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Effects of food type, feeding frequency, and temperature on juvenile survival and growth of *Marisa cornuarietis* (Mollusca: Gastropoda)

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Abstract. The present experiments are part of a larger study designed to investigate the influence of husbandry parameters on the life history of the ramshorn snail, *Marisa cornuarietis,* in order to identify suitable husbandry conditions for maintaining multi-generation populations in the laboratory for use in ecotoxicological testing. In this paper we focus on the effects of a combination of food types and feeding frequencies (i.e., the frequency with which the snails were offered food) on juvenile growth and survival at different temperatures. Offspring produced in the laboratory by wild specimens of *M. cornuarietis,* from Puerto Rico, were used to test the effects of three types of food (lettuce, alginate with fish food, alginate with snail mix) fed at three frequencies (given *ad libitum* on 4/4, 2/4, or 1/4 d) on juvenile survival and growth. The 4-d feeding regimens were repeated four times, giving a total of 16 d for the experiments. The experiments were conducted at two temperatures (22° and 25°C) under a 12 h light:12 h dark photoperiod. Juvenile growth rates increased with increasing feeding frequency for all food types. The most rapid growth rates occurred in the high-frequency lettuce treatments and the slowest growth rates in the low-frequency lettuce and alginate with snail mix treatments. Juvenile snails grew faster at 25° than at 22°C, and mortality was about twice as high at the lower temperature. Growth rates were used to provide a rough estimate of time to maturity, which was determined to take about twice as long at 22° than at 25°C. The results showed that lettuce is the best food if supplied in abundance, but effects on growth are very dependent on feeding frequency and temperature. We conclude that 25°C is a more appropriate temperature for maintaining populations than 22°C, that lettuce provides a suitable food source, and that food should be supplied continuously for husbandry and toxicity testing of populations of *M. cornuarietis*.

Additional key words: diet, ecotoxicological testing, husbandry, life history

The present experiments are part of a larger study designed to investigate the influence of husbandry parameters on the life history of *Marisa cornuarietis* Linnaeus 1758. 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 an earlier paper we focussed on the effects of photoperiod, temperature, and population density on adult fecundity and juvenile growth (Aufderheide et al. 2005). In the present article we focus on the effects of food quality and feeding frequency, at different temperatures, on juvenile growth and survival.

The giant ramshorn snail, *M. cornuarietis,* is a large (adults: 40–50 mm in shell diameter), sexually dimorphic, prosobranch belonging to the family Ampullariidae (Jobin 1970; Robins 1971; Demian & Ibrahim 1972). Populations of *M. cornuarietis* have a widespread distribution in freshwater habitats of the Caribbean, and Central and South America, and

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its natural distribution has been expanded by introductions for parasite control. It has been investigated as a potential weed control agent in both Puerto Rico and Florida (Ferguson & Palmer 1958; Radke et al. 1961), where it was introduced, apparently by aquarists, in the early 19th century (Robins 1971). Subsequent to its introduction to a Puerto Rican stream, the previously stable populations of the snail vector of schistosomiasis, *Biomphalaria glabrata,* declined, suggesting that *M. cornuarietis* could act as a biological control organism (e.g., Åkerlund 1969; Jobin 1970; Robins 1971). Later investigations supported this hypothesis (Hofkin et al. 1991), although there has been some debate as to the effectiveness of *M. cornuarietis* (e.g., Cedeño-León & Thomas 1983). For example, Cedeño-León & Thomas (1983) concluded that the predatorial behavior of *M. cornuarietis* was dependent on many factors (e.g., experience, maturity) and that the snail was effective as a predator at high densities only.

Individuals of *M. cornuarietis* are omnivorous but feed primarily on living and decaying aquatic plants (Ferguson & Palmer 1958; Robins 1971). Furthermore, *M. cornuarietis* has been found to exhibit preferences for certain food types (Grantham et al. 1993). The growth rate and reproductive fitness of snails may be affected by the energetic content, nutrient composition, and edibility (structure and texture) of their food, as well as food digestibility and absorption by the digestive tract (e.g., Thomas et al. 1983; McShane et al. 1994; Foster et al. 1999). The selection of an appropriate diet is therefore a key parameter in successful snail husbandry. In many experimental protocols (e.g., chronic ecotoxicity testing), it is essential to employ food sources that are nutritionally adequate (and so do not exacerbate the effects of toxic stressors) as well as easy to reproduce and control. As food is so important to snail performance, uncontrolled variability in dietary quality or quantity can potentially confound the effects of experimental treatments and/or make treatment-related differences more difficult to detect.

In the present study, a combination of food types and feeding frequencies was tested as growth and reproduction in *M. cornuarietis* are known to depend on both quality and quantity of food (Oehlmann et al. 2000). We compared three food types: fresh lettuce, dried fish food blended with alginate, and "snail mix" (an equal mixture of dried fish food, dried spinach, and dried baby cereal) blended with alginate. Previous work has shown that *M. cornuarietis* can feed and grow on fresh or partially decayed lettuce (Robins 1971). However, the disadvantage of this food type (i.e., lettuce) for experimental purposes is that it is relatively difficult to control in terms of both quantity and quality. Therefore we compared lettuce against two food sources that were prepared as alginate discs. This method of feeding snails has been used previously with success (Thomas et al. 1983: *B. glabrata*), and has the advantage that discs of a relatively precise quality and size can be reproduced. We chose dried fish food for its relatively high protein content and more balanced nutritional composition compared with lettuce. We chose the "snail mix" because we have previously achieved very high growth and fecundity in other snail species, and other aquatic invertebrates, fed this mixture, and we use it routinely in culturing invertebrates in the Roskilde University laboratory (Jacobsen et al. 1996).

We chose to manipulate food quantity by controlling the amount of time that snails were allowed to feed rather than by giving different masses of food, because we judged that the former method would be more practical than the latter for conducting long-term experiments and would give lower within-treatment variability in snail feeding activity.

We conducted feeding experiments at two different temperatures because food preferences of members of *M. cornuarietis* appear to be influenced by temperature, with high water temperatures correlating with a preference for high protein diets (Hofkin et al. 1991). The temperatures selected were 22°C and 25°C, as previous experiments indicated differences between these two temperatures in juvenile growth rates (Aufderheide et al. 2005).

Methods

Culture establishment and maintenance

Wild specimens of *Marisa cornuarietis* were collected from Lake Guajataca, Puerto Rico (for site details and collection methods, see Aufderheide et al. 2005). Approximately 150 snails (referred to as F_0 snails) were sent to Roskilde University by overnight express mail via Brixham Environmental Laboratory and established as stock cultures in the laboratory. Cultures were maintained at a temperature of 25°C, with a photoperiod of 12 h L:12 h D, in artificial freshwater (i.e., Standard Snail Water [SSW2]), which had the following composition in mmol L^{-1}—Ca: 2.0; Mg: 0.13; Na: 0.63; K: 0.09; Cl: 2.0; HCO_3: 0.67; SO_4: 0.13; NO_3: 0.05 (J.D. Thomas, unpubl. data, modified after Thomas et al. 1975)—and a food supply of fresh lettuce (*Lactuca sativa* var. *capitella*) and commercial fish pellets (Spectrum fish pellets, New Life International Inc., Homestead, FL).

All stock cultures were maintained under semi-static conditions, and water was renewed with freshly made SSW2 every 2–4 weeks depending on water quality measures. Fifty liters of water was added to each 63-L aquarium. An external Eheim Model 2231 three-stage canister filter (Eheim GmbH & Co. KG, Deizisan, Germany), a heater, and an air stone were connected to each aquarium. The filter produced a flow rate of 300 L h^{-1}. Each aquarium was illuminated by an overhead lamp. Light levels were measured at the water surface with an Aqualite Check Lux Meter (Trans Instruments Pte, Ltd, Petro Centre, Singapore) to be 4300–9300 lx. Also, two silicone tubes were placed vertically in each aquarium as oviposition sites. Twenty-five adult snails with an ~1:1 sex ratio were added to each aquarium (i.e., 0.5 snail L^{-1}). Snails were sexed based on external head/foot coloration characteristics (males: smooth head/foot; females: mottled head/foot). Waste products, uneaten food, and eggs were removed, and fresh food was supplied three times a week. The amount and type of food, as well as the cleaning/feeding interval, were estimated from observing the cultures over a period of 4 weeks. The final interval ensured that lettuce always was in abundance and that the snails were offered an additional protein source (i.e., fish pellets). In the stock aquaria, fish pellets were periodically replaced with carrots.

Survival of the field-collected adults was close to 100% at the beginning of the first experiment (~8 months after receipt), and snails began reproducing immediately. Eggs were collected from each stock aquarium and transferred to 400 mL glass containers, which were placed completely immersed in 63 L nursery aquaria that were maintained under the same conditions as described for the stock cultures above. Subsequent to hatching, the juvenile snails (i.e., F_1 snails) were transferred to new stock aquaria (without adults) until further use in an experiment. Lettuce was supplied at the same intervals as for the F_0 adults.

Experimental setup

Two sets of experiments were conducted: Experiment 1 at 25°C and Experiment 2 at 22°C. Food quality and feeding frequency were varied similarly in the two experiments, and the photoperiod was 12 h L:12 h D. Two replicate aquaria (200 L), each containing 100 L of artificial freshwater (i.e., SSW2), were employed per experiment, and the experimental duration was 16 d. An external Eheim Model 2231 three-stage canister filter, a heater, and two air stones (one in each side of the aquarium) were connected to each aquarium. Each aquarium contained 50 submerged glass beakers ($d = 10$ cm, $h = 4.5$ cm), each containing one juvenile snail and covered with a polyethylene net held in place by a glass ring. The snail density was 0.5 snail L^{-1}, as in the stock cultures. Five replicate snails from each of 10 different food treatments were placed in each aquarium.

Juvenile snails were collected from nursery aquaria on November 14, 2003 (Experiment 1) and December 3, 2003 (Experiment 2). One hundred juvenile snails were collected at the start of each experiment, gently blotted with tissue and weighed (wet weight of tissue plus shell), filmed using a digital video camera (Sony DCR-PC100E, Sony Corporation, Tokyo, Japan), and allocated randomly to the glass beakers. The snails were weighed and filmed again at experimental termination. Initial and final shell diameters were estimated from measurements of snail images on film using an image analysis program (i.e., SigmaScan Pro software vers. 5.0.0, SPSS, Chicago, IL). Repeated measurements ($n = 10$) of the same snails showed the standard deviation (SD) of shell diameter to be 1.2–0.4% of the mean for snails in the size range 1.4–3.6 cm. Individual specific growth rates were determined following Kaufmann (1981):

$$G = (\ln_{\text{final size}} - \ln_{\text{start size}})\ \text{d}^{-1}$$

where G is the growth rate, ln is the natural logarithm, and size was either snail wet weight (WW, mg) or snail shell diameter (D, mm). Mortality was also recorded during the course of the experiments, and dead snails were removed from aquaria. The initial average snail shell diameters were 5.1 ± 0.5 (SD) mm ($n = 100$) and 5.7 ± 0.6 mm ($n = 100$) in Experiments 1 and 2, respectively.

Food treatments

Three food types were employed, namely lettuce (L), alginate with fish food (F), and alginate with snail mix (M). Rinsed Danish head salad (*Lactuca sativa,* var. *capitata*) was used as the lettuce source, TetraMin fish food (TetraWerk, Melle, Germany) was used as the fish food, and a mixture (1:1:1 by weight) of TetraMin fish food, dried baby cereal (Babymin, Nutricia A/S, Allerød, Denmark), and dried organic spinach was used in the snail mix (see Table 1).

The concentrations of total protein (P), total carbon (C), and total nitrogen (N) were analyzed in all three food types. Briefly, total carbon (C) and total nitrogen (N) concentrations were measured on freeze-dried samples with a Carlo Erba element analyzer (EA 1110 CHNS, CE Instruments, Milan, Italy). Total protein content was measured using

Table 1. Food specifications from suppliers.

	Spectrum fish pellets	TetraMin Fish food	Baby cereal	Spinach
Protein (minimum)	34%	46%	12%	2%
Fat (minimum)	5%	8%	67%	1%
Fiber (maximum)	5%	2%	14%	
Ash (maximum)	9%	11%		
Moisture (maximum)	10%	6%		
Vitamin A	8000 IU kg^{-1}	37,600 IU kg^{-1}	2.9 mg kg^{-1}	
Vitamin B	450 IU kg^{-1}	2000 IU kg^{-1}	5.0 mg kg^{-1}	
Vitamin D			50 μg kg^{-1}	
Vitamin E	200 IU kg^{-1}	125 IU kg^{-1}		
Calcium			3.9 g kg^{-1}	
Carbohydrate				0.1%
L-ascorbyl-2-polyfosphate		265 mg kg^{-1}		
Energy			15,080 kj kg^{-1} 4420 kcal kg^{-1}	200 kcal kg^{-1}

the Danish standard 242 method (Kjeldahl method). Sulfuric acid, copper, iron, and sodium hydroxide were used to transform organically bound nitrogen into ammonium sulfate. Ammonia was transferred into a solution of boric acid by distillation, and finally the total amounts of organically bound nitrogen and ammonium nitrogen were determined by titration with hydrochloric acid.

Small portions (~0.7 g wet weight) of alginate foods were prepared according to methods modified after Thomas (1987); 1 g of sodium alginate (Alginic acid, Sigma-Aldrich, Copenhagen, Denmark, Cat. No. A-2033) was dissolved in 63 mL SSW2, and subsequently 10 g of either TetraMin fish food alone or the snail mix was added. After homogenization, the mixture was poured into a number of small glass petri dishes (*d*: 3 cm). Only the bottom of the petri dish was covered with the homogenate. Gelling was achieved by covering the homogenate with a 2% $CaCl_2$ solution for ~45 s, after which the $CaCl_2$ was removed from the gel. The food alginates were frozen (−20°C) until use.

Given the differences in composition among food types, it was not obvious how to achieve equal quantities of the three food types (i.e., they could be defined by total dry weight, protein, carbon, or some other measure). We concluded that time available for feeding was an acceptable and practical method. Therefore, food quantity was controlled by allowing snails to feed *ad libitum* for a pre-determined time period, after which excess food was removed. The three food types were offered for three durations defined as "low (L)," "middle (M)," and "high (H)." Food quantity was controlled such that snails in "low" treatments were allowed 1 d of feeding followed by 3 d without food, snails in "middle" treatments were fed for 2 d followed by 2 d without food, and snails in "high" treatments were fed continuously. Food was renewed every second day in "high"-frequency treatments to minimize potential problems associated with food decomposition. The glass beakers were wiped with tissue paper upon removal of food (i.e., low- and middle-frequency treatments) and at each food renewal period before the addition of new food (i.e., low-, middle-, and high-frequency treatments) to remove waste products. The 4-d feeding regimens were repeated four times, giving a total of 16 d for each experiment.

An additional group of snails ($n = 5$) called the N-group (i.e., the non-fed group) was added to replicate aquaria. As snails from the different frequency treatments were kept in the same aquaria, and we expected some release of dissolved organic mater (DOM) from the food sources, we included the non-fed treatment to ensure that snails were not able to survive and grow on DOM alone, which could affect the assessment of diet upon snail growth. The N-group was placed by the water outflow where the concentrations of DOM were expected to be highest. Thus, a total of 10 food treatments were employed in each aquarium and a total of 10 snails per food treatment (i.e., five replicates per food treatment per aquarium).

Water chemistry and temperature

Water chemistry and temperature were followed during the experiments because (1) it has been recommended to measure and control the concentration

of calcium in the presence of individuals of *M. cornuarietis* due to their high calcium uptake ($\geq 0.4\,mg\,snail\,h^{-1}$; Meier-Brook 1978; WHO 1982); (2) ammonia toxicity is an important challenge with intensive cultures of organisms with high waste production, especially because ammonia has been recognized as a problematic waste product of snails that may give severe negative effects on animal performance (Russo 1985); and (3) to ensure that the temperature was maintained within the range of $\pm 1°C$ of the desired temperature.

Water chemistry (i.e., pH, ammonium, and calcium) and temperature were measured regularly in both aquaria in Experiments 1 and 2. Ammonium was measured by a photometric test kit (Merck, Darmstadt, Germany, Cat. No. 1.14752.001; measuring range: 0.05–3.00 $mg\,L^{-1}$; detection limit: $\pm 0.08\,mg\,L^{-1}$), and pH and temperature were measured using a pH/temperature electrode connected to an IKS Aquastar computer (IKS Computer Systeme GmbH, Karlsbad, Germany). Calcium ion concentration was measured by titration (i.e., ethylenediaminetetraacetic acid [EDTA] titrimetric method).

Statistics

Student's t-tests were performed to test for differences between replicate aquaria within food treatments using either pooled or separate variances as appropriate (i.e., the latter was employed when variances differed between the groups). Because the replicate aquaria did not differ significantly in snail growth rate within each of the two experiments, the aquaria were pooled giving a total of $n = 10$ snails per treatment. Differences in food composition (i.e., inorganic carbon, total nitrogen, and total protein) among the three food types were tested by analysis of variance (ANOVA), and Tukey HSD pairwise comparisons were used to test for differences among pairs in cases in which ANOVA indicated a significant overall effect. Three-way ANOVA (SYSTAT vers. 10.0, General Linear Model Analyses, Systat Software, Inc., Richmond, CA) was used to test for interaction effects among food type, feeding frequency, and temperature on juvenile growth rate (i.e., snail wet weight or snail shell diameter). Snails that died as a result of the treatment were assigned a growth rate of zero as recommended by Snedecor & Cochran (1967:317). The number of dead snails is presented in Table 4. Data are presented as mean ± 1 SD. A significance level of $p \leq 0.05$ is used throughout. p-Values between 0.05 and 0.1 were defined as marginally significant.

Table 2. Mean ($\pm$SD) temperature and pH in aquaria 1 and 2 of each food experiment. *N*, number of measurements.

Experiment	Aquarium	Temperature (°C)	*N*	pH	*N*
1	1	25.0 (0.1)	4	7.96 (0.37)	4
	2	25.1 (0.1)	4	7.95 (0.41)	4
2	1	21.9 (0.3)	4	7.77 (0.09)	6
	2	21.9 (0.4)	4	7.82 (0.08)	6

Results

Water chemistry and temperature

Temperature and pH did not differ between replicate aquaria in either experiment (Table 2). The temperature was maintained at $25.0 \pm 0.1°C$ ($n = 8$) in Experiment 1 and at $21.9 \pm 0.4°C$ ($n = 8$) in Experiment 2. pH was on average 7.96 ± 0.37 ($n = 8$) in Experiment 1 and 7.80 ± 0.09 ($n = 12$) in Experiment 2. Ammonium concentration was below the detection limit and thus the level of concern ($0.2\,mg\,NH_4\,L^{-1}$) in both experiments, and calcium concentration (Ca^{2+}) was constant throughout both experiments (Experiment 1: 110.0 ± 5.60, $n = 4$; Experiment 2: $108.3 \pm 10.5\,mg\,Ca^{2+}\,L^{-1}$, $n = 2$).

Food content

The protein content per gram dry weight food differed significantly among lettuce, alginate with fish food, and alginate with snail mix (ANOVA: $p = 0.013$). Tukey's HSD pairwise test showed that the protein content was significantly higher in lettuce than in alginate with snail mix (Tukey: $p = 0.011$), and marginally higher than in alginate with fish food (Tukey: $p = 0.090$). There was no significant difference in protein content between alginate with fish food and alginate with snail mix (Tukey: $p = 0.238$) (Table 3). Likewise, the lettuce contained a higher percent of total nitrogen than the two alginate foods, whereas the percent of total carbon content was at about the same level in the three food types (Table 3). The composition of the lettuce is comparable to results published by the Danish Ministry of Food, Agriculture and Fisheries (http://www.foodcomp.dk/fvdb_details.asp?FoodId=0239) as of July 25, 2005: per 100 g wet weight *Lactuca sativa* L. var. *capitata* L, water content = 95.2 g, total protein = 1–2 g, total nitrogen = 0.160–0.320 g, and total carbon = 1.3–5.5 g.

Table 3. Mean (±SD) measured percent (of dry weight) of total inorganic carbon (C), total nitrogen (N), and total protein in the three foods: lettuce, alginate with fish food, and alginate with snail mix. *n*, number of replicates.

Food type	Total C (%)	Total N (%)	*n*	Total protein (mg g dry wt^{-1})	*n*
Lettuce	36.8 (0.07)	5.79 (0.02)	5	402.5 (86.0)	3
Alginate with fish food	39.9 (0.49)	3.33 (0.05)	5	279.4 (48.9)	3
Alginate with snail mix	36.9 (0.18)	2.94 (0.06)	5	192.6 (17.2)	3
Alginate				6.57 (0.90)	2

Mortality

The highest mortality was observed in the N-group, in which 100% of the juvenile snails died in both food experiments within the first 9 d.

Mortality was evenly distributed among all food treatments at 22°C, whereas mortality was mainly restricted to the low lettuce treatment at 25°C (Table 4). Mortality was 100% in both aquaria in the low lettuce treatment at 25°C compared with 80% and 40% in aquariums 1 and 2, respectively, at 22°C (see Table 4). However, the total mortality across all treatments was almost twice as high at 22°C (Experiment 2: 46 dead snails) than at 25°C (Experiment 1: 27 dead snails).

Effects on juvenile growth

There was a significant interaction effect of temperature, feeding frequency, and food type on juvenile growth based on both snail wet weight and snail shell diameter (Tables 5 and 6), indicating that the response of individuals of *Marisa cornuarietis* to each of these factors is dependent on levels of the other two factors.

Within each temperature (Fig. 1), juvenile growth rates increased with increasing feeding frequency regardless of food type, and the increase was fastest (i.e., the slope was steeper) for snails fed lettuce than for snails fed either of the alginate foods. A graphical comparison among slopes on each graph (Fig. 1) shows that at the low food frequency, the highest mean growth is observed for snails fed alginate with fish food, whereas at the high feeding frequency, snails fed lettuce grew the fastest. Furthermore, the slopes appear parallel between the two alginate foods, but a higher growth was observed in snails fed alginate with fish food compared with alginate with snail mix. In both temperature experiments, the highest mean growth rates were observed in the high lettuce (HL) treatments (25°C: $GR_{WW} = 9.4 \pm 1.7\%\ d^{-1}$; $GR_D = 3.1 \pm 0.5\%\ d^{-1}$; 22°C: $GR_{WW} = 5.7 \pm 1.3\%\ d^{-1}$; $GR_D = 1.6 \pm 0.4\%\ d^{-1}$) and the lowest mean growth rates in the low lettuce (LL) treatments (25°C: $GR_{WW} = 0 \pm 0\%\ d^{-1}$; $GR_D = 0 \pm 0$; 22°C: $GR_{WW} = 1.2 \pm 0.2\%\ d^{-1}$; GR_D: $0.3 \pm 0.2\%\ d^{-1}$) and alginate with snail mix (LM) treatments (25°C: $GR_{WW} = 2.8 \pm 0.5\%\ d^{-1}$; $GR_D = 0.9 \pm 0.3\%\ d^{-1}$; 22°C: $GR_{WW} = 1.4 \pm 1.3\%\ d^{-1}$; $GR_D = 0.2 \pm 0.2\%\ d^{-1}$) (Fig. 1). The results show that lettuce

Table 4. Percent observed snail mortality. There were five individual snails per food treatment per aquarium (i.e., 1 snail = 20%).

Food type	Feeding frequency	Abbreviation	Experiment 1 (25°C)		Experiment 2 (22°C)	
			Aquarium 1	Aquarium 2	Aquarium 1	Aquarium 2
Lettuce	Low	LL	100	100	80	40
	Medium	ML	0	40	20	80
	High	HL	0	20	0	20
Alginate with fish food	Low	LF	0	0	80	40
	Medium	MF	0	0	40	40
	High	HF	0	20	0	40
Alginate with snail mix	Low	LM	0	20	80	60
	Medium	MM	0	0	0	40
	High	HM	20	20	20	20
No food		N	100	100	100	100
Total mortality			27		46	

Table 5. Three-way ANOVA. Categorical variables: temperature, food type, feeding frequency. Dependent variable: juvenile wet weight based growth rate. One case deleted due to missing data.

	Sum of squares	df	Mean square	F-ratio	p-value
Temperature	0.019	1	0.019	72.040	<0.001
Food type	0.003	2	0.002	5.667	0.004
Feeding frequency	0.036	2	0.018	66.318	<0.001
Temperature × food type	0.000	2	0.000	0.914	0.403
Temperature × feeding frequency	0.001	2	0.001	1.998	0.139
Food type × feeding frequency	0.015	4	0.004	14.203	<0.001
Temperature × food type × feeding frequency	0.003	4	0.001	3.056	0.018
Error	0.043	161	0.000		

was the best of the tested foods if supplied continuously, but the worst of the tested foods if supplied in limited quantities. Within each food type, growth rates were higher at 25°C than at 22°C regardless of food treatment (Fig. 2). The figures indicate that temperature and feeding frequency interacted to determine snail growth (based on both wet weight and diameter) in snails fed lettuce, whereas the interaction effect was less obvious for snails fed the two alginate diets.

Discussion

Effects of starvation

There seems to be some disagreement as to how well individuals of *Marisa cornuarietis* are able to withstand starvation. Hunt (1958) claimed that this species was able to withstand the effects of starvation very well, whereas Åkerlund (1969) found that they had a poor capacity to survive starvation. Åkerlund (1969) suggested that the snails quickly use up their nutritional stores. During fasting, individuals deplete their carbohydrate stores twice as fast as their protein stores, whereas the decrease in lipid appears to be relatively minor (Horne 1979). In the present study, juveniles of *M. cornuarietis* were unable to survive for >9 d in the absence of food. Although lettuce gave the best growth rates when provided at a high frequency, mortality was extensive if snails were fed lettuce at a low frequency, and this effect was exacerbated at the higher temperature.

Our results suggest that fresh lettuce may be more readily digestible and have a higher nutritional value than the two alginate foods. The latter is supported by Thomas (1987), who suggested that calcium alginate in itself is relatively poor in nutrients (being largely carbohydrate and thus relatively low in nitrogen) because in his study snails fed pure calcium alginate gained little weight or even showed negative growth. However, the presence of alginate was beneficial in the low-frequency feeding regimen, which is consistent with reliance of members of *M. cornuarietis* on carbohydrate reserves during starvation (Horne 1979).

Effects of feeding frequency and food type

In its natural habitat, *M. cornuarietis* is an opportunistic species feeding on a broad range of food items, including other snails, various plant materials, and debris. Laboratory-raised individuals of *M. cornuarietis* have been fed diets ranging from cucumber to synthetic diets (e.g., commercially available fish food)

Table 6. Three-way ANOVA. Categorical variables: temperature, food type, feeding frequency. Dependent variable: juvenile shell diameter-based growth rate.

	Sum of squares	df	Mean square	F-ratio	p-value
Temperature	0.003	1	0.003	113.364	<0.001
Food type	0.000	2	0.000	7.635	0.001
Feeding frequency	0.003	2	0.002	61.056	<0.001
Temperature × food type	0.000	2	0.000	1.850	0.160
Temperature × feeding frequence	0.000	2	0.000	4.185	0.017
Food type × feeding frequency	0.002	4	0.000	16.224	<0.001
Temperature × food type × feeding frequency	0.000	4	0.000	3.084	0.018
Error	0.004	162	0.000		

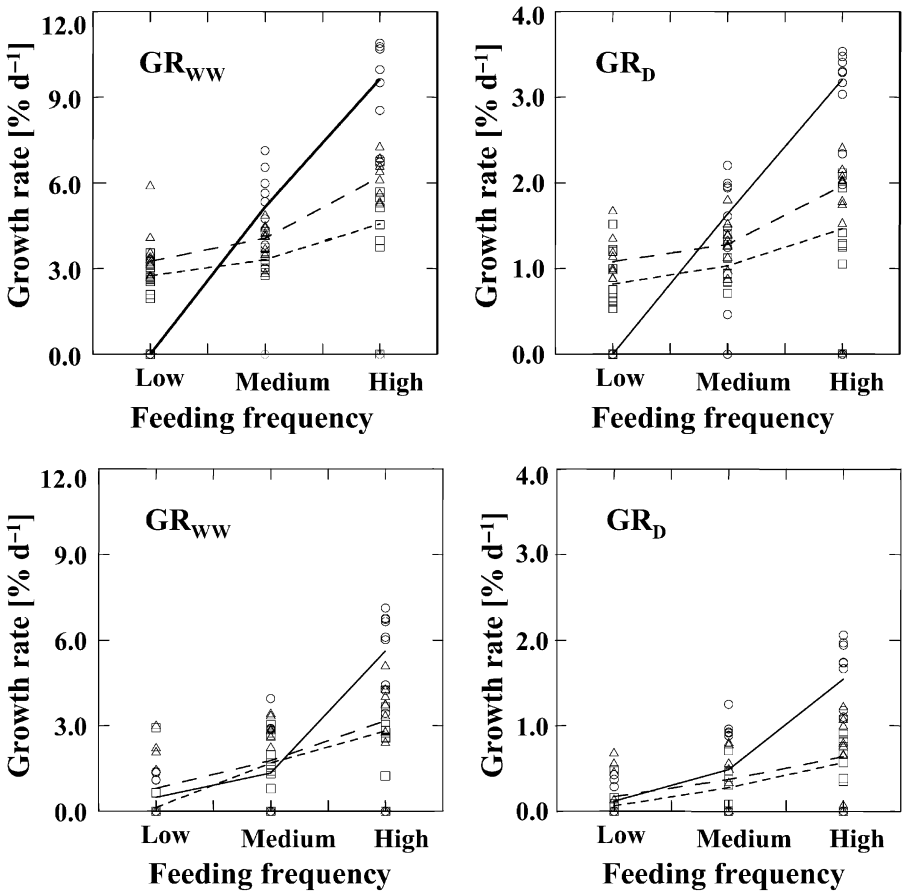

Fig. 1. Individual growth rates for juvenile snails in Experiment 1 (top; 25°C) and Experiment 2 (bottom; 22°C) given a diet of lettuce (circle, solid line), alginate with fish food (triangle, dashed line), or alginate with snail mix (square, dotted line) at low, middle, or high feeding frequencies. No data exist for the low lettuce treatment in Experiment 1 due to 100% mortality. In both cases, the left graph describes growth rate based on snail wet weight (GR_{WW}), and the right graph shows growth rate based on snail shell diameter (GR_D). Lines are locally weighted scatterplot smooths through the data.

and a wide range of aquatic weeds (Robins 1971; Thomas et al. 1983; Thomas 1987; Grantham et al. 1993; Oehlmann et al. 2000).

In the present study, juvenile snails fed at the high feeding frequency showed lower growth rates when fed alginate with either snail mix or fish food compared with juveniles fed lettuce. Lettuce has a higher nitrogen and protein content compared with both alginate foods. Dorgelo et al. (1995) found higher growth rates in a freshwater deposit-feeding snail, *Potamopyrgus jenkinsi* Smith 1889, fed an excess of lettuce (*Lactuca sativa*) compared with a protein source (lamb heart). However, the highest growth rates were found in snails fed both lettuce and lamb heart (Dorgelo et al. 1995). At least in the marine environment, nitrogen is generally considered the limiting nutrient not only for plant production but also for herbivores consuming plant foods (Barile et al. 2004). Increasing evidence suggests that invertebrate grazers select for macroalgae with higher nitrogen contents (e.g., Hauxwell et al. 1998; Yates & Peckol 1993: both cited in Barile et al. 2004) and that the higher nitrogen content is positively related to growth rate (Barile et al. 2004). The snail *Haliotis rubra* Leach 1814 was found to prefer fresh macroalgae (seven species tested) over artificial food (agar), probably because the macroalgae were less tough than the artificial food pellets, and thus easier to break down mechanically. This result may be related to a lack of hardened teeth in the radula of *H. rubra* (McShane et al. 1994). These authors also found that the ingestion rate of agar pellets (i.e., the artificial food) decreased with increasing concentration of agar and thus higher resistance to penetration by the snail. It was also found that the feeding rate of *H. rubra* was positively correlated with the nitrogen content of its food (McShane et al. 1994).

Members of *M. cornuarietis* have been reported to have a preference for high-protein diets (Hofkin et al. 1991). Thus, the higher nitrogen and protein contents in the lettuce than in the two alginate mixtures may have resulted in higher feeding rates. Thus, the higher nitrogen content in the lettuce, compared with the two alginate foods, a higher feeding rate on lettuce, or both may explain the higher growth rates in *M. cornuarietis* in the high-frequency lettuce treatment.

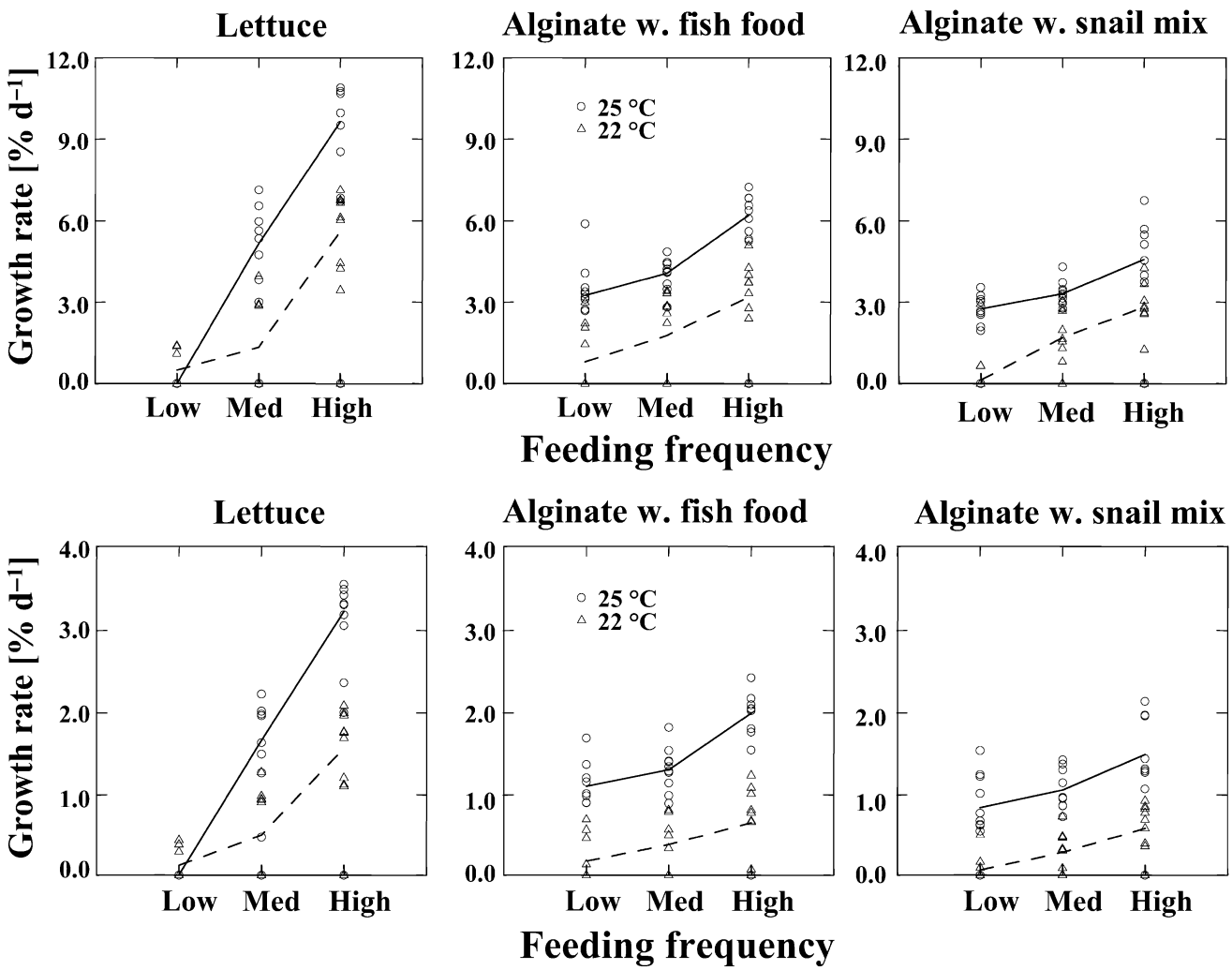

Fig. 2. Individual growth rates for juvenile snails in Experiment 1 (circle, solid line; 25°C) and Experiment 2 (triangle, dashed line; 22°C) given a diet of lettuce (left graph), alginate with fish food (middle graph), or alginate with snail mix (right graph) at low, middle, or high feeding frequencies. No data exist for the low lettuce treatment in Experiment 1 due to a mortality of 100%. Growth rates are based on either snail wet weight (GR_{WW}) (top graphs) or snail shell diameter (GR_D) (bottom graphs). Lines are locally weighted scatterplot smooths through the data.

Effect of temperature

Juvenile snails grew faster at 25°C than at 22°C regardless of what they were fed, and the total juvenile mortality, for all food treatments combined, was almost twice as high at a water temperature of 22°C compared with 25°C. It is widely known that metabolism increases with increasing temperature. This is supported by Åkerlund (1969), who studied the effect of temperature (20°–35°C) and snail size on respiratory rate in *M. cornuarietis*. He found that respiration increased with increasing temperature and was higher in small (10 mg dry weight: range 0.6–4.0 mg O_2 g dw h^{-1}) than in large (700 mg dry weight, range 0.25–0.44 mg O_2 g dw h^{-1}) snails. Consistent with the present results, Aufderheide et al. (2005) found that the growth of juveniles of *M. cornuarietis* was dependent on water temperature, such that snails grew faster at 28°C than at 25°C, and at 25°C than at 22°C.

The effect of temperature and feeding conditions (i.e., food type, feeding frequency) on time to morphological maturity in *M. cornuarietis* can be roughly estimated. The available information is (1) the average shell diameter at experimental start was 5 mm (we estimate that juveniles were 1–2 months of age), (2) adults of *M. cornuarietis* are morphologically mature at a shell diameter of ~20 mm (Aufderheide et al. 2005), and (3) the shell-diameter-based growth rates (G_D) of juveniles of *M. cornuarietis* under different

Table 7. Estimated time (d) to morphological maturity, calculated from the start of the experiment, at a water temperature of 25°C and 22°C using the equation $GR_D = (\ln_{\text{final size}} \ln_{\text{start size}})\ d^{-1}$ (Kaufmann 1981), where start size = initial mean shell diameter (5 mm). Final size = shell diameter at morphological maturity (20 mm; Aufderheide et al. 2005). GR_D, Diameter-based growth rate from Experiments 1 and 2.

Food type	Food level	Experiment 1 (25°C)	Experiment 2 (22°C)	Difference (22°C/25°C)
Lettuce	Low	—	462	–
	Medium	87	139	1.6
	High	45	87	1.9
Alginate with fish food	Low	126	277	2.2
	Medium	107	231	2.2
	High	69	173	2.5
Alginate with snail mix	Low	154	693	4.5
	Medium	139	347	2.5
	High	92	198	2.2

feeding conditions are known (Fig. 1). By using this information and rearranging the Kaufmann equation for days (days = [ln {$\text{diameter}_{\text{morphological maturity}}$} − ln {$\text{diameter}_{\text{experimental start}}$}]/$G_D$), we can calculate the time it will take juvenile snails, under the different feeding conditions, to reach maturity from the start size of 5 mm. According to this calculation, the relative time to morphological maturity in *M. cornuarietis* would be reached about twice as fast at 25°C than at 22°C regardless of food type and feeding frequency (Table 7). Furthermore, morphological maturity seems to be reached fastest by continuously feeding the juveniles lettuce in abundance as the sole food source. Aufderheide et al. (2005) also found that time to morphological maturity was delayed at decreasing temperature and with increasing snail density.

The calculation of time to morphological maturity assumes that it is the size and not the age that determines when snails reach maturity. However, the effect of food level on size and age at first reproduction, as well as on reproductive traits, was examined in the apple snail *Pomacea canaliculata* Lamarck 1819 (Estoy et al. 2002a,b). It was found that food level affected snail size but not age at first copulation in males. In contrast, food level affected both female size and age at first copulation and spawning (Estoy et al. 2002b). Although the frequency of copulation was not affected by food level in either males or females, the spawn production (weight) and number of eggs per spawn were higher for those females fed the highest food level compared with those fed the lowest food level (Estoy et al. 2002a).

Feeding recommendations for snail cultures

Nutrient composition and digestibility influence snail performance (e.g., growth rate and reproductive output), and choosing an appropriate diet is therefore crucial for maintaining successful snail stock cultures in the laboratory and for designing toxicity tests. We found that the food giving the highest growth rate was lettuce supplied at the high frequency, and that both alginate foods resulted in lower growth rates than the lettuce at all feeding frequencies. In this regard, lettuce seems to be the best choice.

Our results suggest that for effective husbandry (1) populations of *M. cornuarietis* should have a continuous supply of food, (2) fresh lettuce (e.g., *L. sativa* var. *capitella*) provides an appropriate food source for achieving high juvenile survival and rapid rates of juvenile growth, and (3) a husbandry temperature of 25°C is preferable to 22°C because it allows juveniles to mature and reproduce more quickly. Together with the results from Aufderheide et al. (2005), which showed better performance of adults of *M. cornuarietis* at 25°C than at 22°C, our results indicate that the population growth rates of *M. cornuarietis* will be higher at the former temperature, which is also consistent with their natural field distribution. This has advantages for both husbandry (allowing more generations of snails to be produced more quickly) and toxicity testing (reducing the duration and hence the effort of tests).

Fresh lettuce is in principle more difficult to control compared with the alginate mixtures and could therefore lead to increases in variability in snail performance. However, we detected no difference in among-snail variability in growth rates in lettuce compared with alginate treatments.

For toxicity testing, the presence of any type of food source in the test systems can potentially influence the rate of degradation and thus bioavailability of added toxicants. It is therefore essential that such tests include multiple measurements of the actual toxicant concentrations to which organisms are

exposed and that necessary steps are taken to keep exposure concentrations as constant as possible throughout the duration of the test.

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References

Åkerlund G 1969. Oxygen consumption of the ampullariid snail *Marisa cornuarietis* L. in relation to body weight and temperature. Oikos 20: 529–533.

Aufderheide J, Warbritton R, Pounds N, File-Emperador S, Staples C, Caspers N, & Forbes VE 2005. The effects of husbandry parameters on the life-history traits of *Marisa cornuarietis:* effects of temperature, photoperiod and population density. Invertebr. Biol. 125: 9–20.

Barile PJ, Lapointe BE, & Capo TR 2004. Dietary nitrogen availability in macroalgae enhances growth of the sea hare *Aplysia californica* (Opisthobranchia: Anaspidea). J. Exp. Mar. Biol. Ecol. 303: 65–78.

Cedeño-León A & Thomas JD 1983. The predatory behaviour of *Marisa cornuarietis* on eggs and neonates of *Biomphalaria glabrata,* the snail host of *Schistosoma mansoni*. Malacologia 24: 289–297.

Demian ES & Ibrahim AM 1972. Sexual dimorphism and sex ratio in the snail *Marisa cornuarietus* (L). Zool. Soc. Egypt Bull. 24: 52–63.

Dorgelo J, Meester H, & van Velzen C 1995. Effects of diet and heavy metals on growth rate and fertility in the deposit-feeding snail *Potamopyrgus jenkinsi* (Smith). Hydrobiologia 316: 199–210.

Estoy GF, Yusa Y, Wada T, Sakurai H, & Tsuchida K 2002a. Effects of food availability and age on the reproductive effort of the apple snail, *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae). Appl. Entomol. Zool. 37: 543–550.

——— 2002b. Size and age at first copulation and spawning of the apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae). Appl. Entomol. Zool. 37: 199–205.

Ferguson FF & Palmer JR 1958. Biological notes on *Marisa cornuarietis,* a predator of *Australorbis glabratus,* the snail intermediate host of schistosomiasis in Puerto Rico. Am. J. Trop. Med. Hyg. 7: 640–642.

Foster GG, Hodgson AN, & Balarin M 1999. Effect of diet on growth rate and reproductive fitness of *Turbo sarmaticus* (Mollusca: Vegigastropoda: Turbinidae). Mar. Biol. 134: 307–315.

Grantham ÖK, Moorhead DL, & Willig MR 1993. Feeding preference of an aquatic gastropod, *Marisa cornuarietis,* effects of pre-exposure. J. N. Am. Benthol. Soc. 12: 431–437.

Hofkin BV, Stryker GA, Koech DK, & Loker ES 1991. Consumption of *Biomphalaria glabrata* egg masses and juveniles by the ampullareed snails, *Pila ovata, Lanistes carinatus* and *Marisa cornuarietis*. Acta Tropica 49: 37–44.

Horne FR 1979. Comparative aspects of estivating metabolism in the gastropod, *Marisa*. Comp. Biochem. Physiol. 64A: 309–311.

Hunt BP 1958. Introduction of *Marisa* into Florida. The Nautilus (Philadelphia) 72: 53–55.

Jacobsen R, Forbes VE, & Skovgaard O 1996. Genetic population structure of the prosobranch snail *Potamopyrgus antipodarum* (Gray) in Denmark using PCR-RAPD fingerprints. Proc. Roy. Soc. Lond. B Biol. 263: 1065–1070.

Jobin WR 1970. Population dynamics of aquatic snails in three farm ponds of Puerto Rico. Am. J. Trop. Med. Hyg. 119: 1038–1048.

Kaufmann KW 1981. Fitting and using growth curves. Oecologia 46: 293–299.

McShane PE, Gorfine HK, & Knuckey IA 1994. Factors influencing food selection in the abalone *Haliotis rubra* (Mollusca: Gastropoda). J. Exp. Mar. Biol. Ecol. 176: 27–39.

Meier-Brook C 1978. Calcium uptake by *Marisa cornuarietis* (Gastropoda: Ampulariidae), a predator of schistosome-bearing snails. Arch. Hydrobiol. 82: 449–464.

Oehlmann J, Schulte-Oehlmann U, Tillmann M, & Markert B 2000. Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part I: Bisphenol A and octylphenol as xeno-extrogens. Ecotoxicology 9: 383–397.

Radke MG, Ritchie LS, & Ferguson FF 1961. Demonstrated control of *Australorbis glabatus* by *Marisa cornuarietis* under field conditions in Puerto Rico. Am. J. Trop. Med. Hyg. 10: 370–373.

Robins CH 1971. Ecology of the introduced snail, *Marisa cornuarietis* (Ampullariidae) in Dade County, Florida. Biologist 53: 136–152.

Russo RC 1985. Ammonia, nitrite and nitrate. In: Fundamentals of Aquatic Toxicology. Rand GM & Petrocelli SR, eds., pp. 455–471. Hemisphere, New York.

Snedecor GW & Cochran WG 1967. Statistical Methods, 6th Edition. The Iowa State University Press, Ames, IA. 317 pp.

Thomas JD 1987. An evaluation of the interactions between fresh-water pulmonate snail hosts of humans schistosomes and macrophytes. Phil. Trans. R. Soc. Lond. B 315: 75–125.

Thomas JD, Lough AS, & Lodge RW 1975. The chemical ecology of *Biomphalaria glabrata* (Say), the snail host of *Schistosoma mansoni* Sambon: the search for factors in media conditioned by snails which inhibit their growth and reproduction. J. Appl. Ecol. 12: 421–436.

Thomas JD, Grealy B, & Fennell CF 1983. The effects of varying the quantity and quality of various plants on feeding and growth of *Biomphalaria glabrata* (Gastropoda). Oikos 41: 77–90.

WHO 1982. Data sheet on the biological control agent *Marisa cornuarietis* (Linn.). Information document produced by the WHO Division of Vector Biology and Control 11 pp.