THERMAL BIOLOGY OF THE SOUTH AMERICAN APPLE SNAIL *ASOLENE PLATAE* (CAENOGASTROPODA: AMPULLARIIDAE)

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ABSTRACT

Asolene platae is a freshwater snail of the Ampullariidae native from La Plata River basin in South America. Water temperature has an overwhelming influence on the biology of apple snails, though there are no systematic studies related to the thermal biology of this species. The effect of temperature on the activity, growth and mortality of *A. platae* was studied through laboratory experiments using constant temperatures of 15, 20, 25, 30 and 35ºC. The lower threshold and cumulative degree-days for the embryonic development were estimated under fluctuating temperature. Water temperature had a significant effect on the activity of *A. platae*, being almost null at 15ºC, reaching its maximum in the range 25-30ºC and decreasing above 30ºC. Regardless of temperature, snails remained resting nearly half of the time, exhibiting less activity than other apple snails across similar temperatures. Mortality was higher for hatchlings than for adults at all temperatures with the exception of 35ºC, which resulted in high levels of mortality for both groups. Growth of hatchlings was significantly affected by temperature, being highest between 25 and 35ºC. The lower temperature threshold for the embryonic development was around 16ºC and the cumulative degree-days to complete development were estimated between 107.5 and 120.5ºC.d. The optimum temperature for *A. platae* would be 25-30ºC. Temperatures of 35ºC and above would be harmful, while temperatures below 20ºC resulted in rapid decreasing in activity and slower growth rates. The information about the thermal requirements of *A. platae* will be useful optimizing rearing conditions and developing predictive models of the potential distribution of *A. platae* in scenarios where it is introduced outside of its native range.

Key words: temperature, growth, mortality, activity, embryonic development; degree-days, threshold.

INTRODUCTION

Ampullariids or apple snails are recognized worldwide as successful invaders, especially *Pomacea* spp. that became serious pests of aquatic crops (Cowie, 2002; Hayes et al., 2008), promoters of ecosystemic changes in natural wetlands (Carlsson et al., 2004, Fang et al., 2010; Horgan et al., 2014) and vectors of parasitic diseases (Lv et al., 2009). For these reasons, *Pomacea* has been the target of most research on ampullariids and hence a vast knowledge of its species is available, whereas little is known about the biology and ecology of other apple snail genera like *Asolene*.

Water temperature has an overwhelming influence on most aspects of the biology of ampullariids, including activity levels, aerial respiration, growth rate, development, reproduction and survival (e.g., Freiburg & Hazelwood, 1977; Albrecht et al., 1999; Estebenet & Martín, 2002; Aufderheide et al., 2006; Gettys et al., 2008; Seuffert & Martín, 2009; Seuffert et al., 2010, 2012). However, there are no systematic studies to date related to the thermal biology of the genus *Asolene*. The information about the thermal limits of ectotherms in general and aquatic snails in particular would be necessary to understand and predict changes in distribution ranges and abundances in native and invaded areas in the face of global climatic changes (Tomanek, 2008; Madeira et al., 2012; Fordham et al 2013).

Asolene platae (Maton, 1811) is an apple snail native from La Plata River basin, whose range extends from Bolivia to northern Buenos Aires province, Argentina (Cowie & Thiengo, 2003). Recently, this species has been considered a senior synonym of *Asolene pulchella* (Maton, 1838) on the basis of their genetic, anatomical and conchological similarity (Hayes et al., 2009a; Leon et al., 2014). The embryonic development of *A. platae* has been determined under a controlled temperature of 25 ± 2ºC, extending for 10.8 days on average (range: 8-16 days; Tiecher et al., 2014). However, the temperature below which embryonic development stops, known as lower temperature threshold (Seuffert et al., 2012), is so far unknown. As temperature increases above the lower threshold, the duration of embryonic development will decrease. However, the combination of temperature above the lower threshold and time required to hatch remains generally constant (Zalom et al., 1983; Seuffert et al., 2012). The estimation of this physiological time (measured in cumulative degree-days) and the lower threshold are essential to estimate the duration of embryonic development in thermally fluctuating environments, since a bigger difference between temperature and the lower threshold implies an earlier accumulation of the required degree-days and hence a faster developmental rate. Among apple snails, these parameters are only known so far for species of *Pomacea* that have aerial egg masses and calcareous eggshells (Seuffert et al., 2012) but not for genera possessing gelatinous and subaquatic egg masses such as *Asolene* (Hayes et al., 2009b).

A series of experiments were designed to study the effect of temperature on the activity of *A. platae* and the relation with some aspects of its life cycle, such as growth and mortality. Additionally, we estimated the lower threshold and cumulative degree-days for embryonic development. These results provide insights into the fundamental biology of ampullariids and contribute to our understanding of the factors influencing its distribution. These data are also informative for useful to developing predictive models of successful establishment and spread of this species if introduced outside of its native range, and which are critical for assessment of invasion potential by regulatory agencies (e.g., EFSA, 2013, 2014). Furthermore, the results of this study and those on thermal requirements of other ampullariids are useful for optimizing culturing conditions for research and aquaculture projects (Seuffert & Martín, 2013).

MATERIALS & METHODS

Origin and Maintenance of Snails

The snails used in this study were descendants of a pool originally obtained from Regatas pond (Ciudad Autónoma de Buenos Aires, Argentina; 34°33'S, 58°26'W). They were raised in 20 L collective aquaria with $CaCO_3$ saturated tap water, kept in a room at 25 ± 2ºC under a 14:10 h (light/dark) photoperiod. The snails were fed with fresh lettuce *ad libitum* and the aquaria were cleaned and renewed weekly. Before each trial, snail shell length (SL, from the apex to the extreme opposite lip of the aperture) was measured with a Vernier caliper to the nearest 0.1 mm.

The laboratory experiments were performed in 10.8 L glass aquaria (20 cm-wide, 27 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. The aquaria were provided with a 1 cm sand layer to allow the snails to bury themselves. The snails were provided with fresh lettuce as entire leaves and also fragmented in floating particles, allowing pedal surface collecting (Saveanu & Martín, 2013). The aquaria were not provided with artificial aeration.

Experiment 1: Effect of Constant Temperatures on Activity

The water temperatures used in this experiment were: 15, 20, 25, 30 and 35ºC and for each temperature three snails (SL: 11.1-31.3 mm) were randomly chosen from the general pool. Before the observation period, the snails were acclimated to the target temperature for 48 h. After, they were observed at five minute intervals for over three hours with all treatments run concurrently. At the end of each observation period snails were evaluated to see if they were dead or alive by checking on the heartbeat through the translucent shell or by stimulating them with a probe; in some cases, it was obvious because of the putrefaction state. For each treatment five replicated aquaria were used and each snail was used only once.

During the observation period, the instantaneous activity or state of each snail was recorded and classified as: feeding (F, considering both shredding and pedal surface collecting), crawling (C), ventilating the lung (V, during which it was also registered the number of pumping movements; Seuffert & Martín, 2009), resting (R, on the bottom or clinging to the sides of the aquarium with the foot extended) and inactive (I, with the head and foot fully or partially retracted inside the shell, either lying on the bottom or buried in the sand).

On the basis of the total records during the 3 h observation period, the percentage of time spent in the different activities per aquarium was calculated as:

$$\frac{\text{number of records for each activity}}{\text{(number of snails per aquarium * total number of observations).}} \times 100$$

Experiment 2: Effect of Constant Temperatures on Mortality of Adult Snails and on Mortality and Growth of Hatchlings

The water temperatures used were as in experiment 1. To analyze the effect of temperature on the mortality of adult snails, five snails (SL: 14.8-31.5 mm) were randomly assigned to each temperature. During a 25 day trial period, aquaria were checked every two to three days to determine if snails were alive or dead. Five replicated aquaria were used for each temperature.

To analyze the effect of temperature on the mortality and growth of hatchlings, one week-old hatchlings were reared in tap water in 500 ml plastic bottles containers held inside 10.8 l aquaria containing water maintained at one of the following temperatures: 15, 20, 25, 30 and 35ºC. During 20 days the number of dead hatchlings was counted every three days. The five temperatures were replicated three times and the total number of hatchlings per container varied according availability at the start of each trial, but were the same for all five temperatures (31, 21 or 19 for each of the replicates).

At the end of the trials, the mortality (percentage of dead snails or hatchlings) was calculated for each temperature. A scaled digital photograph was taken of ten randomly chosen surviving hatchlings to determine their shell lengths.

Experiment 3: Threshold Temperature and Degree-days Requirement for Embryonic Development

The egg masses used in this experiment were laid in 2.8 L aquaria by snails reared under the general conditions described previously. Females were allowed to copulate each week to ensure that sperm reserves were not depleted. Due to the lack of external sexual dimorphism, females were recognized while they were laying eggs in a collective aquaria or when an egg mass was found in an individual aquaria where a putative female was isolated after being mounted by a male (Tiecher et al., 2014). Egg masses were incubated in 7 cm diameter dishes filled with 2 cm of tap water in a room with variable temperature which was recorded hourly with a Hobo® data-logger (T_h, ºC). Water temperature in the dishes followed closely the air temperature but with a delay of approximately one hour. The egg masses were checked daily to record the appearance of the first hatchling. The counting of hatchlings continued until the last egg hatched and the remaining eggs were counted to estimate egg mass viability. The duration of embryonic development (d, days) was calculated as the number of days elapsed between the date of egg laying and the date of first hatching.

Following the results with *Pomacea canaliculata* (Lamarck, 1822) reported by Seuffert et al. (2012), the lower temperature threshold (T_L) for embryonic development was estimated by three different methods: the least standard deviation from the mean observed number of days (SD-day), the coefficient of variation in days (CV-day) and the linear regression coefficient method (REG). The different threshold temperatures were estimated using the equations developed by Yang et al. (1995) based on these methods.

The degree-days (DD, ºC.d) for the embryonic development were calculated on the basis of hourly records of temperature by computing the degree-hours and summing over the 24-h period. Degree-hours were calculated as the difference between the recorded hourly mean temperature (T_h) and the lower threshold (T_L):

$$DD = \Sigma (T_h - T_L)$$

The cumulative DD were estimated by summing the daily DD over the specific period of development of each egg mass (Seuffert et al., 2012). To estimate the possible error due to the use of air temperatures instead of water temperatures we mimicked the smaller variation of the latter using the moving average of the last ten hourly records of air temperature and estimated T_L and the cumulative DD again.

Statistical Analysis

The effect of temperature on the snails' activity and mortality was investigated through one-way ANOVAs. If homogeneity of variances was rejected by Levene's test, the dependent variable was arcsine square root transformed and re-analyzed.

Means were compared with LSD post hoc test. Differences in hatchlings' size among treatments were analyzed with nested ANOVAs, with the three replicates (bottles) being the nested random factor and water temperature the main fixed factor.

RESULTS

Experiment 1: Effect of Constant Temperatures on Activity

Snails spent nearly half of the total time resting with the foot extended regardless of temperature (range: 41.4-68.8%; Fig. 1), without significant differences among treatments. The percentage of time that the snails were inactive (fully or partially retracted) decreased significantly with temperature (Table 1); the mean percentage recorded at 15ºC (44%) was significantly higher than the means at 25, 30 and 35ºC (Fig. 2), temperatures with very low levels of inactivity (between 0.9 and 7.3%). The time spent ventilating the lung increased significantly with temperature (Table 1), from zero at 15ºC to a maximum of 6.7% at 30ºC (Fig. 3). The mean frequency of pumping movements per ventilation event was seven and ranged between 1 and 18. The time spent feeding increased significantly with temperature from 2.5% at 15ºC to a maximum of 28% at 25ºC, and then decreased significantly from 25 to 35ºC (Table 1; Fig. 4). In contrast, the percentage of time spent crawling was not affected by temperature (Table 1).

At 15 and 20ºC the snails were found buried in the sand with a mean frequency of 17.3 and 16.8%, respectively, and at higher temperatures (25, 30 and 35ºC) burying was almost null (< 1% of total time). During the duration of this experiment, no snails were observed mating and no eggs were laid.

Experiment 2: Effect of Constant Temperatures on Mortality of Adult Snails and on Mortality and Growth of Hatchlings

Temperature had a significant effect on the mortality of adult snails ($F_{4,\ 10} = 4.67$, $p = 0.022$), reaching a maximum of 60% at 35ºC (Fig. 5). This mean was significantly higher than the mean mortalities recorded at the other temperatures (LSD test, $p < 0.05$) that were quite low (6-20%). The mortality of hatchlings was not significantly affected by temperature ($F_{4,\ 10} = 3.00$, $p = 0.072$) despite being higher at 35ºC with a mean mortality of 60% as with adults. For all the other temperatures, mortality was 2.0 to 6.6 times higher for hatchlings than for adults.

The mean size of hatchlings after 20 days was significantly affected by temperature ($F_{4,\ 135} = 13.774$, $p < 0.001$) while the nested factor (replicated bottles) was not significant ($F_{10,\ 135} = 0.895$, $p = 0.539$). The minimum size was recorded at 15ºC (mean = 2.42 mm; Fig. 6), being significantly lower than the sizes recorded at all the other temperatures (LSD test, $p < 0.05$). The biggest size was obtained at 30ºC, being 48.7% higher than the minimum and significantly bigger than the size recorded at 20ºC but not significantly different from those of 25 or 35ºC.

Experiment 3: Threshold Temperature and Degree-days Requirement for Embryonic Development

A total of 84 egg masses were used in this experiment. The mean viability of egg masses (100 * number of live hatchlings / total number of eggs) was 57.8% and fluctuated between 1.1 and 100%. For the estimations of thresholds and degree-days we decided to use the data obtained from egg masses with viabilities greater than 10%, leaving a total of 77 egg masses. The duration of embryonic development (d) varied from a minimum of 9 days to a maximum of 26 days (mean ± SD = 14.57 ± 3.18 d). The estimations of the lower temperature threshold for the embryonic development of *A. platae* varied between 15.7ºC and 16.6ºC according to the method used (Table 2), and the corresponding mean cumulative DD estimations ranged between 107.6ºC.d and 120.7ºC.d. The cumulative DD of each egg mass was not related to the mean temperature to which it was exposed during development ($F_{1, 75}$ = 0.029, p = 0.864, estimated slope ± SD = -0.51 ± 2.97) indicating that degree days were constant within the temperature range to which the eggs were exposed (19-28ºC). The estimations obtained using the moving average of water temperatures (whose greater delay reduced the variation in 4.5%) were only 1.5% higher and 2.4% lesser at most for the lower threshold and cumulative degree-day estimations, respectively.

DISCUSSION

Water temperature had a significant effect on the general level of activity of *Asolene platae*. Regardless of temperature, snails remained resting attached to the substratum nearly half of the time. At 15ºC snails were inactive (i.e., fully or partially retracted) the other 44% of time; only during 1% of the time snails were found crawling or feeding. The general level of activity of *A. platae* reached its maximum in the range 25-30ºC, and the time that the snails spent buried was almost null above 25ºC. Above 30ºC activity began to decrease, increasing the percentage of time resting.

The level of inactivity at 15ºC comprised nearly half of the total observation time. In a study of the thermal biology of *P. canaliculata*, it was reported that although the time inactive was lower at 15ºC (25%) it steeply increased to 98% at 10ºC (Seuffert et al., 2010). These results suggest that *A. platae* will also be inactive at 10ºC. On the other hand, *A. platae* snails spent nearly half of the time resting, with the foot extended but motionless, regardless of water temperature. Based on the scarce information available, *A. plate* is apparently a slow growing (MJT, unpublished results) species, much less mobile than *P. canaliculata* which spends only 26% of the time on average resting between 15 and 30ºC (Seuffert et al., 2010).

The time spent feeding increased significantly with temperature, reaching its maximum at 25-3ºC and decreasing at 35ºC. This increase in feeding corresponded with the decrease of inactivity above 20ºC and not to a decrease in resting time as it was reported for *P. canaliculata* (Seuffert et al., 2010). Feeding in this species was maximum at 25ºC and began to decrease with higher temperatures. The level of feeding of *Pomacea maculata* Perry, 1810, was minimum at 15ºC and reached its maximum at 30ºC (Gettys et al., 2008). For ectothermic animals, this increase seems to be essential to fulfill the energetic demands of an increased metabolism. The decrease in feeding recorded at 35ºC may indicate that temperatures above 30ºC begin to be suboptimal and even harmful for *A. platae* in the midterm, since growth decreases and mortality reaches its maximum at 35ºC.

The amount of time that *A. platae* individuals spent crawling (average 17.5%) was not affected by temperature. A similar result was reported for *P. canaliculata*, which spent almost the same proportion of time crawling (20.2%) regardless of temperature variation (Seuffert et al., 2010). As it was previously proposed, a plausible explanation is that the snails can crawl faster with increasing temperature. In consequence, they do not spend additional time to move between different places to perform their routine activities.

Ampullariids have both a functional gill and a lung and are considered as amphibious snails (Andrews, 1965; Berthold, 1991; Seuffert & Martín, 2009). The maximum level of lung ventilation in *A. platae* was recorded at 30ºC, probably related to an increase in metabolic rate (Åkerlund, 1969; Freiburg & Hazelwood, 1977) and to the fact that the levels of dissolved oxygen decrease with increasing temperatures, since the aquaria were not artificially aerated. With increasing levels of hypoxia, the significance of aerial respiration increases, becoming a complementary mechanism to branchial intake of oxygen (Seuffert & Martín, 2010). Whereas *P. canaliculata* breathes air between 15 and 35ºC, irrespective of water aeration (Seuffert & Martín, 2009), *A. platae* ventilates the lung only between 25 and 35ºC in non-aerated water. This indicates a lower degree of dependence on aerial respiration in *A. platae*, which is correlated with the smaller size of its siphon (Seuffert & Martín, 2009) and the late development of its lung (Tiecher et al., 2014). The number of pumping movements per ventilation event varied greatly among replicates and showed no relation to temperature. Similarly, no effect of temperature was detected on the number of pumping movements of *P. canaliculata* (Seuffert & Martín, 2009).

Mortality was higher for hatchlings than for adults at all temperatures with the exception of 35ºC, which resulted in high levels of mortality for both groups. Temperature significantly affected the mortality of *A. platae* adults but no differences were detected with the mortality of hatchlings. By contrast, an effect of temperature on the mortality rate of hatchlings was reported for *Marisa cornuarietis* (Linnaeus, 1758) and *P. canaliculata* (Selck et al., 2006; Seuffert & Martín, 2013). The influence of temperature on the mortality rates of *A. platae* hatchlings was probably masked by their high intrinsic mortality rates (MJT, unpublished data), since the survivorship curves of *A. plata* generally conform to Type III (Deevey, 1947). A different pattern has been reported for *P. canaliculata* (survivorship curves Type I; Estebenet & Martín, 2002), which evidences a very low intrinsic mortality of hatchlings and juvenile snails (Estebenet & Cazzaniga, 1992, 1998), even when exposed to extreme temperatures (only 20% after ten weeks of exposure at 35ºC; Seuffert & Martín, 2013).

The shell length of hatchlings attained after 20 days was significantly affected by temperature, being highest at 25, 30 and 35ºC. The smaller snails were obtained in the treatment of 15ºC. Similarly, the maximum shell length of *P. canaliculata* hatchlings after ten weeks was recorded between 25 and 35ºC, being significantly higher than the sizes attained with 15 and 20ºC (Seuffert & Martín, 2013). Hatchlings of *P. maculata* reared at 15ºC did not grow at all and at 35ºC grew significantly less than at 20-30ºC (Gettys et al., 2008). The growth rate of *M. cornuarietis* (Linnaeus, 1758) juveniles significantly decreased with temperature from 28ºC to 22ºC (Aufderheide et al., 2006; Selck et al., 2006). Based on this information we conclude that the optimum temperature range for growth varies a little among these South American ampullariids, but for all of them temperatures below 20ºC will result in a stunted growth.

The risks of predation, severe hypoxia or prolonged air exposure of subaquatic egg masses (McMahon, 1983; Turner et al., 2007; Tiecher et al., 2014) increase with the duration of development for most oviparous snails inhabiting freshwater habitats, which are highly variable and unpredictable as compared with marine ones. Temperature and oxygen are the main environmental drivers of the duration of development although the effects of temperature may be contradictory, increasing mitotic rates but also reducing oxygen concentrations in water. The analyses of this trait would be important to understand the selective pressures that aquatic snails faced during colonization of inland waters from the sea or terrestrial habitats (McMahon, 1983; Dillon, 2000) and also the evolution of aerial egg masses in some lineages (Hayes et al., 2009b).

The duration of embryonic development of *A. platae* predicted by our degree-day models at different temperatures was compared to the durations previously reported for this species and for other species of ampullariids with subaquatic egg masses and also to the duration estimated for the aerial egg masses of *P. canaliculata* using the same methods (Fig. 7). Due to the small size and flat shape of the dishes used for the incubation of egg masses, the effect of using air temperature instead of water temperature in our present estimations was smaller than the effect of the three different calculation methods. The durations reported by Tiecher et al. (2014) for egg masses incubated under controlled temperature (25 ± 2ºC) in the same laboratory and deposited by snails from the same pool as the present study showed a wide variation but the mean duration (10.8 days) agrees with the predictions from our degree-day models. The lower temperature threshold for the embryonic development of *A. platae* is around 16ºC, coinciding with the values of 15.7-16.2ºC estimated for *P. canaliculata*. The cumulative degree-days estimated for *A. platae* ranged between 107.5 and 120.5ºC.d, being also very close to those obtained for *P. canaliculata* (120.7-132.2ºC.d; Seuffert et al., 2012; Fig. 7). On the other hand, up to 1400ºC.d have been estimated for acroloxid limpets inhabiting a high latitude lake (Shirokaya & Röpstorf, 2003).

The duration of development in the temperature range of 25-30ºC for *M. cornuarietis* (Demian & Yousif, 1973), whose subaquatic egg masses are similar to those of *A. platae* (Tiecher et al., 2014), are quite coincident with our estimations. In contrast, the duration of 20 days reported for *M. cornuarietis* in the range of 15-20ºC is much shorter than that predicted by our model as temperature approaches the lower threshold (30 days below 20ºC). Even though it is a different species, it seems odd that development occurs at such low temperatures, given the tropical origin of the snails used in that study (Puerto Rico). Demian & Yousif (1973) only mentioned the extreme temperatures recorded (15-20ºC) so the actual temperature regime under which the eggs developed is unknown. In another study, *M. cornuarietis* eggs took eight days at 28ºC and 17 days at 22ºC to hatch (Aufderheide et al., 2006), which is consistent with the reports for this species in the range 25-30ºC and only two to three days shorter than our estimates for *A. platae*. In contrast, the only report to date of the development of an Old World ampullariid with subaquatic egg masses (*Lanistes ovum* Peters, 1845) indicates that it requires 6-7 days at 26-28ºC (Heiler et al., 2007), which is faster than the development for *A. platae* and *M. cornuarietis* at similar temperatures. Perhaps this is related to the fact that the eggs of *L. ovum* are disposed in a single layer and hence there is a reduced chance of competition for oxygen within the gelatinous egg mass, which usually delays hatching (Moran & Woods, 2007; Lardies & Fernández, 2002). On the other hand, the cleidoic eggs of *P. canaliculata* develop at all temperatures at a slightly lower rate (Seuffert et al., 2012) than the aquatic eggs of *A. platae* studied here. However, the difference may not be significant when taking into account the high inter- and intra-egg mass variation in the duration of development in *A. platae* (Tiecher et al., 2014) and the consequently high standard deviations in the degree-days estimations (Table 2).

Considering the overall results found in this study, we conclude that the optimum temperature for *A. platae* populations would be the range 25-30ºC. These temperatures were the optimum for the performing of this species' routine activities. Temperatures of 35ºC and above would be harmful for the snails since not only the levels of activity dropped above 30ºC but also mortality rates were higher. On the other hand, at temperatures of 20ºC and below the level of general activity rapidly decreased and the snails grew at a slower rate. *Asolene platae* exhibits a wide range of thermal tolerance, similar to that of *P. canaliculata* from Encadenadas del Oeste basin (Seuffert et al., 2010). However, the lower activity levels at low temperatures and the higher mortalities at high temperatures probably explains the narrower latitudinal range on *A. platae* (25 to 35ºS; Castellanos & Fernández, 1976) as compared to *P. canaliculata* (20 to 38ºS; Hayes et al., 2012).

Even though *A. platae* is not regarded as an invasive species, it is entering in the aquarium pet trade and thus there are chances of dispersal to other regions of America and other continents by this mean in the future (Smith, 2006). Specimens are being obtained from natural populations within the natural range of *A. platae* (Lower Paraná river and the southern shore of La Plata river) and offered as Zebra Apple Snails in pet shops and in aquarist web pages (PRM; personal observations).

Temperature has been considered the main determinant of establishment in models predicting the spread of *Pomacea* spp. (e.g., Lv et al., 2009; Byers et al., 2013; EFSA, 2013). Based only on the thermal regime, the potential range of distribution of *A. platae* in the possible scenario of an introduction would be less extensive than the currently invaded regions with *Pomacea* spp. (southern and eastern Asia, southern USA, Pacific Islands and Spain; Rawlings et al., 2007; Hayes et al., 2008; López et al., 2010), nevertheless it could include many temperate and subtropical regions around the world. However, other factors like predation, competition and feeding need to be considered when developing predictive models of the success of its establishment outside of its native range. Knowing the potential range of distribution of *A. platae* would be useful for detecting and controlling incipient invasions of these apple snails.

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TABLE 1. Summary of one-way ANOVAs for activity variables of *A. platae* at different constant temperatures. Dependent variables: time spent inactive (I), ventilating the lung (V), feeding (F), crawling (C) and resting (R); V and I were arcsine square root transformed before the analysis.

	I	V	F	C	R
$F_{4,20}$	5.335	3.765	8.139	1.410	1.304
p	0.004	0.019	< 0.001	0.267	0.302

TABLE 2. Threshold temperatures (ºC) and cumulative degree-days (DD; ºC.d) for the embryonic development of *A. platae*. Threshold temperatures were estimated by different methods: the least standard deviation in days (SD-day), the coefficient of variation in days (CV-day) and the linear regression coefficient method (REG); the values for the cumulative DD are mean ± SD.

Method	Threshold	Cumulative DD
SD-days	16.1	114.9 ± 23.4
CV- days	15.7	120.7 ± 24.5
REG	16.6	107.6 ± 21.9

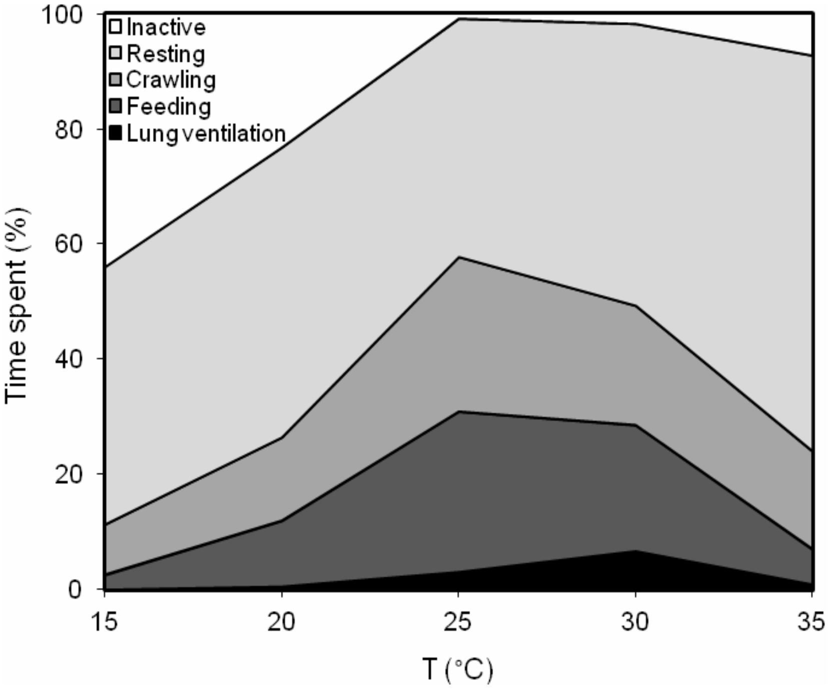
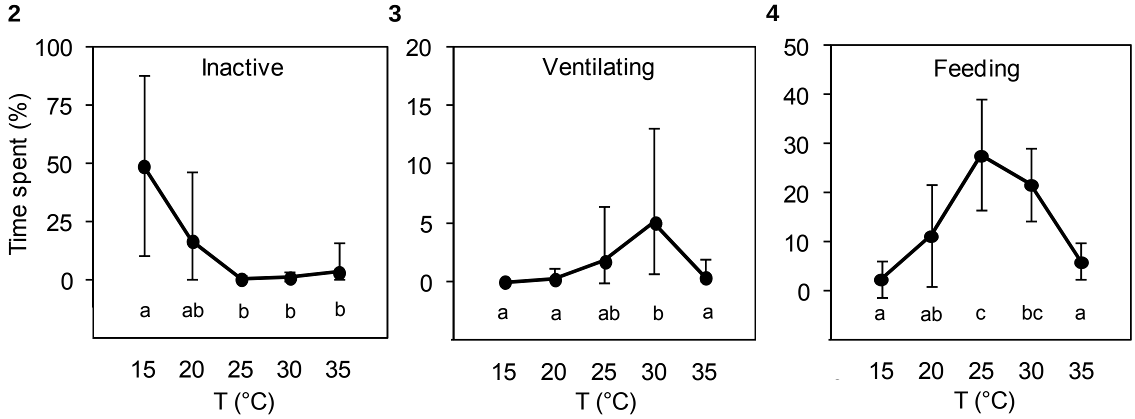


FIG. 1. Time spent (%) in different activities by *A. platae* at different constant temperatures (T).



FIGS. 2-4. Means ± confidence interval (95%) of time spent in different activities by *A. platae* at different constant temperatures (T); different letters indicate significantly different means after LSD test (y-axis are in different scales). Fig. 2: Inactive; Fig. 3: Ventilating the lung; Fig.4: Feeding; for the first two variables back-transformed values are shown.

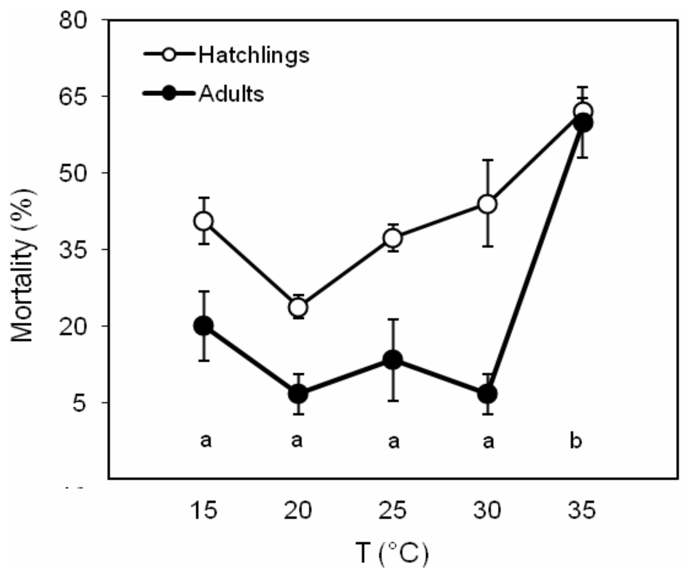


FIG. 5. Means ± confidence interval (95%) of the mortality (percentage of dead snails) of adults and hatchlings of *A. platae* at different constant temperatures (T); different letters indicate significantly different means for adults after LSD test.

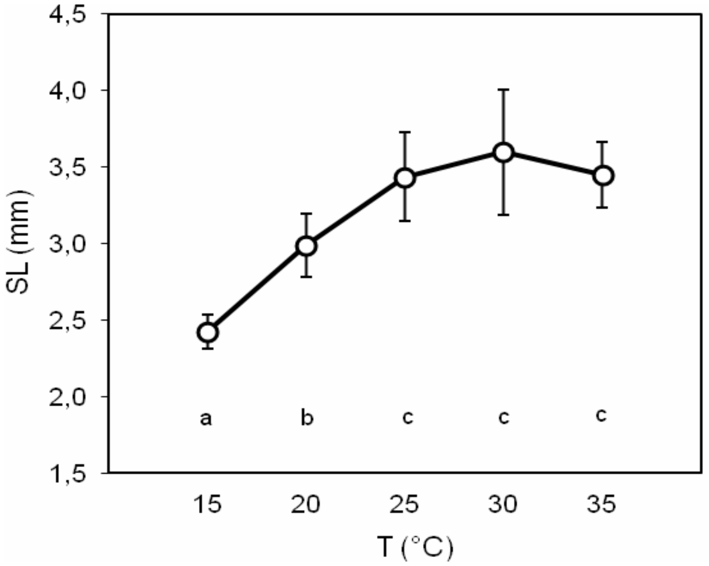


FIG. 6. Means ± confidence interval (95%) of shell length (SL) of hatchlings of *A. platae* attained after 20 days at different constant temperatures (T); different letters indicate significantly different means after LSD test.

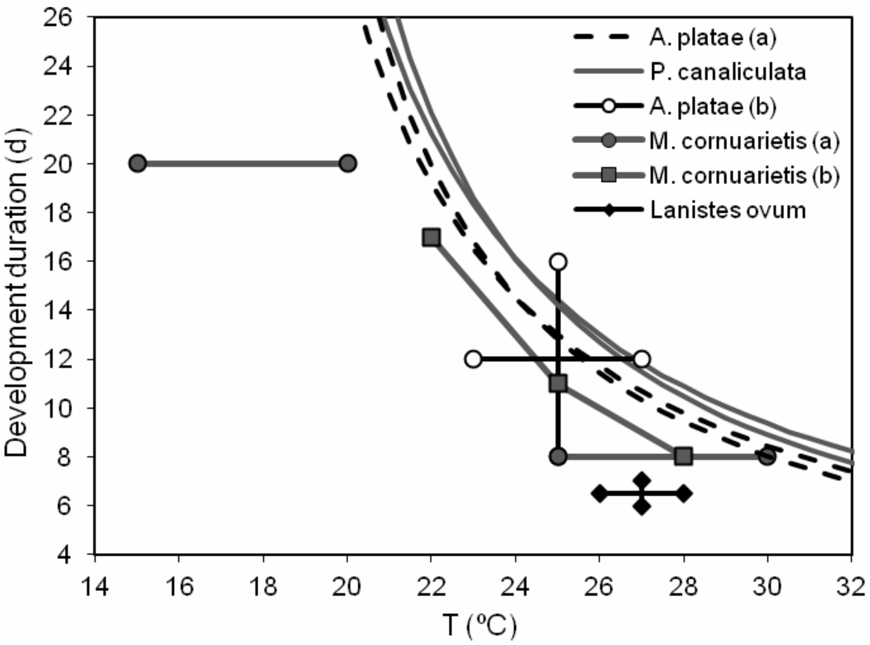


FIG. 7. Duration of embryonic development of *A. platae* at different temperatures (T) [a: calculated with the extreme estimations obtained in this paper (Table 2); b: reported by Tiecher et al. (2014)], durations of two other species with subaquatic egg masses [*Marisa cornuarietis* (a*:* Demian & Yousif, 1973, b: Aufderheide et al. 2006) and *Lanistes ovum* (Heiler et al. 2007)] and those obtained for aerial egg masses of *Pomacea canaliculata* with the same methods used here (bars indicates ranges of temperatures and/or durations).