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Dependence on aerial respiration and its influence on microdistribution in the invasive freshwater snail *Pomacea canaliculata* (Caenogastropoda, Ampullariidae)

María E. Seuffert · Pablo R. Martín

Abstract The invasive Neotropical snail *Pomacea canaliculata* is usually regarded as amphibious, although the relative significance of aerial and aquatic respiration is unknown. To investigate the degree of dependence on aerial respiration and its influences on microdistribution, experiments were performed in the laboratory and under seminatural and natural conditions. Restriction of aerial respiration negatively affected survivorship, activity and feeding, its effects worsening with temperature and water fouling; females were more seriously affected than males although the effect depended on reproductive effort. Snails were unevenly distributed relative to the access to air, both in a stream and in an outdoor tank, being concentrated less than 2–4 m from the nearest emergent substratum. Accessibility to air would be an important trait of waterbodies prone to invasions of *P. canaliculata*, especially in tropical areas. The effectiveness of some control measures could be increased by focusing on areas where snails are concentrated due to their dependence on air.

Keywords Apple snail · Water fouling · Temperature · Surfacing · Survivorship · Activity

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Introduction

Pomacea canaliculata (Lamarck 1822) (Caenogastropoda, Ampullariidae) is a freshwater snail (commonly known as the golden apple snail) native to South America, the range of which extends from the La Plata river basin southwards to the Tandilia and Ventania mountains (Southern Pampas, Argentina; Martín et al. 2001). This macrophytophagous snail was introduced to several southern and eastern Asian countries, becoming a serious pest because of the great damage caused mainly to rice (Cowie 2002; Hayes et al. 2008). Moreover, Carlsson et al. (2004) reported significant ecosystemic changes in natural wetlands resulting from the feeding of apple snails. *P. canaliculata* and other South American apple snails were introduced to North America and Pacific islands where many new areas are susceptible to being invaded (Rawlings et al. 2007; Karatayev et al. 2009). *P. canaliculata* is the only aquatic snail listed among the "100 of the world's worst invasive alien species" (Lowe et al. 2000).

The lack of a clear comprehension of the factors limiting the distribution of invasive species is often an obstacle to the development of efficient management strategies (Guo 2006). The distribution of *P. canaliculata* is still increasing worldwide and many countries in tropical, subtropical and temperate regions are threatened by potential invasion (Baker 1998; Rawlings et al. 2007). Moreover, many potential habitats within the invaded areas have not been reached yet

(Ito 2002; Kwong et al. 2008), so it is crucial to predict the main features of waterbodies where apple snails could produce the greatest damage. For instance, *P. canaliculata* was typically considered as a lentic species but recent surveys in Southern Pampas showed that it inhabits and thrives in lotic habitats too (Martín et al. 2001; Martín and De Francesco 2006). Ecological and behavioral studies are heavily required for a sustainable management of invasive apple snails and to minimize their spread (Ranamukhaarachchi and Wickramasinghe 2006).

Several studies have addressed the influence of environmental factors on the reproductive activity of *P. canaliculata* (e.g. Albrecht et al. 1999, 2005) or its performance and survival (e.g. Yusa et al. 2006a; Matsukura and Wada 2007), but the latter have focused mostly on oversimplified or artificial habitats in invaded areas. Few studies explore the factors determining the presence of *P. canaliculata* across different waterbodies, both in their native range (Martín et al. 2001) and in invaded regions (Kwong et al. 2008). Studies at the microhabitat level are even fewer (Ito 2002) and were performed in irrigation schemes and paddy fields. However, the possible influence of individual behavior on *P. canaliculata* distribution has not been investigated yet.

Ampullariids are regarded as amphibious because they possess both a well developed gill and a lung and they often perform aquatic and aerial respiration simultaneously (Andrews 1965; Berthold 1991; McClary 1964; Seuffert and Martín 2009). However, the relative significance of the two types of respiration varies widely, *Pomacea* being one of the genera most specialized in atmospheric air exploitation (Andrews 1965). The degree of dependence on aerial respiration has not been investigated thoroughly in ampullariids, though Burky and Burky (1977) restricted the access to air in *Pomacea urceus* (Müller 1774) for 5 days in aerated aquaria and observed that all snails survived in apparent good condition.

As long as temperature is high enough to keep it active, *P. canaliculata* routinely contacts the water surface with its siphon to ventilate the lung and the interval between emersions decreases sharply with temperature (Seuffert and Martín 2009). Lung ventilation frequency in *Pomacea* also increases with decreasing dissolved oxygen (McClary 1964; Burky and Burky 1977). Therefore, temperature and dissolved oxygen levels presumably affect the degree of dependence on aerial respiration relative to aquatic respiration. Burky and Burky (1977) reported that *P. urceus* can detach from the bottom and float in order to ventilate the lung; Heiler et al. (2008) described a controlled ascent and descent in the water column in aquaria for *P. canaliculata* but we have never observed this behavior (Seuffert and Martín 2009). According to our observations, the snails crawl in search of access to air every time they ventilate the lung. If a circumstantial lack of access to air negatively affects the performance or interferes with the behavior of these amphibious snails, they would remain near the shore or other emergent substratum. Darby et al. (2002) and Karunaratne et al. (2006) suggested that the need to surface to breathe contributes to restrict the distribution of the Florida apple snail *Pomacea paludosa* (Say 1829).

In previous work (Seuffert and Martín 2009) we studied the effect of temperature, size and sex on several aerial respiration variables. The aims of the present study were to determine the degree of dependence on aerial respiration in *P. canaliculata*, evaluating the effects of restricting access to air on activity and survivorship at different temperatures and water conditions, and subsequently to investigate the possible influence of periodic air replenishment on its microdistribution.

Materials and methods

Origin, maintenance and sexing of the snails

All the snails used in the trials were born and reared in the laboratory; the original snails were obtained from a population in Curamalal stream (37°14′31″S, 62°08′04″W), a watercourse originating on the northern slope of the Ventania mountains, Buenos Aires Province, Argentina. Prior to their use in each trial, 15 snails were maintained in 20 l aquaria with $CaCO_3$ saturated tap water at 25 ± 2°C and fed on fresh lettuce. Males were recognized by observation of the testicle through the translucent shell (Takeda 1999) or a humped operculum (Estebenet et al. 2006); snails without these male traits were considered sexually undifferentiated if smaller than 25 mm and considered as females if bigger than 25 mm.

Effects of short term restriction of aerial respiration

The effects of short term restriction of aerial respiration on the snails' activity and feeding rate were investigated in 24 l aquaria (20 × 30 × 40 cm) divided vertically by a plastic grid (1 × 1 cm holes) into two compartments (one with and one without access to air). Accessibility to air (A) was controlled with another plastic grid but in a horizontal position. In one compartment the grid was fixed at the water surface, allowing the snails to ventilate the lung by extending the siphon through the holes; in the other compartment the grid was fixed 10 cm below the water surface, a depth known to be greater than the reach of the siphon (Seuffert and Martín 2009). The trials were performed simultaneously at five different temperatures (15, 20, 25, 30 and 35°C). The total duration of the trials was 5 days and they were repeated on three consecutive weeks, using different snails each week.

Five differentiated snails (randomly chosen from a stock with shell lengths (L) ranging from 27 to 47.5 mm) individually marked with synthetic polish were placed in each compartment. Every morning a pre-weighed slice of the same carrot was put in both compartments of each aquarium and weighed the next morning to estimate the amount ingested. The aquaria were observed for a period of 30 min twice a day (once in the morning and once in the afternoon), recording the time spent in different activities and the condition of each snail. Snails were considered active when feeding, crawling, mating, clinging to the sides of the aquarium or ventilating the lung (only for compartments with access to air) and inactive when withdrawn inside the shell or comatose (see below).

The food ingestion rate of each compartment was calculated as the carrot consumed divided by the number of snails alive in the morning. To reduce the variability due to possible differences in water gain between different carrots, the difference in ingestion rates (DIR, g snail^{-1}) between both compartments of the same aquarium were calculated for the 5-day period. The time spent active (TA, %) was calculated as the accumulated time active over the last 4 days relative to the total observation time (the first day was considered an adaptation period) and the values were averaged for the five snails in each compartment. Some snails died during these trials, so the average

survival time (ST, days) was calculated for each compartment; dead snails were retrieved from the aquaria as soon as death was confirmed (see below). The time spent in the upper half of the aquarium (up to a depth of 15 cm) relative to the total time active (TU, %) and the time spent crawling in the upper part relative to the total time in the upper part (TC, %) were also estimated. For the compartments with access to air, lung ventilation frequency (VF, ventilations h^{-1} snail^{-1}) was calculated for the entire 5 days as the average of the number of ventilations per compartment divided by the number of live snails during each observation period.

The same scheme (five temperatures × two accessibility conditions × three weeks) was repeated under two different conditions: with and without artificial aeration, provided through an electric pump with a diffuser fixed to the lower side of the vertical division of the aquaria.

Effects of long term restriction of aerial respiration

The effects of long term restriction of aerial respiration on the survival and morbidity of snails differentiated as males or females ("differentiated" snails) was explored in 3 l individual aquaria kept in a room at 25°C. Accessibility to air was controlled in the same way as in the previous trials and the aquaria were not provided with artificial aeration.

Twelve males and twelve females were maintained under each accessibility condition for 3 weeks; they were checked daily to record their behavior and condition according to the following categories: normal (crawling, feeding, clinging to the lateral walls of the aquarium and withdrawn inside the shell) and comatose (lying motionless with the foot curled ventrally and facing up; McMahon 1983). The comatose snails were touched with a probe pushed through the grid holes in order to check if they were actually dead, in which case the survival time (ST, days) was recorded. The frequency of comas (FC, %) was calculated as the number of observations in which each snail was found comatose relative to the total number of observations.

The same factorial design (two sexes × two accessibility conditions × twelve replicates) was repeated for three different lots of snails. The snails in the first and second lots (males: L = 35.5–42.7 mm; females:

$L = 38.7–48.3$ mm) were all virgin (reared in individual aquaria since they could be sexed) and were fed lettuce daily (ad libitum) or not fed, respectively. The snails form the third lot (males: $L = 33.1–41.5$ mm; females: $L = 29.5–44.0$ mm) were not virgin (reared in groups of 15–20 snails in 20 l aquaria; females had been laying eggs for at least 3 months) and no food was provided during the trials. Half of the water in the aquaria with food was changed once a week by siphoning (with the snails prevented from making contact with the air) to avoid an excessive fouling of water which would have overemphasized the effect of restriction of aerial respiration.

Dispersal and microdistribution relative
to access to air

To predict the distance a snail could move away from a substratum suitable for lung ventilation before returning back to renovate the air supply (distance to the point of no return) we used both the speed of locomotion and the time between emersions.

The speed of locomotion of snails (15–50 mm of shell length) was measured at four temperatures (15, 20, 25 and 30°C) in rectangular aquaria (37 × 58 cm) provided with artificial aeration and filled to a depth of 7 cm with tap water above a 1 cm sand layer. For 72 h prior to the trials the snails were acclimatized at the desired temperature; they were not fed in order to promote a maximum locomotion speed when lured with a lettuce leaf. Since *P. canaliculata* is capable of detecting food by chemoreception (Estebenet 1995), the bait was moved at a constant rate in a straight line in front of the snails, inducing them to follow it. The distance covered by each snail and the time spent to do it were measured; only distances greater than 10 cm were considered. This procedure was repeated two to five times for each snail and the maximum speed (MS, mm s^{-1}) was calculated. Values of lung ventilation frequency at different temperatures for snails of different sizes were obtained in trials described by Seuffert and Martín (2009).

The point of no return was estimated using the regression coefficients of maximum speed of locomotion and lung ventilation frequency for the different temperatures and snail sizes. The distance to the point of no return (D, m) depends on the time

between emersions (the inverse of VF) and MS; this was estimated by the following equation:

$$D = 0.5 \cdot VF^{-1} \cdot MS.$$

The predicted distances of the point of no return were tested outdoors in a circular experimental tank with a radius of 14 m and a maximum depth of 1 m; the bottom slope was 33% in the first 3 m from the shore and nil in the central part. The concrete bottom was covered with a 2–3 cm-deep layer of very fine sediments with a high content of organic matter and microscopic algae. A total of 60 snails, 15 males, 15 females (shell length 35–40 mm) and 30 undifferentiated (20–25 mm) were marked with different colors (painting the whole shell to facilitate detection) and released randomly in the bottom of the tank. After a day of acclimation, the whole bottom was systematically explored from the water surface (with the aid of a small raft and a diving mask); the distance to the shore of each snail was recorded over 2 weeks (thrice a day during the first 2 days and once every 2 days thereafter); water temperature was recorded on each occasion.

The influence of the dependence on aerial respiration on microdistribution was also investigated in a natural population of *P. canaliculata* inhabiting a slow flowing reach in Pigüé stream (37°24′32″S, 62°35′46″W, originated on the northern slope of the Ventania mountains, Buenos Aires Province, Argentina). The reach was 56 m long, 11–15 m wide and 1.1–1.5 m maximum depth; water velocity was below the threshold measurement of our flowmeter (0.1 m s^{-1}; Global Water® FP101). Water temperature was 22.8–24.8°C (mean 22.9°C), pH was 8.0, conductivity 0.78 mS cm^{-1}, turbidity 91 NTU and dissolved oxygen was near or above saturation levels (mean 101.35%, range: 98.9–104%; all variables measured with a multimeter Horiba® U10). The plants present in the reach were mostly a water primrose (*Ludwigia* sp.) and in the shallower areas a small sedge (*Cyperus* sp.) and water pennywort (*Hydrocotyle bonariensis*). Snails were searched for by breath-hold diving along transects perpendicular to the shore sequentially located in an upstream direction. For each snail found ($n = 40$) the distances to the shore and to the nearest emergent substratum and the substratum type (mud, limestone, macrophyte, etc.) were recorded. The same variables were recorded at systematic points ($n = 160$) spaced 1.5 m

apart on 18 transects evenly distributed along the reach, to estimate the availability of places with different characteristics.

Statistical analysis

To investigate the effects of short term restriction of aerial respiration on both time spent active and survival time at different temperatures, split-plot ANOVAs were performed (main factor: temperature, secondary factor: accessibility to air, blocks: weeks). In cases in which time spent active was significantly affected by accessibility to air, the time spent in the upper part of the aquarium and the time spent crawling relative to that time were investigated using similar analyses. To test for differences in ingestion rates between the two compartments of the same aquarium at different temperatures, one-way ANOVAs were performed, as well as for the effect of temperatures on lung ventilation frequency (only for snails with access to air). In all cases, separate analyses were performed for each aeration condition. These variables proved to be homoscedastic when investigated with Bartlett tests.

The effect of temperature and size on maximum speed of locomotion and lung ventilation frequency was explored through stepwise linear multiple regression. Dependent variables were square root transformed ($\sqrt{Y}$) and a surface response model (Weisberg 1985) including linear, quadratic and interaction terms for the independent variables was applied:

$$\sqrt{Y} = B_0 + B_1 \cdot T + B_2 \cdot T^2 + B_3 \cdot L + B_4 \cdot L^2 + B_5 \cdot T \times L.$$

To test for the effect of sex a similar analysis was performed including only differentiated snails and incorporating a term for sex as a dummy variable (Weisberg 1985).

To test for differences between the environmental variables recorded at points with snails and the systematic points in the Pigüé stream (Utzinger et al. 1997), Kolmogorov–Smirnov tests and X^2 Independence tests were performed for continuous and discrete variables, respectively.

Results

When artificial aeration was provided the mean values of time spent active were slightly lower for the snails without access to air at all temperatures but the lowest (15°C). Deaths were recorded only at the two highest temperatures for snails without access to air; however, no significant effect of accessibility to air, temperature or their interaction was detected either on time spent active or survival time (*P*-values ranged from 0.0641 to 0.3231). The difference in ingestion rate between snails with and without access to air was not significantly different from zero and was not affected by temperature ($F_{4,10} = 0.7005$, $P = 0.6090$).

In the aquaria without artificial aeration the time spent active and survival time were significantly affected by accessibility to air and by temperature but the effect of the former was not the same at the different temperatures tested (Table 1; Fig. 1a, b). A significant decrease in activity in snails without access to air, relative to those with it, was detected for temperatures above 15°C (one tail LSD tests, $P < 0.001$ in all cases) while survival time decreased above 20°C (one tail LSD tests, $P < 0.001$ in all cases). For both variables, the differences between snails with and without access to air increased gradually with temperature. The difference in ingestion rate between snails with and without access to air showed a significant effect of temperature (Fig. 1c) but was different from zero only between 20 and 30°C, attaining a maximum at 30°C.

Under non-aerated conditions, the percentage of time spent in the upper part of the aquarium was generally higher for the snails with access to air (Table 1; Fig. 2a) but only proved to be significant at the highest temperature (one tail LSD test, $P = 0.0042$) The snails without access to air increased the time spent crawling as temperature increased while for those with access to air this activity was less important and almost constant relative to temperature (Fig. 2b).

Lung ventilation frequency for the compartments with access to air increased 15.5- and 7-fold when temperature increased from 15 to 30°C both under non-aerated and aerated conditions, respectively, though it was significant only in the former ($F_{4,10} = 10.2648$, $P = 0.0014$; $F_{4,10} = 2.8886$, $P = 0.0791$, respectively; Fig. 3). Frequencies were two- to seven-fold

Table 1 Summary of statistical analysis for the trials of short term restriction of aerial respiration in *Pomacea canaliculata* without artificial aeration: split-plot ANOVAs for time spent active (TA), survival time (ST), time spent in the upper part of the aquarium (TU) and time spent crawling relative to the time in the upper part of the aquarium (TC) and one-way ANOVAs for difference in ingestion rate (DIR)

Variables	MS error	T	A	T × A
TA	98.6359	$F_{4,8} = 7.3077$	$F_{1,10} = 165.5496$	$F_{4,10} = 20.5908$
		$P = 0.0088$	$P < 0.0001$	$P = 0.0001$
ST	0.0447	$F_{4,8} = 174.0437$	$F_{1,10} = 272.2463$	$F_{4,10} = 50.3060$
		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
DIR	0.2740	$F_{4,10} = 21.3047$		
		$P = 0.0001$		
TU	162.2182	$F_{4,8} = 2.2133$	$F_{1,10} = 6.3774$	$F_{4,10} = 2.3761$
		$P = 0.1575$	$P = 0.0301$	$P = 0.1217$
TC	82.5399	$F_{4,8} = 4.7449$	$F_{1,10} = 33.4105$	$F_{4,10} = 7.4936$
		$P = 0.0295$	$P = 0.0002$	$P = 0.0047$

Fixed factors: temperature (T, main factor), accessibility to air (A, secondary factor) and interaction (T × A)
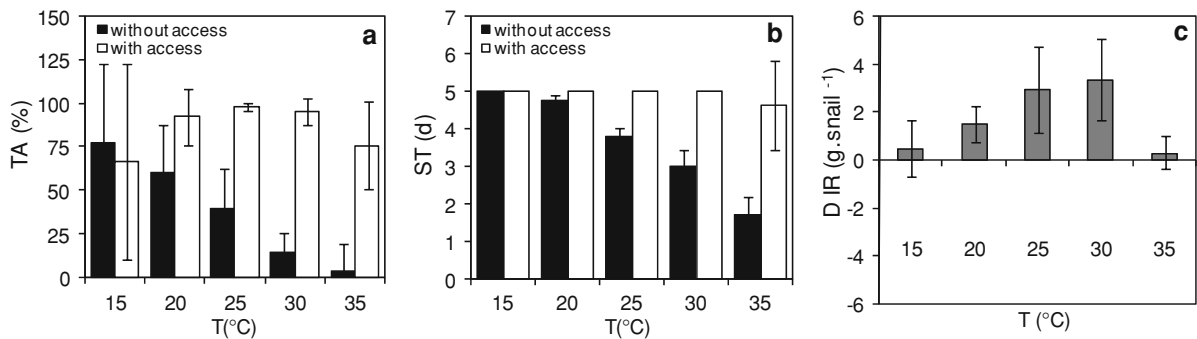

Fig. 1 Performance of *Pomacea canaliculata* at different temperatures (T) in the trials of short term restriction of aerial respiration without aeration; **a** time spent active (TA), **b** survival time (ST) and **c** difference in ingestion rate (DIR); TA and ST were recorded in the compartments with and without access to air while DIR was calculated between both compartments (bars are means ± 95% confidence intervals)
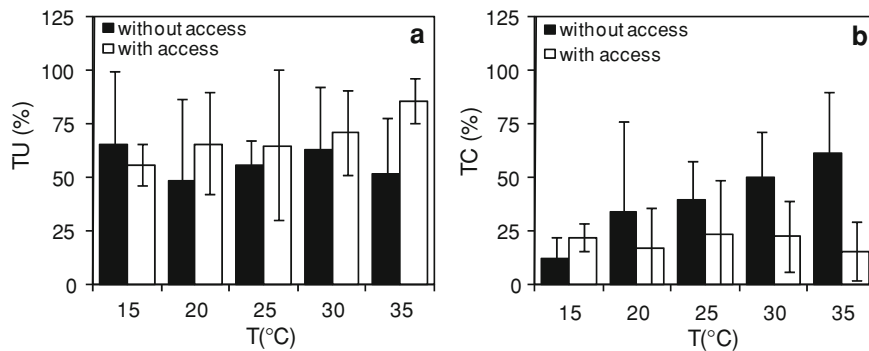

Fig. 2 Performance of *Pomacea canaliculata* at different temperatures (T) in the trials of short term restriction of aerial respiration without aeration; **a** time spent in the upper part of the aquarium (TU), **b** time spent crawling relative to the time in the upper part of the aquarium (TC); bars are means ± 95% confidence intervals

higher under non-aerated conditions at all temperatures except 15°C; under non-aerated conditions, ventilation frequency dropped significantly at 35°C (one tail LSD test: $t = 1.32$, $P = 0.0184$).

In the long term trials no snail with access to air died or entered coma in any of the three lots (Fig. 4), independently of whether water was partially changed (lot fed on lettuce) or not. In the trials with virgin snails, both with food (Fig. 4a) or without it (Fig. 4c), all females died within 2 and 4 days with restriction of access to air, respectively; half of the males in the trial with food died during the first 6 days and two of the males without food died on the ninth day. Only one female died after 3 days in the trial performed with mated snails (Fig. 4e). The relative time in coma was not different between sexes in the trials with virgin snails, being higher in those with food (59.2%; Fig. 4b) than in those without it (12.1%; Fig. 4d). Mated snails spent on average 14.2% of the duration of the trial in coma but in this lot values for females were significantly higher than for males (23.9 and 4.4%, respectively; Fig. 4f; LSD test for unequal variances: $t = 3.12$, $P = 0.007$). In several cases the snails alternated repeatedly between comatose and normal states. After the end of the trials, the survivors were returned to common tanks with access to air and they behaved normally (i.e. no further comas were observed).

The stepwise regression models developed for maximum speed and lung ventilation frequency for differentiated snails did not include sex and,
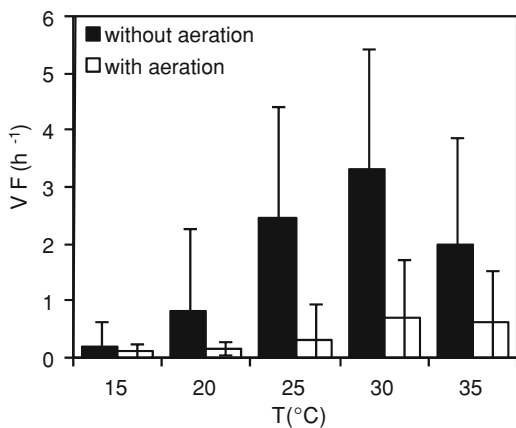
accordingly, it was not considered in the estimation of the distance to the point of no return. The back-transformed equations for these variables were:

$$MS = (0.0031 \cdot T + 0.0004 \cdot T \times L - 0.450)^2$$

$$VF = (0.0020 \cdot T^2 + 0.0015 \cdot T \times L - 0.767)^2.$$

Both maximum speed of locomotion and lung ventilation frequency increased with temperature (Fig. 5a, b), its effect being more important in big snails, as indicated by the positive interaction term. The model predicts that the distance to the point of no
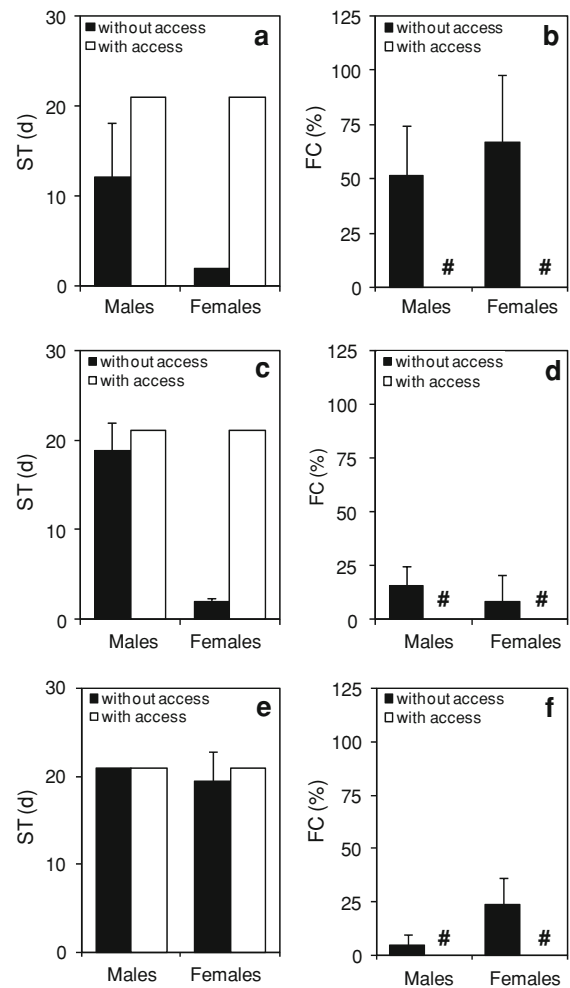

Fig. 4 Performance of males and females of *Pomacea canaliculata* in the trials of long term restriction of aerial respiration (survival time [ST] and frequency of comas [FC]); **a**, **b** virgin snails with food; **c**, **d** virgin snails without food; **e**, **f** mated snails without food; bars are means ± 95% confidence intervals (#: none of the snails with access to air entered coma)
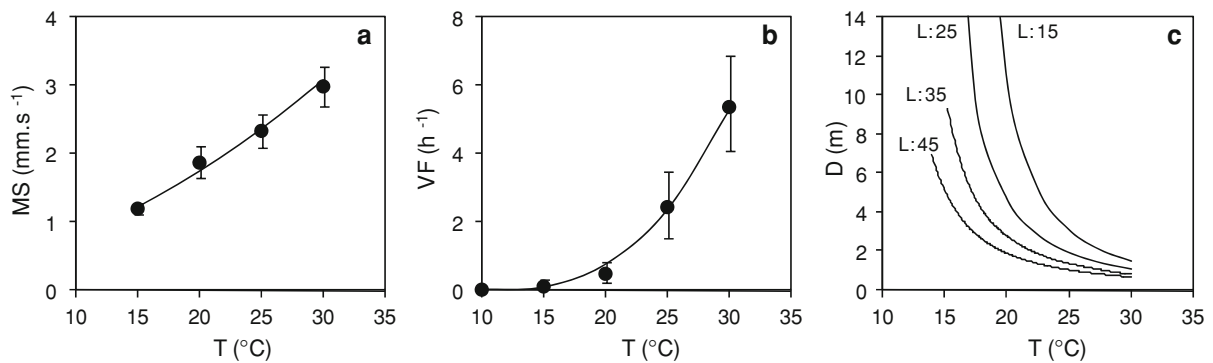
Fig. 5 Locomotion and aerial respiration of *Pomacea canaliculata* at different temperatures (T); **a** maximum speed (MS), **b** ventilation frequency (VF) (means ± 95% confidence intervals, curves were estimated with the correspondent regression models), **c** distances to the point of no return (D) predicted by the quantitative model generated with MS and VF for different temperatures and shell lengths (L)

return decreases with temperature and snail size (Fig. 5c).

No significant differences were found in the experimental tank between males and females in the mean distance to the shore (LSD tests, $P > 0.65$ in each of the inspections, after Bonferroni correction for 13 tests) so both sexes where pooled in a single category (sexually differentiated snails). During the experiment, the distances to the shore of both differentiated and undifferentiated snails changed considerably relative to the initial distribution. The median distance for both groups decreased during the

first week, being more evident in the differentiated snails (Fig. 6), although these differences were significant only for the first day after Bonferroni correction (Mann–Whitney test: $U = 13$, $P = 0.004$). The undifferentiated snails grew considerably, as exhibited by the extent of new, unpainted shell, approaching the smaller differentiated snails in size. The distances to the point of no return predicted by the model according to water temperature, were lower than those observed during the first week for both groups of snails but afterwards only for differentiated ones.
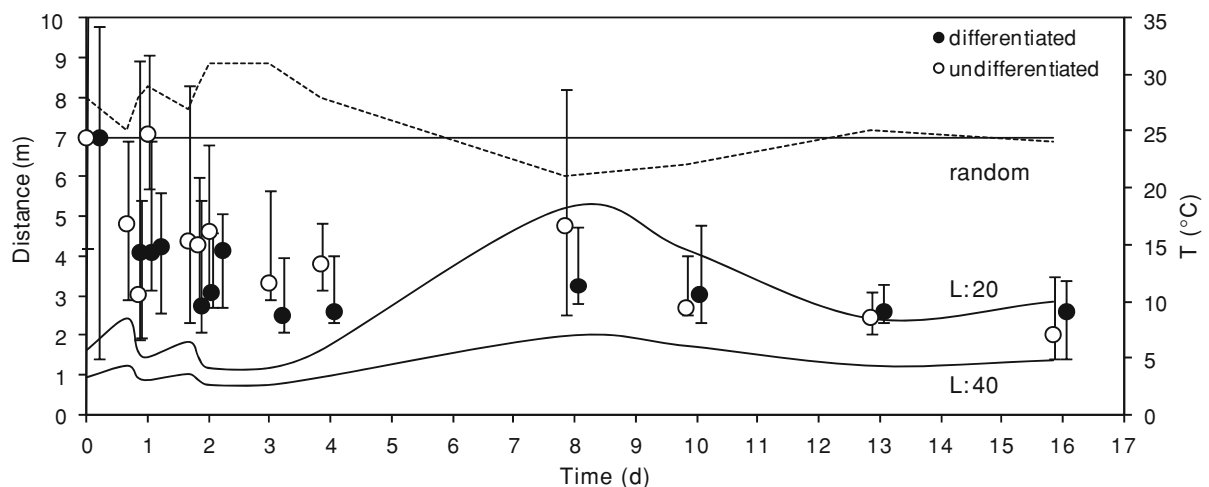

Fig. 6 Distances to the shore of differentiated and undifferentiated *Pomacea canaliculata* over time in an outdoor tank (medians ± 1 quartile). *Solid lines* correspond to the distances predicted by the point of no return model (for shell lengths [L] of 20 and 40 mm) and expected in case of random distribution relative to the shore. *Dotted line* shows temperature (T) in the tank

Fig. 7 Frequency distribution of distances to the shore of *Pomacea canaliculata* after their random release in an outdoor tank; **a** after 2 days, **b** after 16 days
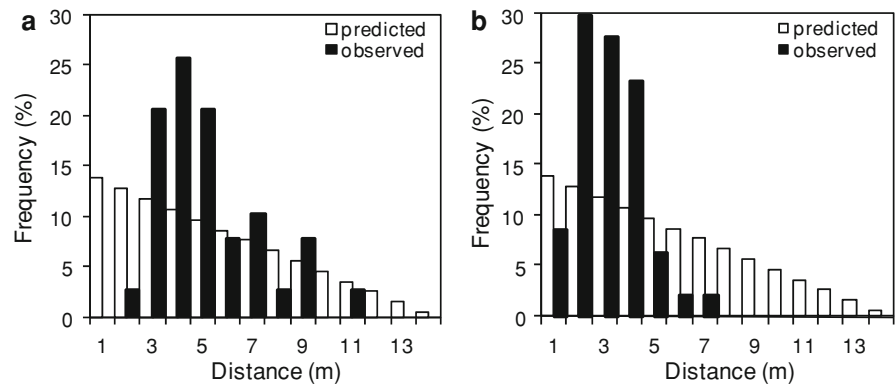
Considering the relative area of the different concentric rings of the tank and assuming independence of snail movement relative to access to air, the predicted relative frequencies decrease linearly with the distance to the shore (Fig. 7). During the first few days frequencies were much higher than predicted at short and intermediate distances from the shore (2–5 m) although snails were very rare in the two outermost meter rings (e.g. day two; Fig. 7a). By day 16 most snails were crowded within the first 4 m from the shore (89.4% as compared to the expected 49.0%; Fig. 7b) although they were still relatively sparse in the first meter.

In Pigüé stream the frequency distribution of distances of snails to the shore (Fig. 8a) and to the nearest emergent substratum (Fig. 8b) were significantly different from those corresponding to the systematic points ($Z = 1.556$, $P = 0.016$ and $Z = 1.98$, $P = 0.001$, respectively). The snails (mean shell length: 40.36 mm, range: 15–59 mm) were more frequent at distances from the shore of 2–3 m and 74% of them were nearer than 3 m; however the first meter was considerably less used relative to its availability. Thirty-four percent of the bottom of the

reach was located at more than 4 m from the nearest shore but only 7.5% of the snails were located in this zone. Half of the snails were located at less than 1 m from the nearest emergent substratum, which was a macrophyte in 77.5% of cases; nevertheless, only 15% of the snails were actually crawling on a plant (*Ludwigia* sp. in all cases). The distribution of snails relative to the bottom types (mud vs. limestone) was proportional to their availability ($X^2 = 0.137$, $P = 0.711$), as was their distribution relative to presence versus absence of plants ($X^2 = 0.286$, $P = 0.593$).

Discussion

Short term trials showed that under unaerated conditions restriction of aerial respiration negatively affects survivorship, activity and ingestion rates and that its effect increases with temperature; however, these effects were almost negligible under aerated conditions. Lung ventilation frequency increased with temperature and lack of aeration for snails with access to air; for those snails without access to air,

Fig. 8 Frequency distribution of distances of *Pomacea canaliculata* in the Pigüé stream (Buenos Aires Province, Argentina); **a** distances to the shore, **b** distances to the nearest emergent substratum (available distances correspond to the series of systematic points)
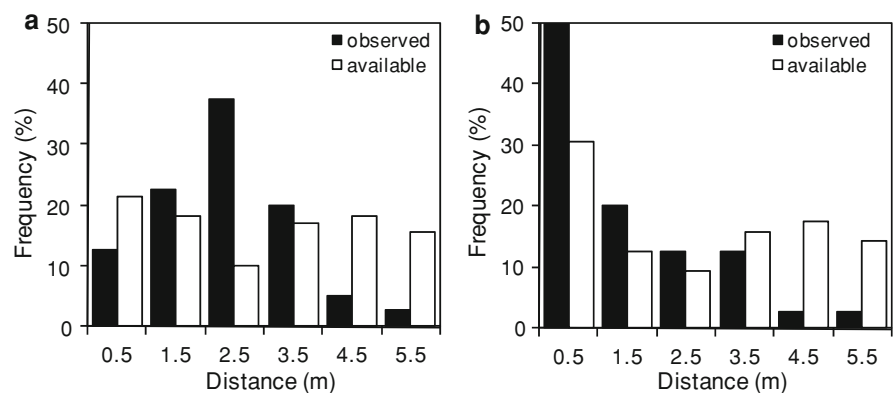
crawling near the surface increased. Most snails without access to air died or entered coma in the long term trials, although most deaths occurred during the first week. Females were more seriously affected than males; however, the effect of restriction of aerial respiration was also influenced by water fouling (by faeces and food remains) and by previous reproductive effort. Neither mortality nor morbidity was observed in the snails with access to air in any of the trials. Snails were unevenly distributed relative to access to air, both in the stream and the outdoor tank, being concentrated at less than two and four meters, respectively, from the nearest emergent substratum.

The influence of restriction of aerial respiration on *P. canaliculata* performance in short term trials was almost negligible under aerated conditions as compared to non-aerated ones. The independence of snails from atmospheric air supplies under aerated conditions can be attributed to a high level of dissolved oxygen (DO) or to a low fouling of water. However, DO saturation levels were not very different in our aquaria with and without aeration (94 and 89% on average at 25°C, respectively) and, in addition, apple snails are able to maintain their oxygen consumption down to DO saturation levels of 21–50% solely by branchial respiration (Hanley and Ultsch 1999; Ramakrishnan 2007). In *Pomacea paludosa* the intensity of aerial respiration increases when irritating substances or suspended particles are present in water (McClary 1964); similarly, in our study, a difference of less than 6% in DO saturation was correlated to a two- to seven-fold higher lung ventilation frequency in non aerated aquaria relative to aerated ones. This evidence suggests that branchial ventilation or perfusion are negatively affected by water fouling. Hence, the compensation by the gill for the reduced oxygen intake when access to air is not possible is hindered. Even though the degree of fouling in the long term trials was much lower, its effect on snails without access to air was also apparent since virgin adults without food showed higher survivorship than those with it.

Under non-aerated conditions without access to air, either with or without food, deaths and comas were frequent. The reduced oxygen intake by the gill, when access to air is not possible and water is fouled or stagnant, seems to be responsible for the lethal and sub-lethal effects observed; these effects worsen with increase in temperature due to a decline in dissolved oxygen and, specially, to an increase in metabolic rate (Seuffert and Martín 2009), highly correlated with an increase in activity levels (our study; Heiler et al. 2008). In *Pomacea lineata* exposure to severe hypoxia (DO saturation levels between 22.12 and 4.42%) for 1 h leads to an oxygen debt that is recovered in an equivalent period, even when both branchial and aerial respiration are allowed (Santos et al. 1987). Dissolved oxygen was not so low in our trials but the reduced oxygen intake due to water fouling and restricted aerial respiration probably provoked the harmful effects reported here. Ramakrishnan (2007) suggested that in *Pomacea insularum* (d'Orbigny 1835) the increase in metabolic rate with temperature cannot be fulfilled due to respiratory functional limitations, leading to a heavier reliance on anaerobic pathways instead of a metabolic depression. The increase in time spent crawling near the surface suggests that metabolic depression is not the strategy adopted to endure a few days of reduced oxygen intake by *P. canaliculata* either. *P. paludosa* also tends to crowd under a barrier located below the surface (McClary 1964). In *P. canaliculata* the lesser time spent active in snails without access to air is apparently the result of an increase in the duration of resting periods after periods of active crawling or feeding. Probably the time spent crawling near the surface trying to access to air limited the time devoted to feeding on the bottom, hence decreasing the ingestion rate.

In long term trials females always suffered stronger noxious effects of restricted aerial respiration than males, dying or entering coma more frequently. The massive albumen gland of females protrudes from the pallial cavity's floor (Andrews 1965) and probably interferes with branchial ventilation currents (Seuffert and Martín 2009). Also, virgin females performed worse than mated ones, despite the fact that virgins were reared individually and mated ones under crowded conditions, the latter being smaller probably due to feeding interference and accumulation of inhibitory substances (Estebenet and Martín 2002). On the other hand, aerial respiration demands, and hence the stress resulting from restriction of access to air, would be expected to be greater in bigger snails since lung ventilation frequency increases with size in *P. canaliculata* (Seuffert and Martín 2009). However, in the overlapping size range of mated and virgin females

without food (38.7–44 mm) all virgin snails died while only one out of nine died among the mated ones. This lack of an evident effect of size renders the reproductive female function as the most probable cause for these differences.

In the experimental tank the distance of snails to the shore decreased during the first week. The general change in their distribution towards the shore was relatively slow, taking place only after 3 days, perhaps due to a lack of orientation cues. *P. paludosa* follows depth gradients to orientate to deep waters (Darby et al. 2002), but the tank's slope was nil in more than 67% of its bottom. Chemical cues (Estebenet 1995) released by riparian or emergent vegetation could be used to locate an access to air in the wild, but this was not the case, since algae and detritus were homogeneously distributed in the tank. A more likely mechanism of orientation to water surface or to the shore is the following of its own mucus trail or that of a conespecific, as described by Deliagina and Orlovsky (1990) in *Planorbis corneus*. *P. canaliculata* is able to detect conespecific mucus trails (Takeichi et al. 2007) but, during the first days, the trails on the tank's bottom were probably scarce and mostly random.

The point of no return model predicts that the degree of restriction of *P. canaliculata* distribution will increase with temperature since its effect on speed of locomotion is linear whereas that on ventilation frequency is quadratic. Compared to the tank's results, the model overestimated the constraints that the dependence on aerial respiration can impose on snails' microdistribution. A feasible explanation may be that the lung ventilation frequency equation used in this model was estimated in experiments performed in small aquaria (20 × 40 × 30 cm) where access to air was very much easier (Seuffert and Martín 2009) and snails were probably able to follow their own trails. On the other hand, the model assumes that a snail will not survive if it fails to locate an access to air before it is time to ventilate the lung again, possibly overestimating the detrimental effects of aerial respiration restriction. The comatose state observed in our long term trials is similar to the reversible "heat coma" of freshwater pulmonates (McMahon 1983) and *P. insularum* (Ramakrishnan 2007). In our trials the snails alternate frequently between this state and crawling or feeding, suggesting that *P. canaliculata* can make use of this

behavior to endure a circumstantially noxious situation (e.g. hypoxia, high temperatures and water fouling) when access to air is not possible; the recurrence of comas during 3 weeks has apparently no subsequent negative effects.

In the Pigüé stream the snails were more concentrated near emergent substrata (mostly plants) than near the shore itself. Three main reasons can explain this pattern: the use of plants as food, as an oviposition substratum or as a means to access the surface to breathe. However, neither snails eating the plants nor the conspicuous aerial egg masses were observed, suggesting that at least in this situation the snails remain close to the plants to use them as a breathing substratum. The snails were rarely found in the stream shore (where most plants were located) and in the outermost portion of the tank, probably due to high temperatures or the activities of birds that may have triggered escape responses (e.g. dropping-off from substrata; Bourne 1993). In fact, empty shells left by *Pomacea*-specialized predators (Limpkins *Aramus guarauna* and Snail Kites *Rostrhamus sociabilis*; Tanaka et al. 2006), in the riparian zone suggest that the low density of snails may be the result of the concentration of predation in that fringe.

In stagnant water at 25°C adult snails routinely ventilate the lung every 25 min, but they can certainly resist for very much longer if they have to. The acute consequences of the lack of access to air recorded here were an increase in crawling in search of the water surface, an increase in resting time and, in some cases, entering a reversible coma. Our experiments best simulate the situation of a snail that is dislodged from the substratum, loosing its orientation to the access to air, or which is entangled beneath a dense mat of macrophytes. These behavioral changes would increase the chances of snails regaining access to air, without suffering excessive stress. Our laboratory evidence indicates that the need to ventilate the lung would restrict the distribution of *P. canaliculata* to locations near emergent substrata in stagnant waters with heavy detritus loads and high temperatures. Nevertheless, our outdoor and field evidence showed that a restricted distribution can appear even under not so extreme conditions (e.g. slow running streams in temperate regions). The need of an access to the surface to breathe in *Pomacea paludosa* has been related to a restriction to zones shallower than 50 cm (Darby et al. 2002) and to

higher densities in habitats with emergent macrophytes than in those without them (Karunaratne et al. 2006).

Pomacea canaliculata is able to resist a wide range of environmental conditions (Martín et al. 2001; Kwong et al. 2008; Yusa et al. 2006b) though only a number of habitats would be suitable for its populations to thrive and eventually spread out. According to our results, access to the water surface would be an important attribute of waterbodies prone to catastrophic invasions of *P. canaliculata*, especially lentic waterbodies in tropical areas. Carlsson et al. (2004) reported a total depletion of aquatic vegetation in natural wetlands dominated by floating macrophytes. In waterbodies lacking suitable substrata to crawl to the surface (i.e. rooted plants or stems), the apple snails' impact would be limited to a few meters from the shoreline. On the other hand, in very shallow or narrow habitats or those with abundant floating or scattered emergent macrophytes, their destructive effects would spread over the whole waterbody.

The success of apple snails in paddy fields and the serious difficulties found in trying to control this pest (Cowie 2002; Wada 2004) suggest that their eradication would be an almost impossible task in natural wetlands (although the eradication of new local foci has been accomplished; Cowie 2002). However, our present results indicate that the effectiveness of some control measures could be increased by focusing the efforts on areas where apple snails are concentrated due to their dependence on access to air. For instance, quinoa (*Chenopodium quinoa*) saponins, recently proposed as an effective and safe molluscicide against apple snails in paddies (Joshi et al. 2008; San Martín et al. 2008), could also be effective in some natural wetlands (e.g. deep waterbodies with only marginal emergent vegetation) since it should be applied only in the littoral zone.

A diverse array of control methods has been suggested against apple snails (e.g. Cowie 2002; Wada 2004; Yusa et al. 2006b). Although the possibility to asphyxiate apple snails has not been considered before, our results suggest that it is possible to do so under certain conditions and especially for females with high reproductive value. Different types of "benthic barriers" deployed over the bottom have been used to control submersed weeds (e.g. Mayer 1978; Ussery et al. 1997) and are commercially available (e.g. Aquascreen®). Underwater screens with holes of appropriate size can impede the access of adults to air (and the spawning of the aerial egg masses) without causing too much damage to the natural vegetation and small organisms. Their effect could be enhanced combining them with low doses of quinoa saponins that apparently damage the gills of apple snails (Joshi et al. 2008; San Martín et al. 2008) and would increase their reliance on aerial respiration.

On the basis of their different palatabilities to *P. insularum*, Burlakova et al. (2009) suggested that emergent macrophytes, instead of submersed ones, should be selected for wetland restoration when this invader is present. However, the effect of plants' habit on aerial respiration and egg laying should also be considered in restoration plans when either invasive (our study) or native apple snails (Karunaratne et al. 2006) are involved.

Because of their very big maximum sizes, adults constitute the most conspicuous stratum of apple snails' populations and appear at first sight to be responsible for most of the damage to aquatic crops and vegetation (Boland et al. 2008). However, small snails have been pointed out recently as the potentially most destructive population stratum due to their higher grazing rates (Carlsson and Brönmark 2006; Boland et al. 2008; Tamburi and Martín 2009). Furthermore, our results suggest that hatchlings and very small snails would also show a less restricted distribution than adults and hence the damage to vegetation would be more extensive during recruitment periods.

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