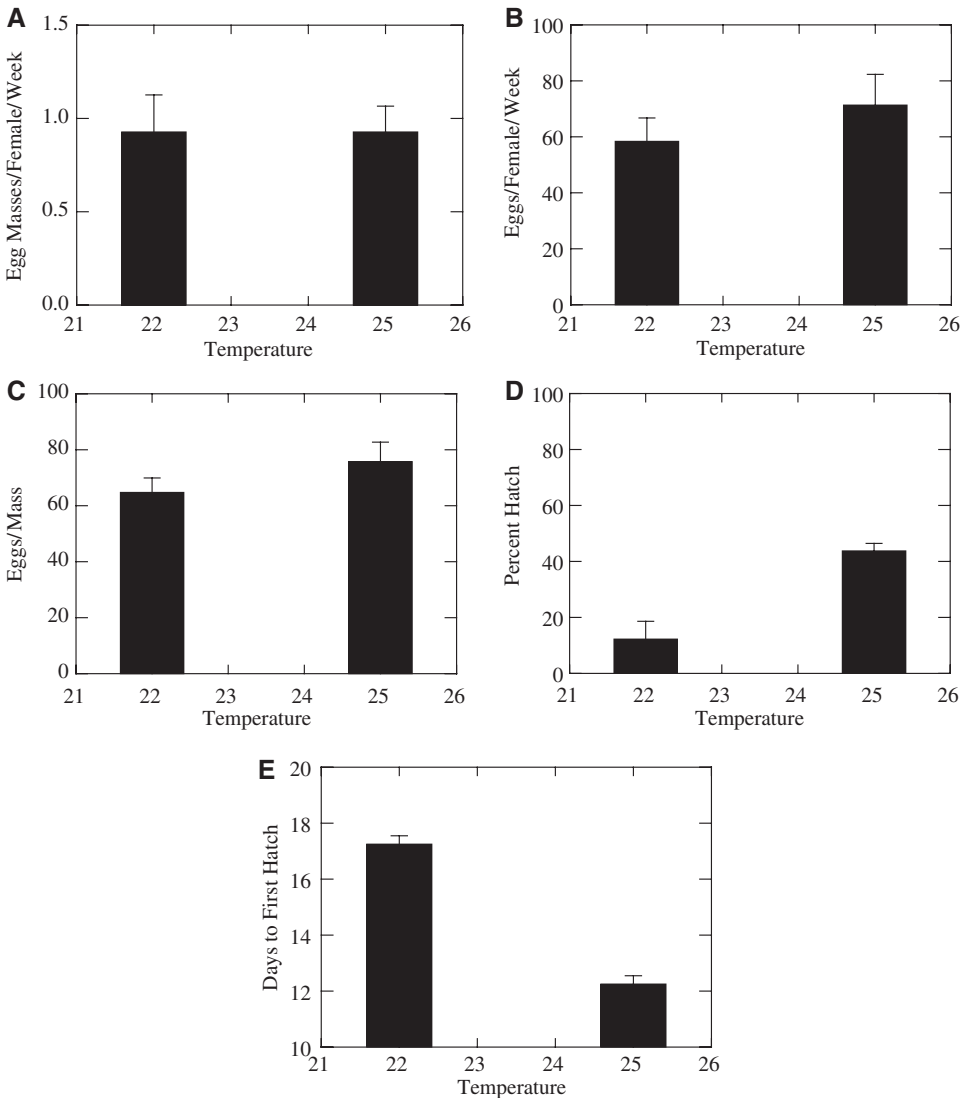

Fig. 3. Experiment 3. Effect of temperature (22°C vs. 25°C) on adult reproductive traits. Data are presented as means ± SEM. **A.** Egg masses/female/week. **B.** Eggs/female/week. **C.** Eggs/mass. **D.** Egg-hatching success. **E.** Number of days for first eggs in a clutch to hatch.

average period for embryonic development in individuals of *Marisa cornuarietis* of 8 d at a temperature range of 25°–30°C and of 20 d at 15°–20°C. Robins (1971) reported hatching times in laboratory aquaria at uncontrolled temperatures varying 14°–31°C to be 11–24 d. In our experiments, eggs took between 8 d (at 28°C) and 17 d (at 22°C) to hatch, which is entirely consistent with previously published observations.

A number of earlier studies have reported on the effect of temperature on fecundity in *M. cornuarietis*.

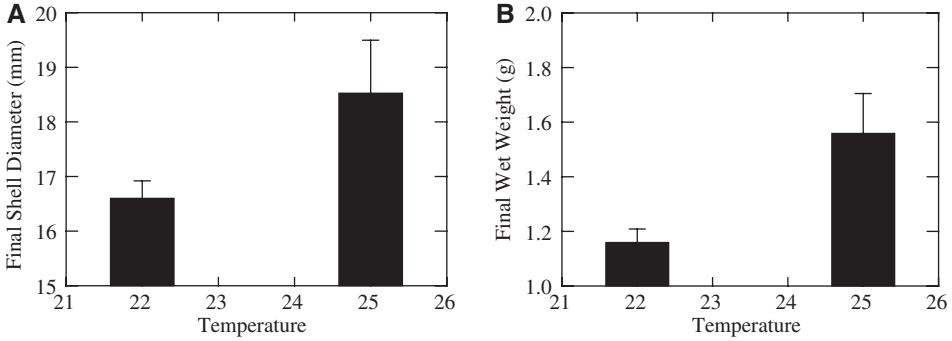

Fig. 4. Experiment 4. Effect of temperature (22°C vs. 25°C) on juvenile growth rate measured in terms of wet weight (**A**) and shell diameter (**B**). Data are presented as means ± SEM.

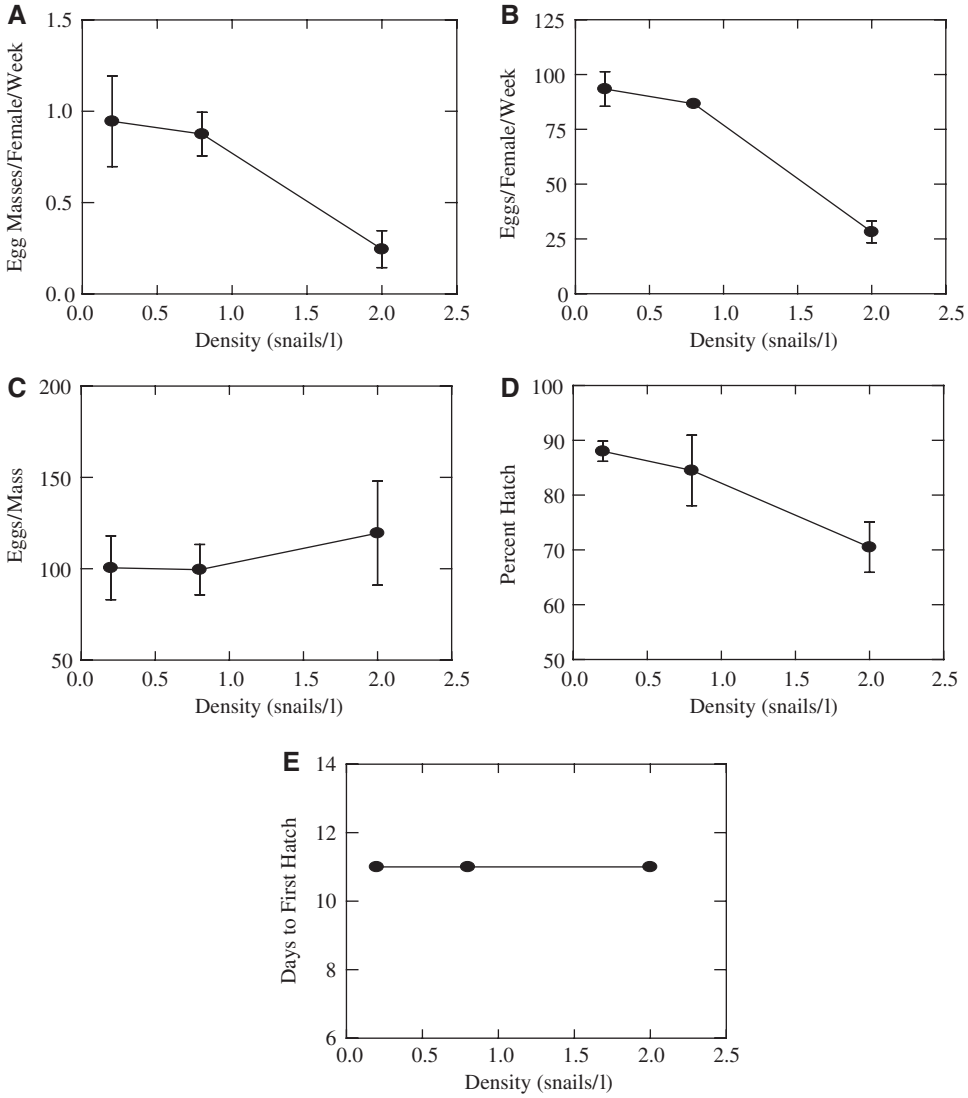

Fig. 5. Experiment 5. Effect of snail density (0.2, 0.8, and 2.0 snails L^{-1}) on adult reproductive traits. Symbols are means; error bars are SEM. **A.** Egg masses/female/week. **B.** Eggs/female/week. **C.** Eggs/mass. **D.** Egg-hatching success. **E.** Number of days for first eggs in a clutch to hatch.

Robins (1971) reported that field populations of *M. cornuarietis* from canals in Dade County, Florida, reproduced in all months of the year except May and June, with reproductive activity concentrated in November, and February through April. In contrast, Demian & Ibrahim (1971) found laboratory populations of *M. cornuarietis* of Puerto Rican origin, maintained at ambient room temperature, to breed from March to early December at temperatures varying 20°–33°C. In this population, egg production was reported to increase gradually, with increasing temperatures from March through July, and then to

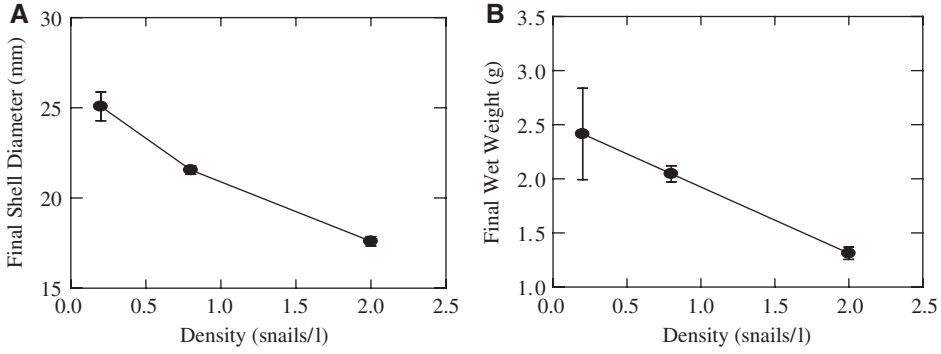

Fig. 6. Experiment 6. Effect of snail density (0.2, 0.8, and 2.0 snails L^{-1}) on juvenile growth rate measured in terms of wet weight (**A**) and shell diameter (**B**). Symbols are means; error bars are SEM.

decrease again until ceasing during January and February, when room temperatures fell within the range 14°–20°C. This appears to be largely a temperature effect, as the authors reported that snails could be induced to breed during January and February if temperatures were raised to summer levels. In addition, Blackburn et al. (1971) reported egg-hatching success in *M. cornuarietis* to be reduced by 50% when eggs were exposed to temperatures of 14°–16°C for 8 h, and even exposure to 16°C for 2 h produced a measurable reduction in hatching success.

Juvenile snails grew significantly faster at 28°C that at 25°C under a 12L:12D photoperiod, but not under a 16L:8D photoperiod. Juvenile growth rates were more substantially reduced at 22°C, with time to maturity delayed by 13 d and time to first reproduction delayed by 21 d compared with that at 25°C.

The sex of laboratory populations of *M. cornuarietis* used in the present study is distinguishable at a DIAM of ~20 mm, which occurred at an age of 89–101 d at 25°C, with some variability between Experiments 1 and 3. At this same temperature, snails reproduce for the first time at a size of ~30 mm, which occurred at an age of 127–145 d (i.e., 4–5 months). At 22°C (Experiment 3), developmental rates were at the slow end of this range and about 15% slower than the 25°C treatment group in this experiment. In contrast, Demian & Ibrahim (1972) characterized individuals of *M. cornuarietis* collected from a Puerto Rican field population as newly mature at a DIAM of 25 mm, which they reported occurred at an age of ~1 year. Whether "maturity" as defined by Demian & Ibrahim (1972) refers to morphological maturity or first reproduction, the age at which it occurred is substantially longer than that observed in our laboratory populations. It could be expected that lower temperatures during part of the year, and possibly food limitation or other density-dependent factors, may contribute to the slower development of field populations. A generation time of 4 months was reported for field populations established in the Sudan at sites with an average temperature of ~26°C (Haridi et al. 1985), which is consistent with the present results.

Thus, on the basis of our own and published results, we conclude that 25°–28°C lies centrally within the tolerance range of individuals of *M. cornuarietis,* but that at temperatures of 22°C, snails begin to show signs of reduced reproductive performance.

Effects of photoperiod

It is widely acknowledged that, for most seasonal breeders living in temperate climates, photoperiod is the primary environmental signal that regulates the timing of reproduction (Wayne 2001). Temperate gastropod species have been observed to show increased egg-laying with increasing photoperiod. For example, Bohlken et al. (1986) reported an increase in egg-laying with increased photoperiod in *Lymnaea stagnalis*. For tropical species, such as *M. cornuarietis,* the role of photoperiod in controlling the reproduction of field populations is less clear. Robins (1971) claimed that *M. cornuarietis* is primarily nocturnal and most reproductively active at night. Demian & Ibrahim (1971) observed laboratory-reared adults of *M. cornuarietis* to lay eggs more often at night and in the early morning than at other times. Therefore, we expected photoperiod to have a significant effect on adult fecundity in our laboratory-reared adults. However, we observed no effects of photoperiod on any of the adult fecundity traits or on egg-hatching. Although under a 12L:12D photoperiod juvenile snails grew faster at 28°C than at 25°C, there were no significant differences in juvenile growth or development between the two temperatures under the 16L:8D photoperiod. Photoperiod had a marginal effect on juvenile growth at 25°C, but no effect at 28°C.

Thus, despite suggestions from the literature of an effect of photoperiod on snail fecundity, we could detect no effect on any fecundity trait (and only limited effects on juvenile growth), by increasing the duration of the light period from 12 h to 16 h.

Effects of density

Increasing rearing density from 0.2 to 0.8 snails L^{-1} had little effect on adult fecundity traits, but increasing density to 2.0 snails L^{-1} resulted in significant reductions in egg clutches/female/week and eggs/female/week (but not eggs/clutch). Whereas time to hatch was not affected by density, hatching success was reduced at the highest adult density. Juvenile growth and development were very sensitive to density effects, with a reduction in growth already apparent at 0.8 snails L^{-1}. It has been suggested that metabolites produced by the snails may inhibit growth or reproduction at increasing densities (Thomas et al. 1975). However, as we used a flow-through system (with a renewal rate of 5.8 tank volumes day), it is unlikely that there could have been a build-up of metabolites, even at the highest densities.

Other studies have suggested that density-dependent declines in snail growth, reproduction, and survival can be a direct result of food limitation (O'Keeffe 1985). Because snails in the present study were fed in excess at all densities, it is not likely that

food limitation can explain the negative density effect. However, as pointed out by Thomas et al. (1983), snails may increase their feeding rate as the amount of available food/snail increases, and it cannot be assumed that various uptake sites involved in growth are saturated simply because food remains uneaten. Also, even though snails may not consume 100% of the available food, the quality of the remaining food may decline with increased snail density, suggesting that at higher densities snails consume a higher proportion of lower quality material.

Williamson et al. (1976) reported a slowing of juvenile growth rates with increasing field densities of the land snail *Cepaea nemoralis* L., which they could not attribute to density-dependent limitation of food quality or quantity. They concluded that interactions between snails, either chemical or behavioral, were responsible for slowing juvenile growth rates in high-density populations. Brown & Carman (1994) found increasing levels of behavioral interference with increasing density in the pulmonate *Physella virgata,* which they suggested contributed to decreased grazing rates at higher densities. Their results suggested that neither dissolved metabolites nor substrate-borne cues could explain the density-dependent decline in grazing rate of this species.

Regardless of the specific mechanism, our results suggest that snail density is an important factor to control in rearing populations of *M. cornuarietis* in laboratory cultures.

Differences among experiments

We noted particularly large differences in fecundity on comparing Experiment 1 with Experiments 3 and 5, even among snails reared under near-identical temperature and light conditions. Comparing the 25°C, 12L:12D, 0.7 or 0.8 snails L^{-1} treatments among experiments shows that the number of eggs/female/week averaged only 28 in Experiment 1, whereas it was 71 in Experiment 3 and 87 in Experiment 5. The egg clutches produced by Experiment 1 snails were the largest of the three, but there were fewer of them produced per week, compared with Experiment 3 or 5 snails. Whereas Experiments 3 and 5 were conducted with laboratory-bred adults that were <1 year of age, Experiment 1 was conducted with field-collected adults of unknown age. It is possible that the latter individuals were much older and possibly approaching senescence when the experiment was conducted. The golden apple snail, *Ampullarius* sp., was reported to show a reduction in egg clutch number and size, as well as egg-hatchability, with increasing adult age (Lacanilao 1990). Estoy et al. (2002) found that the number of egg clutches/day and total egg clutch weight, but not number of eggs/clutch or egg weight, decreased with increasing age in the ampullarid *Pomacea canaliculata.*

There have been conflicting observations on seasonality of egg production patterns in cultured populations of *M. cornuarietis* reared under constant laboratory conditions. The University of Sussex (D. Thomas, unpubl. data) has observed no annual cycling of sexual performance in *M. cornuarietis* cultured under constant light and temperature (i.e., 12 L:12D, ~25°C). A slight depression of sexual activity during the winter months, but no sexual repose during spring and summer, was observed in cultures of *M. cornuarietis* of the Danish Bilharziasis Laboratory (H. Madsen, unpubl. data). In contrast, Oehlmann et al. (2000) reported that reproduction in cultures of *M. cornuarietis* maintained under somewhat lower temperatures (e.g., ~21°–23°C) is seasonally controlled with peak copulation and spawning activity occurring in November, December, and January, the other months of the year being the sexual repose phase (with reduced egg-laying activity). In the present study, egg production rates in Experiment 1, conducted from May 16 to June 17, were lower than in Experiment 3 (conducted from December 4, 2003 to January 15, 2004) and Experiment 5 (conducted from October 17 to November 12). From the present results it is not possible to determine whether this difference is due to effects of snail age or effects of season. However, experiments are ongoing to quantify reproductive output in laboratory-reared snails of known age during an entire year, to determine whether there is any seasonal and/or age-dependent variability in fecundity traits.

In the present study, egg-hatching success in Experiment 3 was about half that in Experiment 1 or Experiment 5 for snails reared under identical conditions (44% vs. 93%). Because of time constraints in the initiation of Experiment 3, it was not feasible to allow the same time for acclimation to the test conditions as in the other experiments. Therefore, the adult snails were collected from a stock culture that had a density exceeding 1.0 snails L^{-1}. Adult density was demonstrated to effect egg-hatchability at a density of 2.0 snails L^{-1} (Experiment 5). Thus, it is possible that a poorer condition of the adults at the start of Experiment 3 resulted in the low hatchability of the egg clutches from this experiment, compared with Experiments 1 and 5.

A large variability in clutch sizes in *M. cornuarietis* has been reported within and among published studies. For example, Robins (1971) found clutch sizes in

M. cornuarietis to vary by 22–191 eggs/clutch. Demian & Ibrahim (1971) observed egg clutches of laboratory-reared adults of *M. cornuarietis* to vary by 12–200 eggs/clutch, with most in the range 40–80 eggs/clutch. The World Health Organisation (1982) reported an average of 100 eggs/clutch. In our experiments, average clutch sizes ranged 65–140 eggs/clutch between experiments, with coefficients of variation within experimental treatments ranging 11–23%. There were no effects of temperature, photoperiod, or density on this trait, indicating that most of the variability was among replicates, within experimental treatments.

In conclusion, on the basis of the present results we conclude that rearing populations of *M. cornuarietis* at a temperature of 25°C, a photoperiod of 12L:12D, and a density of <0.8 snails L^{-1} (with lower densities for juvenile snails) should provide favorable husbandry conditions for maintaining multi-generation populations of this species in the laboratory.

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