



Influence of water temperature on the activity of the freshwater snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae) at its southernmost limit (Southern Pampas, Argentina)

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ABSTRACT

1. The effect of temperature on the activity of the freshwater snail *Pomacea canaliculata* was investigated through field surveys and laboratory trials.
2. During winter most snails in the field were inactive but not in a deep lethargic state; the temperature at which half of the snails were active was 13–15 °C.
3. The time spent active and feeding increased with temperature between 10 and 30 °C, exposure time being unimportant except in foul water at 35 °C, while time spent crawling remained constant above 10 °C.
4. Activity decreased above 30 °C but no heat coma was observed with temperatures raised to 36.2 °C.
5. Under fluctuating temperatures, the rate of change in the percentage of active snails during cooling was higher than during warming, whereas the temperature at which half of the snails were active was lower.

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1. Introduction

Pomacea canaliculata (Lamarck 1822) is a freshwater snail (commonly known as the golden apple snail) native to South America. It was introduced to several Asian countries, North America and Hawaii, where it established and became a serious pest of aquatic crops (Cowie et al., 2006; Rawlings et al., 2007; Hayes et al., 2008; Karatayev et al., 2009). It has recently been recognized that *P. canaliculata* has facilitated the expansion of the parasite *Angiostrongylus cantonensis* in mainland China, probably leading to the emergence of angiostrongyliasis, a previously rare disease (Lv et al., 2006, 2009). Furthermore, apple snails are able to promote major ecosystem changes in natural wetlands resulting from the total depletion of macrophytes (Carlsson et al., 2004). *P. canaliculata* is included in the International Union for the Conservation of Nature's list of the 100 of the World's Worst Invaders, being the only freshwater snail in that list (Lowe et al., 2000). The ongoing colonization of new habitats and the expansion of its potential habitat caused by global warming could increase the impacts of *P. canaliculata* (Lv et al., 2006), thus

making it crucial to increase the understanding of its thermal biology.

The natural range of *P. canaliculata* extends from La Plata river basin southwards to the Tandilia and Ventania mountains and reaches its southernmost limit in the Encadenadas del Oeste basin (Southern Pampas, 37°S), making it the southernmost apple snail (Ampullariidae) species in the world (Martín et al., 2001). Water temperature has an overwhelming influence on freshwater snails' behavior in general (Costil and Bailey, 1998) and on most aspects of the biology of *P. canaliculata* in particular, including growth rate, aerial respiration, reproduction and survival (e.g. Estebenet and Cazzaniga, 1992; Estebenet and Martín, 2002; Ito, 2002; Albrecht et al., 2005; Matsukura and Wada, 2007; Seuffert and Martín, 2009a). During the coldest months in temperate climates the snails remain inactive and overwintering buried in muddy bottoms has been frequently reported (Hylton-Scott, 1958; Bachmann, 1960; Damborenea, 1996); however, the physiological state and the mechanism behind this phenomenon are not clear (Estebenet and Martín, 2002; Seuffert and Martín, 2009a).

Some studies, reviewed by Cowie (2002), reported the temperatures tolerated by different species of Neotropical ampullariids. Lethal limits reported for *P. canaliculata* are rather variable, probably due to different experimental conditions (exposure time, aquatic or aerial conditions, etc.) or uncertain

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species identity (e.g. Mochida, 1988; Matsukura and Wada, 2007), with tolerance to temperatures between 4 and 35 °C being reported by most authors. For the closely related *Pomacea insularum* (Rawlings et al., 2007), Ramakrishnan (2007) determined that the lethal chronic limits are 15.2 and 36.6 °C. However, little information exists about the effect of temperature on apple snail activity: an outdoor investigation of the Florida apple snail, *Pomacea paludosa* (Stevens et al., 2002), and laboratory trials performed within a narrow temperature range (20 to 32 °C) with an aquarium strain of *P. canaliculata* (Heiler et al., 2008). Furthermore, the temperatures to which *P. canaliculata* is normally exposed in its natural habitat have not been systematically explored.

The aims of this work were to determine the effect of temperature and exposure time on the activity of *P. canaliculata* from Southern Pampas in the laboratory and to investigate temporal patterns of activity in two of the southernmost natural populations of this snail.

2. Materials and methods

2.1. Temporal patterns of activity in natural populations

Temporal patterns of activity of *P. canaliculata* were investigated in natural populations in two slow flowing watercourses originating on the northern slope of the Ventania mountains (Buenos Aires Province, Argentina) that belong to the Encadenadas del Oeste basin and have been the subject of previous studies (Martín and Estebenet, 2002; Estebenet and Martín, 2003). The climate of the region is temperate, with a mean annual temperature and amplitude of 13.8 and 15.1 °C, respectively; mean annual rainfall is 733 mm, mainly concentrated in fall and spring, with marked fluctuations between dry and wet years (Scian and Donnari, 1997).

The Curamalal Grande stream is a permanent watercourse 91 km long. The stream section investigated (37°14'31"S, 62°08'04"W), is 25 m long, 3–8 m wide and with a mean depth less than 75 cm, though it can reach up to 1.5 m. Cochicó Chico stream is a short creek (20 km) with intermittent flow along most of its course. The reach investigated (36°57'37"S, 62°16'57"W) is 5–7 m wide and with a mean depth near 20 cm. The bottom is mainly mud in both streams but limestone beds and stones are also present.

The survey lasted three years and the two sites were visited every two to three weeks in summer and four to five weeks in winter, from July 5th 2004 to April 8th 2006 for the Curamalal Grande and from October 26th 2005 to 14th April 2007 for the Cochicó Chico. On each occasion, two people searched visually and by touch for snails among the submerged vegetation, under stones or buried in the substrate along the reach. All snails found were immediately inspected *in situ* and categorized (Fig. 1) as: FR (fully retracted) with the operculum deeper inside the shell than the plane of the aperture, PR (partially retracted) with the operculum in the plane of the aperture, PE (partially extended) with the foot and operculum up to 45° from the plane of the aperture and FE (fully extended) with the foot extended and the operculum forming an angle greater than 45° with the plane of the aperture. The proportion of active snails (P) was calculated for each sampling date as PE+FE relative to the total number of snails collected.

On each sampling date, water temperature was recorded upstream of the reach before searching. Also, patterns of daily and seasonal variation in water temperature were obtained from hourly readings of a submersible data-logger (Hobo®) anchored to the bottom of the stream, from July 5th 2004 to June 11th 2006

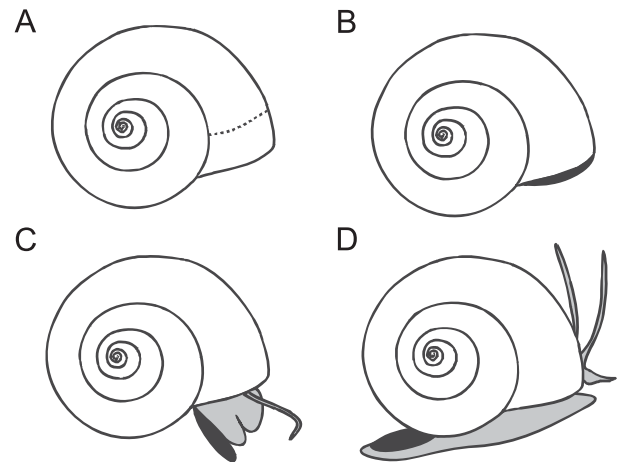


Fig. 1. Activity categories of *Pomacea canaliculata* (body: gray, shell: white, operculum: black); (A) fully retracted (FR); (B) partially retracted (PR); (C) partially extended (PE); (D) fully extended (FE).

in the Curamalal Grande and from June 11th 2006 to July 1st 2007 in the Cochicó Chico.

2.2. Origin and maintenance of experimental snails

Snails used in the trials were obtained from eggs laid in the laboratory by mature snails obtained from the Curamalal Grande stream population. Prior to their use in the trials, they were reared in 20 L aquaria (up to 15 individuals) with CaCO₃ saturated tap water at 25 ± 2 °C, under 14 h light/day photoperiod and fed on fresh lettuce. Before each trial snail shell length (from the apex to the extreme lip of the aperture) was measured with a Vernier caliper and the snails were categorized according to externally observable characters as undifferentiated, male or female, following the criteria used in Seuffert and Martín (2009b); the snails were chosen so that the sexes within each aquarium were as balanced as possible.

2.3. Recording of activity

The three laboratory experiments (see below) to estimate the levels of different activities relative to temperature were performed in 24 L glass aquaria (20 cm wide, 40 cm long and 30 cm deep). Water temperatures (*T*) were kept constant with electric thermostats located at the bottom of each aquarium to reduce thermal stratification.

All snails were marked with synthetic polish on the shell to identify them individually and facilitate recording of activity. The activities or states recorded in the trials were: feeding, crawling, mating, clinging (to the sides of the aquarium), resting (on the bottom with the foot extended), ventilating the lung and retracted inside the shell, either lying on the bottom or buried in the sand (see below). For consistency with field data, snails were considered inactive only if they were retracted with the operculum in the same plane as or deeper than the shell aperture; snails found in any of the other states were considered active. The starting and ending times of the different activities or states were recorded during a pre-established observation periods (see below).

On the basis of the total records during the observation period, different activity variables were calculated. The time spent active (TA, %) was obtained as the accumulated time active relative to the total observation time and the values were averaged for all

snails in each aquarium. The time spent feeding (TF, %) and the time spent crawling (TC, %) were calculated in the same way.

2.4. Experiment 1: effects of constant temperatures on activity

The water temperature was slowly changed (1 °C/h) from 25 °C to the desired temperature (10, 15, 20, 25 and 30 °C) and the snails were then acclimated to the new temperature for 72 h. The aquaria were provided with a sand layer deep enough to allow the snails to bury themselves (3 cm) and water was continuously aerated with electric air pumps. One leaf of lettuce was supplied to each aquarium every day. Six snails (shell length 13.5–47 mm, two undifferentiated, two males and two females) were used in each trial. The trials were repeated three times for the 10 and 15 °C treatments and six times for the others; each snail was used only once.

The observation period in these trials lasted 2 h and before starting water temperature was recorded, lettuce remains were carefully removed and a new lettuce leaf was provided; the air pumping system was disconnected to avoid interference with the observation. No negative responses (sinking, retraction, etc.) to these procedures were observed in the snails.

2.5. Experiment 2: combined effects of constant temperatures and exposure time on activity

At 10 °C there was almost no activity (see Results). Therefore, the effects of temperature and exposure time on different activities were investigated at 15, 20, 25, 30 and 35 °C. The trials were performed in the same 24 L aquaria with artificial aeration, but in this case they were divided vertically by a plastic grid (1 cm × 1 cm holes) into two compartments (20 cm × 20 cm × 30 cm one with and one without access of the snails to the surface to breathe air), as part of a study on the effects of restriction of aerial respiration (Seuffert and Martín, 2009b). Five differentiated snails (shell length 27.0–47.5 mm) were placed in each compartment but only the data from the compartment with access to air are considered here.

Snails were fed daily with a slice of carrot (to facilitate the estimation of ingestion rate; (Seuffert and Martín, 2009b) that was renewed every morning. The observation period lasted 30 min and was repeated in the morning (8.00 am) and in the afternoon (6.00 pm). Each set of five trials (one for each temperature) lasted five days and was repeated during three consecutive weeks, using different snails each week. Once this experiment was completed, it was repeated but without artificial aeration.

2.6. Experiment 3: effects of fluctuating temperature on activity

On the basis of the results from the two previous experiments and the field survey, we simulated the daily natural fluctuations of water temperature in the streams of the Encadenadas del Oeste basin during the seasons (spring and fall) in which it fluctuates above and below the temperature at which 50% of the snails are active, estimated as the $p=0.5$ value of the logistic regression (see below).

Snails were acclimated for a period of 24 h at 20 °C in aquaria kept in a refrigerated (4–6 °C) dark room (to avoid any interference of photoperiod with the effects of temperature fluctuations), provided with a 3 cm deep sand layer as a burying substrate and fed with fresh lettuce. Ten differentiated snails (shell length 29 to 45.5 mm) were put in each aquarium. Each trial started with the water temperature at 20 °C and it was let to cool gradually down to 8 °C at a rate of 1 °C/h on average, by the

disconnection of the thermostats; then the thermostats were reconnected and the water was warmed at the same rate up to 20 °C. Four aquaria were simultaneously observed on three different days, for a total of 12 replicates. Every hour during the 24 h duration of each trial, the activity of each snail was recorded as in the field (FR, PR, PE and FE) and water temperature was registered in each aquarium.

The frequency of active snails per aquarium (AS, %) was calculated in the same way as in the field survey. The hourly records of water temperature and the frequency of active snails were averaged for the 12 aquaria; logistic equations were fitted to the data corresponding to both the cooling and the warming phases of the experiment. The two equations were then used to estimate the frequency of active snails in Curamalal Grande and Cochicó Chico streams during the year, using the hourly temperature data provided by the data-logger and selecting one or other equation depending on whether temperature was decreasing or increasing.

3. Statistical analysis

The proportions of active snails (P) from Curamalal Grande and Cochicó Chico streams were investigated through logistic regression analysis, with instantaneous water temperature as an independent variable and activity as the binary dependent variable (active or inactive). For the Curamalal Grande stream, the same analysis was also performed using the minimum temperature recorded from the data-logger on the sampling day; there were too few of these records to perform the same analysis for Cochicó Chico stream.

The effect of constant temperatures on the activity variables was investigated through one-way ANOVAs. If homogeneity of variances was rejected by Levene's test, the dependent variable was transformed and re-analyzed. If it was impossible to attain homoscedasticity, a non-parametric test (Kruskal-Wallis) was performed.

The effects of constant temperatures and exposure time on the activity variables were explored through repeated measures ANOVA, with temperature as the main factor and exposure time as the repeated measure or within-subject factor (five consecutive days). When Mauchly's W test was significant, indicating non-sphericity of the covariance matrix, the degrees of freedom were corrected by Greenhouse–Geisser's Epsilon (Crowder and Hand, 1990).

4. Results

4.1. Temporal patterns of activity in natural populations

The proportion of active snails in Curamalal Grande and Cochicó Chico ranged from 0 to 1, with an abrupt change between water temperatures of 10 and 15 °C and 12 and 18 °C, respectively (Figs. 2A, B). Most inactive snails were on the bottom, either lying on the surface or partially covered by very fine sediments; in the Cochicó Chico stream inactive snails were also frequently found beneath stones. During winter, the percentage of partially retracted snails varied between 25% and 71% of the inactive snails in the Curamalal Grande and from 7% to 100% in the Cochicó Chico.

The logistic regression model adequately fitted the data on proportion of active snails for the Curamalal Grande (Hosmer and Lemeshow test: $X^2_7=11.38$, $p=0.123$) but did not for the Cochicó Chico ($X^2_8=1670.97$, $p<0.0001$). The lack of fit in the latter was probably due to the relatively low level of activity at the highest

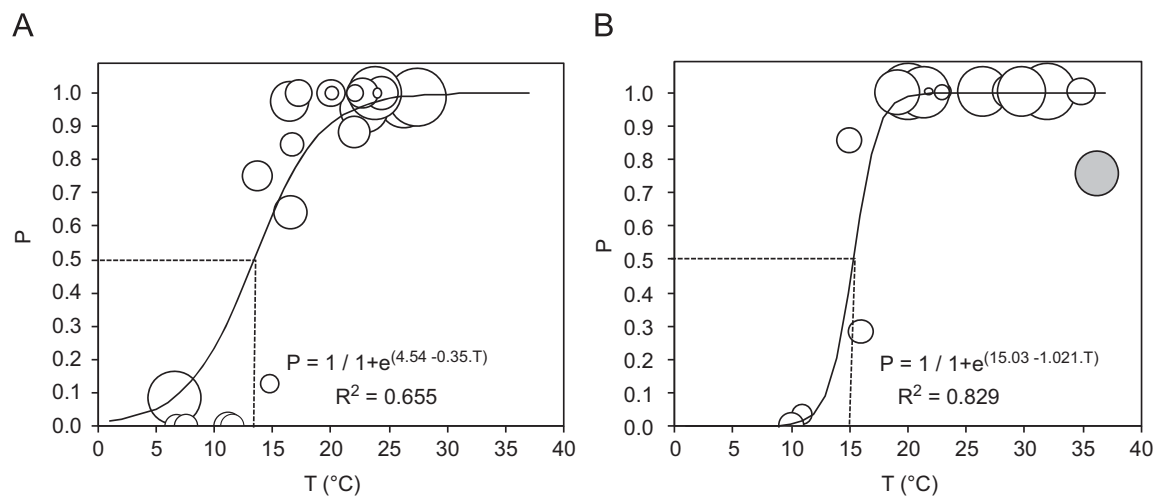


Fig. 2. Proportion of active snails (P) at different temperatures (T) for two populations of *Pomacea canaliculata*: Curamalal Grande (A) and Cochicó Chico (B) streams. The area of the circles indicates the number of snails found in each survey; it ranged from 2 to 87 in Curamalal Grande and 4 to 254 snails in Cochicó Chico (the gray circle was excluded from the analysis). Solid lines correspond to the proportions estimated with the parameters from the logistic regression; dotted lines indicate the temperature at which 50% of the snails are active.

temperature (36.2 °C); the same analysis was performed without this point, improving the fit greatly ($X^2_8=4.92$, $p=0.766$). In both cases the coefficient of temperature was significantly different from zero (Wald test: $X^2_1=172.52$, $p<0.0001$ and $X^2_1=101.97$, $p<0.0001$, respectively). The temperature at which 50% of the snails were active ($p=0.5$) was 12.95 °C for Curamalal Grande and 14.93 °C for Cochicó Chico; the specific rate of change in activity with temperature was also lower in Curamalal Grande than in Cochicó Chico (0.35 and 1.02, respectively). The logistic regression for the proportion of active snails from Curamalal Grande vs. the minimum water temperature of the sampling day showed a worse fit ($X^2_8=28.62$, $p<0.0001$) than that with the instantaneous temperature.

4.2. Effects of constant temperatures on activity

At 10 °C there was almost no activity: only one snail crawled for a few minutes and the remaining snails (94.5%) were retracted into their shells over the sand bottom. Therefore, both the time spent feeding and crawling were analyzed excluding the 10 °C treatment. The percentage of time spent active increased significantly with temperature (Kruskal-Wallis $H_4=21.50$, $p<0.0001$), attaining a maximum of 100% at 25–30 °C (Fig. 3). The percentage of time spent feeding increased significantly with temperature ($F_{3,17}=4.08$, $p=0.0236$), reaching a peak of 60.7% at 25 °C and decreasing to 41.3% at 30 °C. On the other hand, the percentage of time spent crawling (square root transformed) was not affected by temperature ($F_{3,17}=0.66$, $p=0.5852$), averaging 20.2% of the observation period.

4.3. Combined effects of constant temperatures and exposure time on activity

Under aerated conditions none of the variables analyzed was affected by the exposure time to a certain temperature (Table 1). Only the percentage of time spent active showed a significant effect of temperature, while remaining almost constant at ca. 94% between 25 and 35 °C; the percentage of time spent feeding and crawling showed little variation with temperature and was lowest at 35 °C (Fig. 4A).

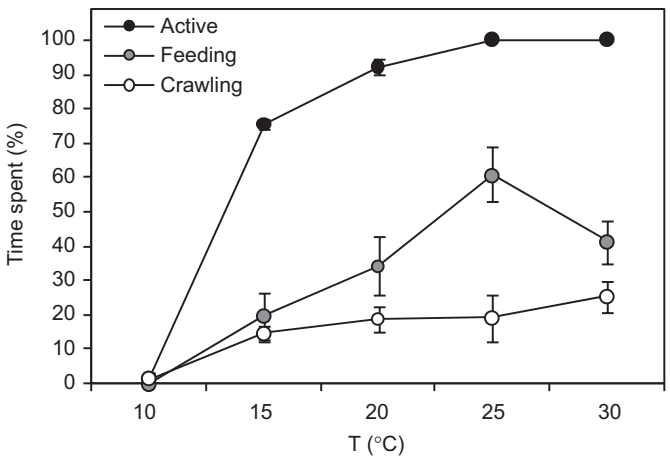


Fig. 3. Activity of *Pomacea canaliculata* in the laboratory at different temperatures (T) after 72 h of acclimation; bars are means \pm standard errors (for an observation period of 2 h).

Table 1
Summary of repeated measures ANOVAs for activity variables of *Pomacea canaliculata* during five days of exposure at constant temperatures. Dependent variables: time spent active (TA), time spent feeding (TF) and time spent crawling (TC) for two water conditions, with aeration (above) and without aeration (below). Factors: exposure time (E, repeated measure or within-subject), temperature (T, between subjects) and interaction (E \times T). All dependent variables were arcsine transformed before the analysis.

Variables	E	E \times T	T
TA	$F_{4,40}=0.714$ $p=0.588$	$F_{16,40}=1.242$ $p=0.281$	$F_{4,10}=4.829$ $p=0.020$
TF	$F_{4,40}=1.540$ $p=0.209$	$F_{16,40}=0.403$ $p=0.974$	$F_{4,10}=1.786$ $p=0.208$
TC	$F_{4,40}=0.581$ $p=0.678$	$F_{16,40}=1.336$ $p=0.224$	$F_{4,10}=1.126$ $p=0.398$
TA	$F_{4,40}=3.852$ $p=0.010$	$F_{16,40}=1.025$ $p=0.453$	$F_{4,10}=7.155$ $p=0.005$
TF	$F_{1.8,18.4}=1.209$ $p=0.318$	$F_{7.4,18.4}=1.857$ $p=0.133$	$F_{4,10}=8.502$ $p=0.003$
TC	$F_{4,40}=1.694$ $p=0.170$	$F_{16,40}=1.151$ $p=0.346$	$F_{4,10}=2.451$ $p=0.114$

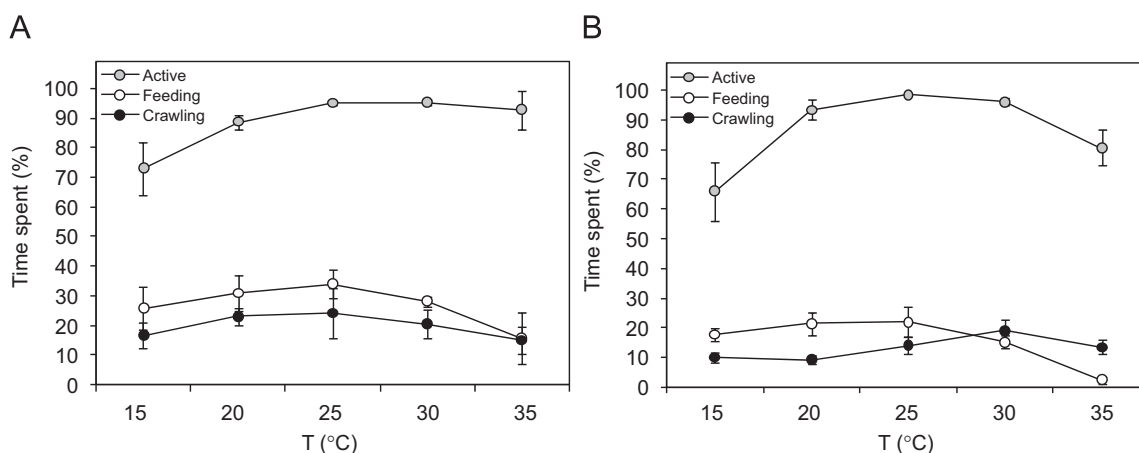


Fig. 4. Activity of *Pomacea canaliculata* at different constant temperatures (T) during five days of exposure, under aerated (A) and non-aerated (B) conditions; bars are means \pm standard errors (means were obtained by averaging the values of the five days).

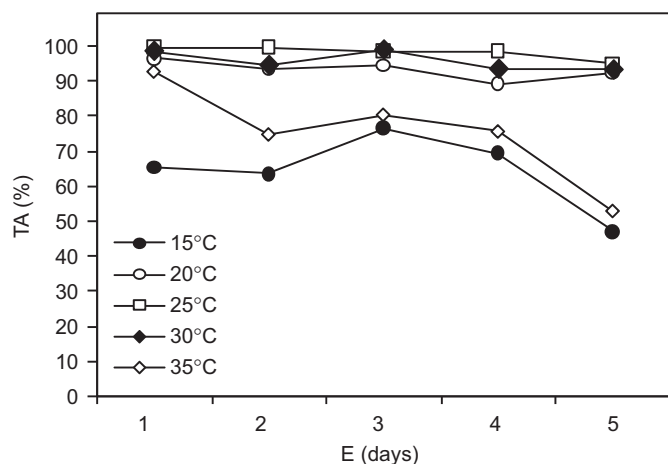


Fig. 5. Time spent active (TA) by *Pomacea canaliculata* at different constant temperatures vs. exposure time (E) under non-aerated conditions.

The time spent active under non-aerated conditions showed a significant effect of exposure time (Table 1); despite the fact that the interaction term $E \times T$ was not significant, different temporal patterns were observed at different temperatures (Fig. 5). Between 20 and 30 °C the snails remained active almost all the time during the five days but showed a decreasing trend over time at the highest temperature of 35 °C (from 92.8% to 52.6%); the activity at 15 °C was clearly lower and showed a further decrease on the fifth day. Temperature affected significantly both the time spent active and feeding but not the time spent crawling (Table 1). The percentage of time spent active attained a maximum between 20 and 30 °C (96.1% on average) whereas the time feeding was quite constant up to 30 °C (19.1%) but dropped to near zero at 35 °C (Fig. 4B).

4.4. Effects of fluctuating temperature on activity

In the experiment with fluctuating temperature the frequency of active snails per aquarium showed a positive relationship to temperature during both the cooling and warming phases (Fig. 6), although the rate of change was higher during cooling (0.72) than during warming (0.44); the temperatures at which 50% of the snails were active were 10.88 and 13.85 °C, respectively. The minimum frequency of active snails (15.83%) was at 8.67 °C

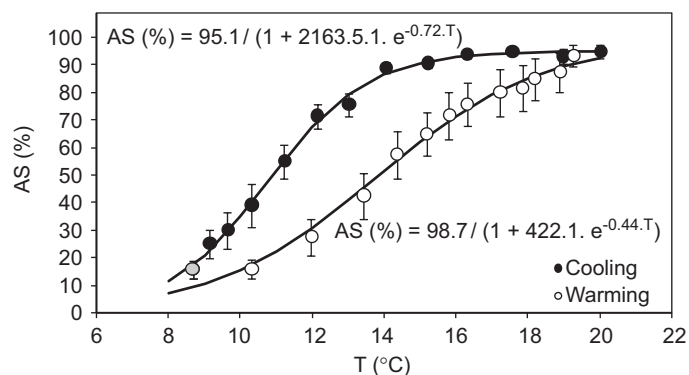


Fig. 6. Frequency of active snails (AS) at different temperatures (T) in the trials of fluctuating temperature for *Pomacea canaliculata*. The cooling and warming phases lasted 12 h and the gray dot indicates the ending of the cooling and the beginning of the warming (i.e. it is on both curves); bars are means \pm standard errors. Solid lines correspond to the frequencies estimated with the parameters from the logistic equations.

at the end of the cooling phase and remained at that minimum despite a ca. 2 °C increase in the first hour of warming. The frequency of active snails was always higher during the cooling phase (up to 31.67% at 14 °C).

The frequency of active snails estimated using the above equations in Curamalal Grande and Cochicó Chico streams showed great fluctuation, especially between late fall and early spring (May to October), being more constant during the rest of the year (Fig. 7). The predicted frequencies closely matched the observed frequencies of active snails in both streams. Two unusual situations occurred during the sampling: a relatively high frequency of active snails (64.3%) in Curamalal Grande stream in late fall (coincident with an instantaneous temperature of 16.5 °C) and a relatively low frequency (75.7%) in Cochicó Chico in early summer (December), coincident with an instantaneous temperature of 36.2 °C.

Water temperature in Curamalal Grande fluctuated between 0.29 and 31.12 °C (5% quantiles=6.22–26.34 °C), while in Cochicó Chico it varied from –0.16 to 35.7 °C (5% quantiles=4.15–28.31 °C). In both streams the “zero” records (–0.16–0.29 °C) were very infrequent (0.02% and 0.13%, respectively) and lasted only 2–4 h; in contrast, records under 5 °C were more common (2.97% and 7.99%, respectively) and persisted for up to two days.

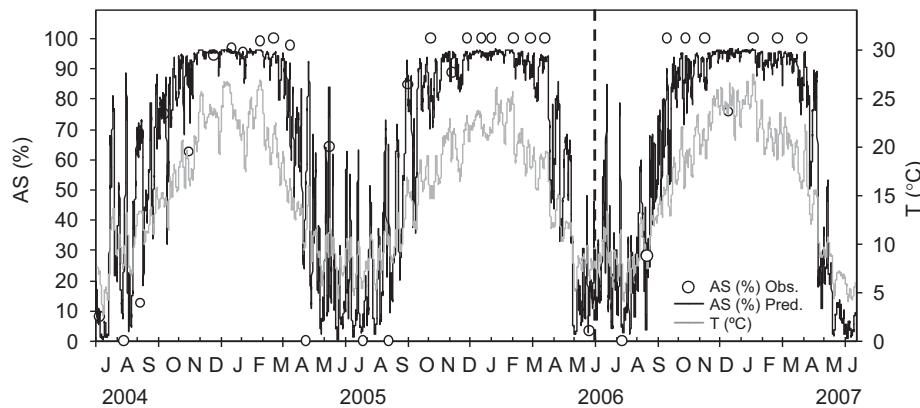


Fig. 7. Temporal patterns of frequency of active snails (AS) of *Pomacea canaliculata* in Curumalal Grande (2004–2006) and Cochicó Chico (2006–2007) streams during three years: AS predicted (moving averages of 24 consecutive hourly readings), AS observed and temperature (T , moving averages of 72 consecutive hourly readings); the dotted line indicates the date when the data-logger was moved from Curumalal Grande to Cochicó Chico.

5. Discussion

P. canaliculata has been reported to overwinter buried in the bottom sediments but also under boulders or entangled in submersed plants (Estebenet and Martín, 2002). In southern Japan *P. canaliculata* shows a daily cycle of burrowing in the soil of shallow (depth less than 8 cm) paddy fields, the percentage of buried snails increasing during fall (Wada and Yoshida, 2000). In our field surveys, most inactive snails found were lying on the bottom or partially covered by fine sediments; besides, burying in the sand was never observed in our trials. Stevens et al. (2002) suggested that in *P. paludosa*, snails resting during winter maintain their opercula slightly protruded beyond the shell to allow water circulation. In the two streams we surveyed, the percentage of snails only partially retracted reached maximum values of 71% and 100% of the inactive snails during winter, suggesting that pallial water renovation is common. This evidence supports the conclusion that, especially in streams, the snails eventually end up buried as a result of siltation and not by active burrowing (Seuffert and Martín, 2009a).

Many studies have proposed that apple snails hibernate during winter in temperate climates, both in their natural range (e.g. Bachmann, 1960; Damborenea, 1996; Martín et al., 2005) and in invaded regions (e.g. Ito, 2002; Wada and Matsukura, 2007; Karatayev et al., 2009), although the physiological processes involved in this behavior are not well understood, except for the acquisition of cold hardiness (Matsukura et al., 2008, 2009). In our field surveys during winter, inactive snails reactivated after a few minutes out of water, even though the air temperature was usually less than 15 °C. Moreover, on one occasion in late autumn (June 2nd 2005) most snails (64.3%) were active in Curumalal Grande (coincident with a sudden increase in water temperature from 6.7 to 16.8 °C in two days), despite the fact that in the previous and the following surveys there was no activity (Fig. 7). Therefore, the snails apparently do not enter a deep lethargic state that persists during the whole cold season but show a rather quick response to instantaneous temperature changes, being able to reactivate as soon as conditions are favorable.

During the warm season, and in coincidence with very low water levels and almost nil flow, only 75.7% of snails were recorded as active when water temperature reached 36.2 °C in the Cochicó Chico population (gray point, Fig. 2), all the inactive snails found being partially retracted and located on the bottom surface in sunny shallow areas. In contrast, *P. canaliculata* males began to bury at temperatures above 35 °C in outdoor experimental plots, almost all being buried at 40 °C (Wada and Yoshida, 2000). In our

study, under non-aerated conditions the time spent retracted showed a considerable increase from 3.8% at 30 °C to 19.4% at 35 °C; moreover, non-retracted snails were mostly attached to the aquarium walls and remained motionless, except for frequent lung ventilation. Ramakrishnan (2007) reported that all *P. insularum* enter heat coma (remaining unattached with no head-foot movement) at 38.2 and 40.6 °C when temperature is raised quickly from acclimation levels of 20 and 30 °C, respectively. No snail exhibited this response to heat stress at the highest temperatures we used (35 °C), even though a similar comatose state has been observed in *P. canaliculata* at 25 °C if aerial respiration is impeded (Seuffert and Martín, 2009b). An increase in temperature from 26 to 36 °C provoked unusual foot detachment and shell twisting in *P. paludosa* (McClary, 1964). Apparently, quite different responses to heat stress are possible in *Pomacea*, at both interspecific and intraspecific levels, probably due in the latter to interacting factors like sex, dissolved oxygen and insolation.

In the trials mimicking spring and fall conditions of the southernmost populations of *P. canaliculata*, the shift to higher temperatures of the curve of warming with respect to that of cooling (Fig. 6) suggests that, it takes at least 1 h for underwater snails' bodies to reach the water temperature. A similar delayed response to temperature changes has been suggested for lung ventilation frequency in *Pomacea* (Seuffert and Martín, 2009a). In addition, during these trials the percentage of active snails at the lowest temperature recorded (8.67 °C on average) did not reach zero, despite the fact that after 72 h of exposure at 10 °C there was almost no activity. This indicates that short exposures are probably insufficient to induce inactivation of all snails. Apple snails of the genus *Pomacea* include the biggest freshwater snails (Cowie et al., 2006), so considerable thermal inertia was to be expected in our snails, which ranged from 4.5 to 17.2 g live weight (estimated with an empirical length–weight equation; Nicolás Tamburi, pers. comm.). The different inactivation and reactivation rates could be related to the fact that during cooling low temperatures physiologically force the inactivation of the snails whereas when temperature surpasses the inactivation limit the snails have a behavioral alternative to reactivate or not; therefore, the reactivation rate will be lower if at least some snails delay the beginning of activity.

A decrease in activity with exposure time was evidenced only in the aquaria without aeration at the highest temperature tested and over five days of exposure (Fig. 5). Together with the results discussed above, this suggests that the response of activity to temperature is very rapid (within a few hours) and that the long

term response of activity is due to harmful effects that only appear at temperatures above 30 °C and in foul water, a condition that hinders the normal perfusion of the gill (Seuffert and Martín, 2009b) and almost precludes feeding (Fig. 4B).

The temperature at which 50% of the snails were active and the specific rate of change in activity with temperature was lower for Curamalal Grande than for Cochicó Chico (Fig. 2). These differences in the estimated parameters could be related to the fact that the two sets of data were not completely overlapped in time and that the streams differed in their thermal regime, Cochicó Chico showing wider and faster fluctuations in water temperature. Besides, a great variation in life history traits exists between both populations, which is in part attributable to genetic differences (Martín and Estebenet, 2002) thus, it is difficult at present to find a straightforward explanation for the differences in the activity curves.

Heiler et al. (2008) also reported a positive response of activity to temperature for an aquarium strain of *P. canaliculata*, though the results are quite different from ours. First, in our study activity reached its maximum in the range of 25–30 °C (Figs. 3 and 4) while they found an almost linear increase from 20 to 32 °C, the highest temperature tested. Second, the proportions of active snails were much higher in our study; for instance, we recorded values of ca. 90% at 20 °C while Heiler et al. (2008) recorded only 18%. This is partially due to the fact that resting but not retracted snails were considered inactive by them. However, even considering these snails as inactive, our values were still higher (60%); the differences between these two strains of *P. canaliculata* may be related to a distinct region of origin or to artificial selection by aquarists.

The time spent crawling remained nearly constant with temperature in our trials with *P. canaliculata* (Fig. 4) though crawling speed increases linearly with temperature in this species (Heiler et al., 2008; Seuffert and Martín, 2009b). Similarly, for the freshwater pulmonate snail *Planorbis corneus*, Costil and Bailey (1998) did not find any change with temperature in the time spent crawling but recorded a significant increase in crawling velocity. Apparently, as these snails can crawl faster as temperature increases, they do not devote additional time to moving between different places to perform their routine activities (e.g. aerial respiration at the surface and food searching at the bottom).

The time spent feeding increased with temperature above 10 °C and attained a maximum at 25 °C but we recorded quite different values in our three trials; for instance, at 25 °C feeding was 60.7% in the trials with lettuce leaves (Fig. 3) but only 22–34% when carrot slices were provided (Fig. 4). These differences are probably not explained by different food preferences or palatabilities but by their different exposed surfaces, feeding interference (Estebenet and Martín, 2002) being more significant in the more compact food (carrot slices) that did not allow all snails to graze simultaneously. Regardless of these differences, in all cases the increase in feeding was related to a decrease in the time spent motionless but attached to substrata. Costil and Bailey (1998) also reported for *P. corneus* an increase in feeding from 17.1% to 56.2% between 10 and 25 °C with a correlated decrease in resting time. Probably these snails operate at suboptimal rates, thus allowing for compensatory increases in feeding rates when confronted with stressful conditions, as suggested by Rollo and Hawryluk (1988) for other freshwater snails.

The increase in the time spent feeding with temperature in *P. canaliculata* is probably essential to fulfill the energetic requirements imposed by higher metabolic rates. The specific oxygen consumption in other *Pomacea* sp. increases between 2.2 and 3.4 times when temperature raises from 15 to 25 °C (Freiburg and Hazelwood, 1977; Santos et al., 1987; Ramakrishnan, 2007), these values being comparable to the increase in feeding observed

here (3.1 times in the same temperature range). As the respiratory demands continue increasing beyond 25 °C up to lethal or harmful temperatures, the decrease in feeding time that we observed within that range implies a potential nutritional deficit if these conditions are maintained. In coincidence, the increase in weight in *P. insularum* after 10 days at 35 °C was smaller than that in the range 20–30 °C despite the fact that the ingestion rates showed no differences between these temperatures (Gettys et al., 2008).

P. canaliculata from temperate Japan (33°N) are able to develop cold hardiness before winter (Matsukura and Wada, 2007; Wada and Matsukura, 2007) but unable to tolerate freezing (Matsukura et al., 2009). Recent molecular studies (Hayes et al., 2008) indicate that Asian *P. canaliculata* populations are the result of multiple introductions from northern Buenos Aires Province (34–35°S) where minimum air temperatures in winter are only 2 °C higher than in our study area (37°S). Wada and Matsukura (2007) posed the question as to whether the development of this cold hardiness has evolved in its native range or in temperate Japan. The similar latitudes of these Japanese populations and those from the region of origin suggest that cold hardiness could have developed in the native range. *P. canaliculata* is not usually exposed to subzero temperatures, neither in its southernmost natural populations (our study) nor in the Japanese paddy fields (where snails seek shelter under rice straw; (Matsukura et al., 2009)), which may explain the lack of freezing tolerance reported for Japanese populations.

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