Life cycle of *Pomacea americanista*, a poorly known apple snail endemic to the Iguazú and Alto Paraná Rivers, southern South America

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ABSTRACT

The Ampullariidae (apple snails) are freshwater snails of tropical and subtropical regions. Recent research has concentrated on a few species that are invasive or potential biocontrol agents, but the basic life history traits of most species remain unknown. One such poorly known species is *Pomacea americanista*, endemic to the Alto Paraná and Iguazú Rivers in southern South America, which may be vulnerable to environmental degradation. We studied the growth, survivorship, maturation and reproductive activity of *P. americanista* to help evaluate its resilience under different scenarios of environmental change. Fifty snails that hatched in the laboratory from two egg masses collected in San Francisco Stream (Misiones Province, Argentina) were reared under controlled conditions until their natural death. Shell growth was almost linear up to the 20th week of life, subsequently decreasing smoothly. Growth rate, asymptotic sizes, time to reach asymptotic size and age at maturity were greater for females than males. Mortality patterns were similar for both sexes, with no deaths up to 38 weeks and steady mortality thereafter, the maximum longevity being 143 weeks. The fecundity of *P. americanista* is lower than that of other apple snails under similar conditions. Under laboratory conditions males and females underwent a single extended reproductive period, but under the seasonal climate in their natural habitats their life cycle would probably extend for more than 3 years, with two or more reproductive periods. The low fecundity and the late maturity of *P. americanista* indicate slow population growth and low resilience relative to invasive apple snails. These traits, combined with a restricted geographical range and an association with hard substrates in swiftly flowing waters, suggest that *P. americanista* could be vulnerable.

INTRODUCTION

The Ampullariidae (apple snails) are medium- to large-sized freshwater snails distributed in tropical and subtropical America, Asia and Africa (Hayes *et al.*, 2015). The aquarium trade and biocontrol or aquaculture projects have dispersed a few species of apple snails to new regions around the world (Horgan, Stuart & Kudavidanage, 2014). In recent decades research has concentrated on a few species, especially those that are invasive or have potential for biocontrol, while most species and genera remain poorly studied (Hayes *et al.*, 2015).

Pomacea americanista (Ihering, 1919), endemic to the Alto Paraná and Iguazú Rivers in Argentina, Paraguay and Brazil, is an especially little studied species; no information about its anatomy and natural history has been published since its description, with the exception of the characteristics of egg masses, hatchlings and mating behaviour (Gurovich, Burela & Martín, 2017). The calcareous eggshells, terrestrial oviposition, precocial hatchlings and the long-lasting copulations suggest that this species belongs to the genus *Pomacea* (Gurovich *et al.* 2017). *Pomacea americanista* is usually found

on hard substrates in swiftly flowing waters (Hylton Scott, 1958; Gurovich, 2016).

The seemingly narrow habitat requirements and restricted geographical range may make *P. americanista* particularly vulnerable to environmental stressors (Martín, Burela & Tiecher, 2013), including the impoundment of most of the 450-km long Alto Paraná River by the Yaciretá and Itaipú dams (Gurovich *et al.*, 2017). Additional threats may be posed by changes in land use and the spread of invasive species such as the byssate golden mussel, *Limnoperna fortunei* (Martín *et al.*, 2013).

The life histories of Neotropical apple snails are diverse (Hayes *et al.*, 2015) and some traits differ profoundly between invasive and noninvasive species (Tiecher, Burela & Martín, 2016a). The study of their life cycles and life history traits may help to forecast the fate of the invasive and vulnerable species under different scenarios of environmental change (Seuffert & Martín, 2017). Our aim was to characterize the life cycle of *P. americanista* through study of the growth, survivorship, sexual maturation and reproductive activity of a laboratory cohort, and to investigate the degree of sexual dimorphism in these life history traits.

MATERIAL AND METHODS

Collection site

On 12 January 2014 we collected two egg masses of *Pomacea americanista* from San Francisco Stream (Comandante Andresito Municipality, Misiones Province, Argentina, 25°36.7′S, 54°2.07′W), a small watercourse, 12–15 m wide and less than 50 cm deep, that discharges into the Iguazú River 83 km upstream of the Iguazú Falls. The local climate is humid subtropical with 1731 mm of annual precipitation and no dry season (Climate-Data.org, 2017); the mean annual temperature is 21.2 °C with monthly means between 26 °C (January) and 16 °C (July).

Rearing of P. americanista *in the laboratory*

Incubation of egg masses and rearing of snails took place at 26 ± 1 °C and under a 14:10 h light/dark photoperiod (from 06.00 to 20.00). The hatchlings were transferred to plastic trays (25 cm long, 17 cm wide and 4 cm deep) with 1700 cm^3 of water and reared with fishfood flakes (VitaFish Premium®). After 2 weeks, 50 hatchlings were randomly selected and transferred to individually numbered plastic aquaria (7 cm diameter, 5.2 cm deep) with 200 cm^3 of CaCO$_3$-saturated tap water; a perforated plastic lid was used to prevent escape of hatchlings. Hatchlings were fed *ad libitum* with fresh lettuce; food availability was checked daily except during weekends. After 11 weeks the snails were transferred to similarly numbered glass aquaria (14 cm diameter, 15 cm deep) with 2300 cm^3 of CaCO$_3$-saturated tap water. Once a week, the water was changed and debris and faeces were removed. The experiment lasted 144 weeks.

The shell size of each snail was measured when the water was changed. To avoid damage due to manipulation, a scaled digital photograph was taken of hatchlings (dorsal view) and small juveniles while they rested on the bottom of the plastic aquaria (Tiecher *et al.*, 2016a). Since the apex of adult shells is often eroded, we measured shell width (SW; Gurovich *et al.*, 2017). Older specimens (in the glass aquaria) were measured with callipers in the same way (Fig. 1A) and were also sexed. Specimens having a convex operculum (Estebenet, Martín & Burela, 2006), a penis sheath in the mantle cavity or a white testis (visible through the translucent shell) were identified as males (Burela & Martín,

2007, 2009), while snails lacking these traits were considered females and later confirmed by oviposition and postmortem examination of soft parts. Each snail was identified by means of its aquarium number painted on the shell with an enamel marker, which allowed us to sex them retrospectively.

Copulation trials were performed weekly from the 17th week until the death of the last female at the 130th week (totalling 113 trials) to determine the time of maturation of males and females. After shell measurement, the 50 snails (34 females and 16 males) were placed in a single circular plastic arena (90 cm diameter and 14 cm deep) with 90 l of water to allow them to copulate freely (Fig. 1B). The snails were checked every 20 min for 7 h to record precopulatory and copulatory behaviour (Burela & Martín, 2009), which were categorized as: copulation attempts (mounting by the male without penis sheath intromission), short copulations (penis sheath intromission lasting less than 2 h) and long copulations (penis sheath intromission lasting more than 2 h). The 2-h limit was used since this is the minimum copulation duration for insemination in *P. canaliculata* (Burela & Martín, 2011). After each copulation trial the snails were returned to their individual aquaria. The female aquaria were checked twice a day (at 9.00 and 18.00) for egg masses on the aquarium walls. All of the males and 24 females were allowed to participate in all the copulation trials until their natural death; ten females were removed from the trials after their first oviposition in order to investigate their oviposition pattern as part of a separate study on sperm storage.

The egg masses from each female were collected from the glass aquaria and incubated at 26 ± 1 °C until hatching. The hatchlings and eggs that did not hatch after 45 d of incubation were counted to obtain the number of eggs in each egg mass.

The original 50 snails were studied until their natural death, which was confirmed by the lack of reaction to touching the foot. As the apices of the shells of the dead snails were intact at the end of the experiment, shell length (SL) was also measured (following Estebenet & Martín, 2003), to compare the growth curves with those previously published for other apple snails.

Measurements and statistical analyses

For males, the age at maturity was identified as the week in which the first copulation attempt was observed; for females the week of the first oviposition was considered as the age at which they were
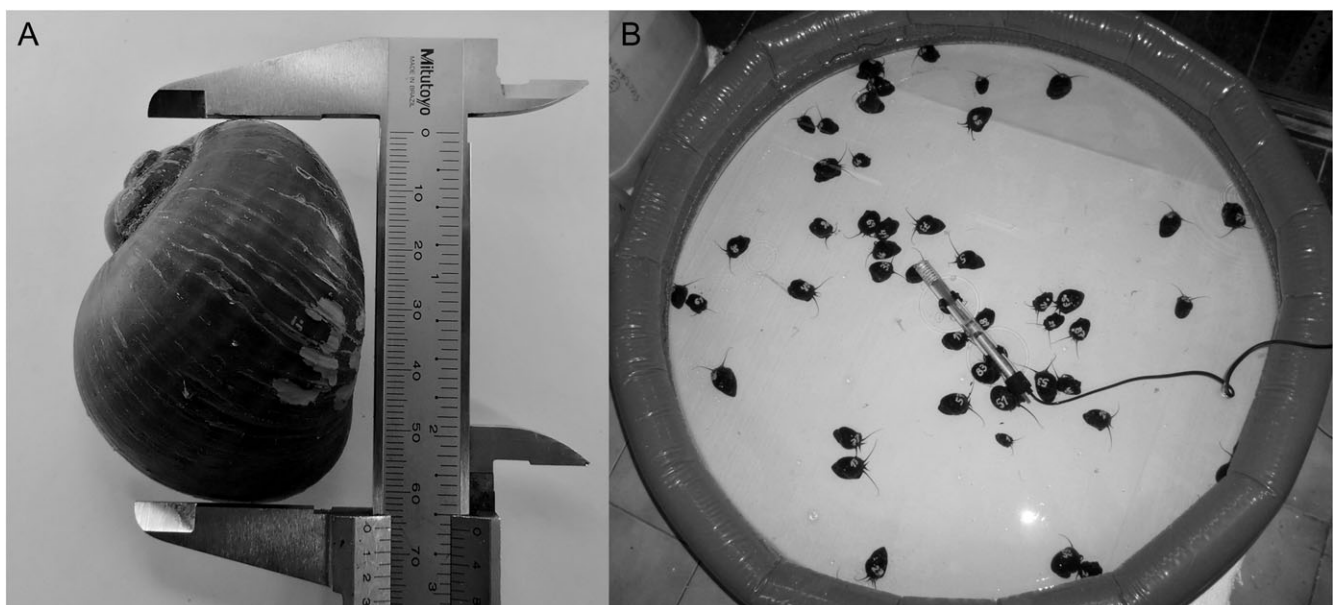
Figure 1. A. Measurement of shell width (SW) of a *Pomacea americanista* with caliper. **B.** Copulation arena with *P. americanista*.

fully mature, while the week of the first short copulation and the week of the first long copulation were considered as initial stages of maturation. The age and size at death were those of the last week in which a snail was observed alive and measured.

To evaluate possible differences between sexes in the age and size at maturity and at death, t tests for equal and unequal variances were performed after testing the homoscedasticity with Levene's test. The coefficients of variation (CV%) of each of these traits was calculated: CV% = $100 \times$ SD/mean. The possible deviation of the sexual proportion from 1:1 was evaluated by a χ^2 test. A von Bertalanffy growth curve was fitted to the means of maximum sizes attained by males and females at each week of age. The parameters of the von Bertalanffy model, SW_∞ (asymptotic shell width), K (specific growth rate) and t_0 (intercept), were estimated for each sex separately using the maximum-likelihood method (Aubone & Wöhler, 2000).

The sexual activity of males in a given week was estimated as the percentage of males showing any copulation behaviour relative to the total number of mature males (i.e. the total number of males that had already shown any type of copulation behaviour). For the females this parameter was estimated as the percentage of females that laid an egg mass in a given week relative to the total number of fully mature females (i.e. the total number of females that had already laid at least one egg mass).

RESULTS

The increase in shell width was almost linear up to the 20th week of life (Fig. 2A). Thereafter, growth rate decreased smoothly and became almost imperceptible after 70 weeks. The opercula of males began to be distinguishable from those of females at 15 weeks of age. The growth patterns exhibited sexual dimorphism from the 13th week onwards; specific tests showed that at the 11th week SW did not differ significantly between the sexes, but that it did so at the 12th week ($t_{48} = 1.711$, $P = 0.093$ and $t_{48} = 2.529$, $P = 0.015$, respectively). Females grew faster and attained larger sizes than males. The parameters of the von Bertalanffy model reflected the differences in the growth curves (Table 1): K and SW_∞ were higher for females than for males. Males and females reached 95% of their asymptotic sizes at 50 and 56 weeks, respectively. The maximum SW (at death) attained by both sexes was significantly different ($t_{48} = 6.57$, $P < 0.0001$; Fig. 3A). The correlation between SW and SL at death was significant ($r = 0.98$, $n = 48$, $P < 0.0001$) and the magnitude of SW was on average 94% (± 2.6) of SL.

Mortality first occurred in the 38th week (Fig. 2B) and from then onwards the decrease in the number of survivors was almost linear and similar for both sexes. The maximum divergence of survivorship curves was observed from weeks 101 to 103, when 43.75% of males and only 17.65% of females were alive. Half of the males and females survived until weeks 91 and 84, respectively, and the maximum longevities were 143 and 130 weeks, respectively. However, the age at death was not significantly different among the sexes ($t_{48} = 1.097$, $P = 0.27$; Fig. 3B).

The pattern of maturation was sexually dimorphic (Fig. 2C). Males first attempted to copulate in week 18 and the percentage of mature males rose quickly, reaching 50% at week 24 and 100% at week 78. The copulation attempts of males did not progress further in many cases, because of the active shaking of the females' shell; the females only allowed shell mounting by males and intromission of the penis sheath some weeks later. In some cases the males gave up their attempts to copulate without any rejection behaviour from the female. After the 25th week copulations lasting more than 2 h were observed and the percentage of females that had engaged in such long copulations increased slowly but steadily. Females began to lay egg masses at most 4 d after their first long copulation was observed and the percentage of females laying

egg masses followed the same pattern; half of the females were fully mature at 74 weeks of age but only 79.4% of females attained that state before dying. The sex ratio in the 50 snails was 2.13 females to 1 male, deviating significantly from 1:1 ($\chi^2 = 6.48$, $P = 0.011$).

Both age and size at maturity were significantly greater for females than for males ($t_{41} = 5.274$, $P < 0.001$ and $t_{41} = 13.469$, $P < 0.001$; Fig. 3A, B). Females began to oviposit at 63.07 $\pm$ 19.59 weeks and 61.21 $\pm$ 4.76 mm SW, while males began to attempt copulations at 31.94 $\pm$ 15.80 weeks and SW 41.73 $\pm$ 3.95 mm. For both sexes the coefficients of variation of age at maturity and death (31.7% and 22.9% for females and 51.1% and 29.2% for males, respectively) were larger than those for size (7.9% and 9.0% for females and 9.8% and 16.4% for males, respectively). For all traits, the coefficient of variation of males was higher than that of females.

The percentage of snails showing sexual activity showed no clear pattern during the experiment (Fig. 2D). In a given week, 21.25% of females laid at least one egg mass while 46.43% of males attempted to copulate at least once; in 10.6% of these attempts, a male tried to copulate with another male. Once reproductive activity had started, it was in general continuous up to the snail's natural death, especially in males; three out of 13 females that copulated and oviposited more than once interrupted their oviposition for more than a month and then restarted. The number of egg masses per female (mean $\pm$ SD) was 10.50 $\pm$ 7.68 and the total number of eggs per female was 1164.93 $\pm$ 903.82, excluding a female that deposited 53 egg masses totalling 6101 eggs.

DISCUSSION

Our experiment showed that *Pomacea americanista* can live for up to 144 weeks, with no mortality until week 38 and with all the males and 79.4% of the females reaching maturity. This species is sexually dimorphic in size and age at maturity, and in size at death, but the survivorship curve was similar for both sexes.

Procedures used by various authors to measure shell size of apple snails vary and are often ill defined (Youens & Burks, 2008). The high correlation ($r = 0.98$) between SW and SL in *P. americanista* and their closely similar absolute magnitudes (SW = 94% of SL) indicate that these two variables are approximately equivalent measures of shell size. Hence in the following discussion we will use shell size to refer to both shell width and to shell length as used in other studies about South American apple snails.

The growth pattern of *P. americanista* resembles that of other ampullariids studied under similar conditions. In terms of the von Bertalanffy model, the specific growth rates of *P. americanista* (0.086–0.094 week^{-1}) are almost twice those of *Asolene platae* (0.047–0.057 week^{-1}; Tiecher *et al.*, 2016a) but only slightly higher than those of *Pomacea patula catemacensis* (0.062–0.073 week^{-1}; Espinosa-Chávez & Martínez-Jerónimo, 2005). The specific growth rates of *P. americanista* fall within the range of 0.056–0.118 week^{-1} estimated at 25 °C for small aquatic pulmonate species (asymptotic sizes 7.6–20.3 mm), but are much higher than the value of 0.016 week^{-1} estimated for the medium-sized (31.8 mm) caenogastropod *Melanoides tuberculata* (Pointier, Toffart & Lefevre, 1991 and references therein). The growth of *P. americanista* is therefore remarkably fast for a large snail (49.45–62.11 mm), yet it is slower than that of the invasive *P. canaliculata*: the shell sizes (attained at 65 d) of these two species were 16.87 mm and 23.63 mm (Martín & Estebenet, 2002), respectively, despite the latter being reared at a higher density.

Pomacea americanista females mature later than those of most apple snail species that have previously been studied under controlled conditions. They oviposit for the first time at 63.07 weeks on average, which is much later than *P. canaliculata* females (15.6 weeks; Tamburi & Martín, 2009) but earlier than *A. platae* females

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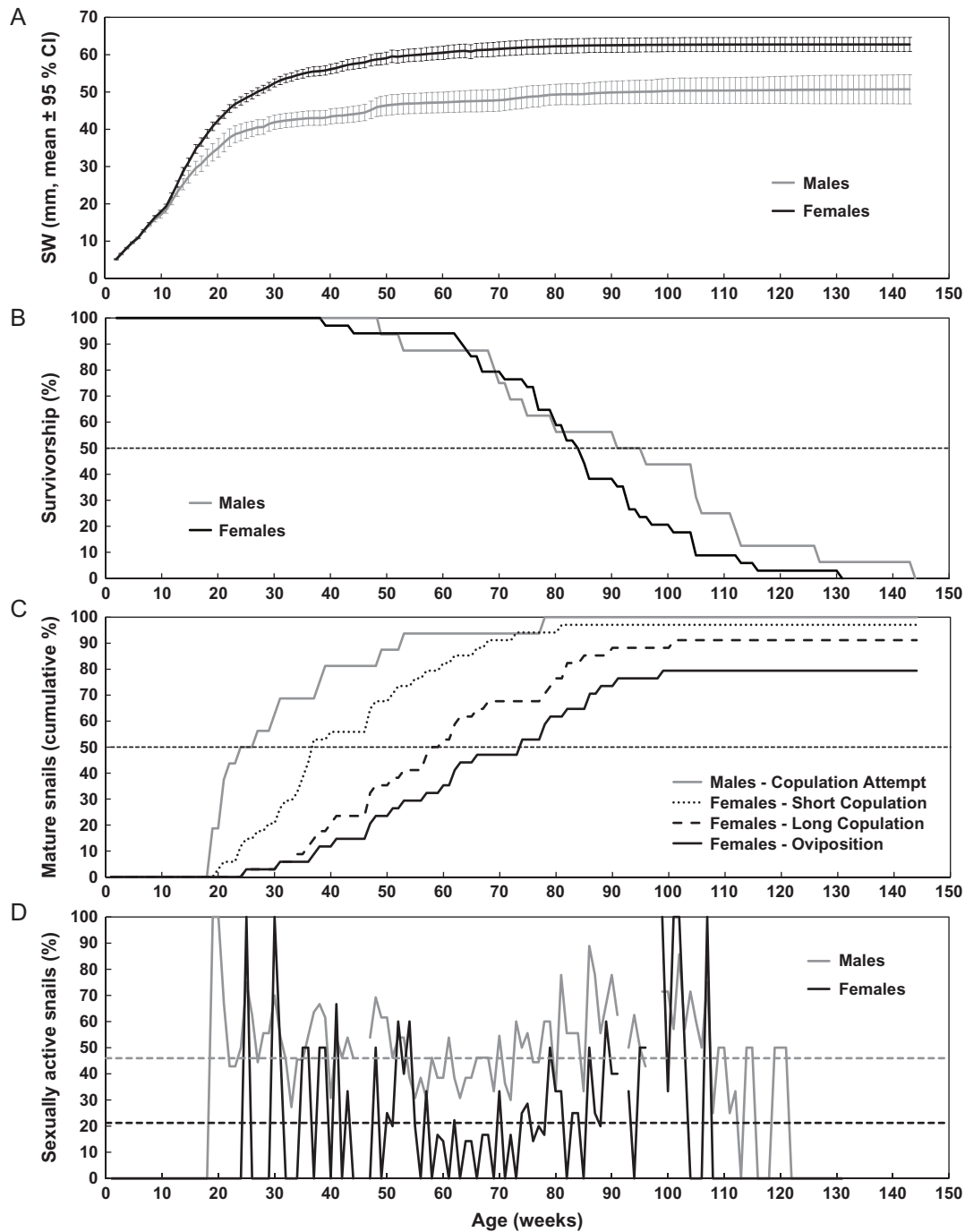
Figure 2. Life cycle of males and females of *Pomacea americanista* in the laboratory. **A.** Growth curves for shell width (SW). **B.** Survivorship curves. **C.** Maturation curves. **D.** Reproductive activity. The horizontal lines indicate 50% survivorship (**B**) and maturity (**C**) or the means of males and females (**D**).

Table 1. Von Bertalanffy growth model parameters for *Pomacea americanista*.

	SW∞ (mm)	K (week^{-1})	t$_0$ (week)	r
Females	62.11	0.094	11.31	0.998
Males	49.45	0.086	9.76	0.993

The correlation coefficients (*r*) between the observed values of SW and those predicted by the model are also given. Abbreviations: SW∞: asymptotic shell width; K: specific growth rate; t$_0$: intercept.

(85 weeks; Tiecher *et al.*, 2016a) under similar conditions, although the first *P. americanista* egg mass was observed at week 25 in the present study. Under crowded conditions *P. bridgesii* (in fact probably *P. diffusa*) matures at 27 weeks (Coelho, Calado & Dinis, 2012). The first egg mass of mass-reared *Marisa cornuarietis* was observed at 18–21 weeks (Aufderheide *et al.*, 2006). Late maturity partly determines the generation time of a population and also its potential growth rate (Tiecher *et al.*, 2017).

As in most apple snails (Estebenet *et al.*, 2006; Hayes *et al.*, 2015), the growth of *P. americanista* is sexually dimorphic.
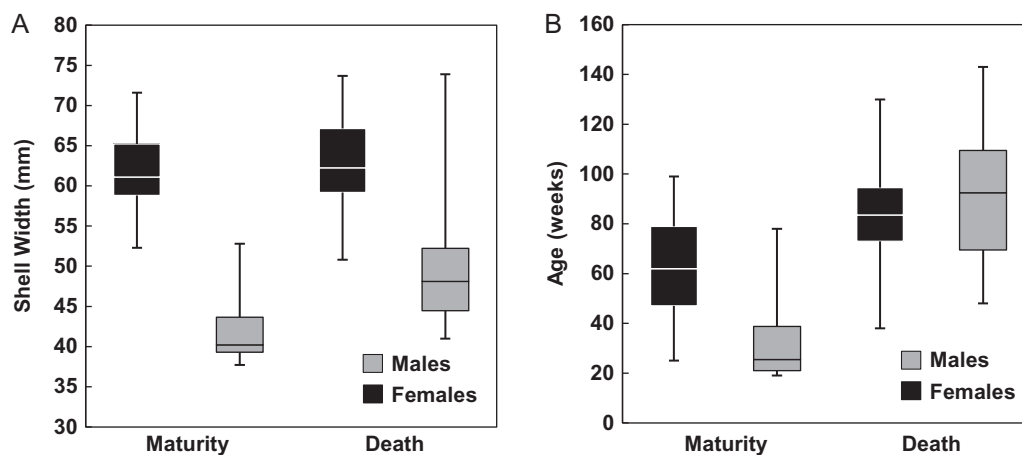
Figure 3. Box-plots of life history traits of males and females of *Pomacea americanista* in the laboratory. **A.** Size at maturity and at death. **B.** Age at maturity and at death. The horizontal bar indicates the median, the box the interquartile range and the vertical bars the extreme values for each trait.

However, the degree of sexual dimorphism is much higher in *P. americanista* than in other apple snails: females are 26% larger than males, whereas in previously studied species the degree of dimorphism varies from 8% (*A. platae*; Tiecher *et al.*, 2016a) to 6.2–9.5% (*P. canaliculata*; Estebenet & Cazzaniga, 1998; Estebenet & Martín, 2003). In *P. canaliculata* the sexual size dimorphism is highly variable, but only in one population have values similar to those for *P. americanista* been reported (23%; Estebenet & Martín, 2003). The age at which size dimorphism becomes significant was similar in *P. americanista* (12 weeks) and *P. canaliculata* (10.7–17.6 weeks), but much lower than in *A. platae* (134 weeks and in only one out of three cohorts). Age at maturity is also sexually dimorphic in *P. americanista* and even more markedly so than size: *P. americanista* females matured 97% later than males. Data for age at maturity obtained independently for males and females are available only for *P. canaliculata* and *A. platae*, females of which mature 30% and 54% later than males (Tamburi & Martín, 2009; Tiecher *et al.*, 2016a), respectively, suggesting that dimorphism in age at maturity is widespread among apple snails. The sexual dimorphism in secondary traits like size and age may reflect divergence in the reproductive roles or in the ecological niches of both sexes and has important consequences in their ecological performance (Fairbairn, Blanckenhorn & Székely, 2007).

The maximum longevity in *P. americanista* was 143 weeks, which is greater than the maximum observed for four *P. canaliculata* cohorts at 25 °C (45–107 weeks; Martín & Estebenet, 2002; Seuffert & Martín, 2017), but less than the minimum reported for *A. platae* (184 to >200 weeks; Tiecher *et al.*, 2016a). In contrast to growth and age at maturity, mortality patterns were similar to those found in several studies of *P. canaliculata* (Martín & Estebenet, 2002; Seuffert & Martín, 2017) and *M. cornuarietis* (Arsuffi *et al.*, 1993) and almost the inverse of the pattern in *A. platae*, which exhibits the highest mortality in the first 8 weeks (Tiecher *et al.*, 2016a).

The lifetime fecundity of *P. americanista* females is lower than that of other apple snails reared under similar conditions. The mean of 10.50 egg masses per female is roughly half that of *A. platae* (20.61; Tiecher *et al.*, 2016a) and *P. canaliculata* (23.22; Martín & Estebenet, 2002). The total number of eggs per female was 1165 in *P. americanista*, which is similar to the values for *A. platae* (1430; Tiecher *et al.*, 2016a), but much less than in the invasive *P. canaliculata* (4534; Martín & Estebenet, 2002). However, as the eggs of *A. platae* are gelatinous and smaller (2.25 ± 0.28 mm; Tiecher, Burela & Martín, 2014) than those of *P. americanista* (2.91

± 0.25 mm; Gurovich *et al.*, 2017); the reproductive effort of the latter is probably greater than in *A. platae*, but nevertheless less than in *P. canaliculata*, the eggs of which are similar in size and structure to those of *P. americanista* (Estebenet & Cazzaniga, 1993).

Under the controlled and constant conditions in our study both male and female *P. americanista* had only one extended reproductive period, which usually lasted from maturity to their natural death. This pattern is similar to that of other apple snail cohorts under constant temperatures (Martín & Estebenet, 2002; Tiecher *et al.*, 2016a; Seuffert & Martín, 2017). However, the life cycle of apple snails can change from semelparity to iteroparity (one or more than one reproductive periods in the lifetime, respectively) when water temperature fluctuates during the year, as was demonstrated for *P. canaliculata* (Estebenet & Cazzaniga, 1992). The occurrence of cold seasons lasting several months, during which water temperature drops to levels at which the snails become inactive (<15 °C; Seuffert, Burela & Martín, 2010), as occurs in Southern Pampas, lengthened the life cycle of this species from 14 months to almost 4 years (Estebenet & Cazzaniga, 1992). Assuming a similar effect of low winter water temperatures for *P. americanista*, the life cycle of this snail in the Iguazú River basin would probably extend for more than the 3 years observed in the present study. The low water temperatures (down to 15 °C; Gutierrez Gregoric, Núñez & Rumi, 2010; Nardelli *et al.*, 2016) in the Iguazú River during the winter may be sufficient to interrupt the activity of *P. americanista*, as in *P. canaliculata* (Seuffert *et al.*, 2010), and thus two or more reproductive seasons are likely to occur.

The sexual dimorphism in age at maturity in *P. americanista* is likely to be greater under natural seasonal conditions. Half of the males matured in less than 6 months compared to 2.94% of the females, most of which (76.5%) took more than 1 year to mature. In the Iguazú River basin most males born at the beginning of the reproductive season may mature in the same season, whereas almost all the females would mature during the next season. A similar pattern has been hypothesized for *P. canaliculata* populations under a regime of seasonal climate and low food availability (Tamburi & Martín, 2009). The life cycle proposed here for *P. americanista* in its native range is iteroparous and multiannual, with most females maturing in their second summer. This falls into life cycle category 'G' of Dillon (2000: 157) for "populations that mature in 12–23 months and reproduce iteroparously". This category and other types of multiannual and iteroparous life cycles were quite infrequent among those reviewed by Dillon (2000) for freshwater caenogastropods as a whole, but are prevalent among

families composed of large snails such as the Ampullariidae (Hayes *et al.*, 2015; Tiecher *et al.*, 2016a).

Life history traits may play a role in the invasive success of some species of *Pomacea* (Keller, Drake & Lodge, 2007; Seuffert & Martín, 2017), but also in the vulnerability of others (Tiecher, Burela & Martín, 2016b). High pre-reproductive survival, fast growth and prolonged lifespan rank among the traits that could be considered to favour the potential invasiveness of *P. americanista*. On the other hand, *P. americanista* females show substantial delays in maturity relative to *P. canaliculata* females, and their fecundity and egg hatchability (39 ± 36% in *P. americanista*; Gurovich *et al.*, 2017) are lower. Late maturity and hence delayed production of progeny, especially if productivity is low, are responsible for low intrinsic rates of population increase in freshwater snails (Tiecher *et al.*, 2017). On the whole, the combination of life history traits in *P. americanista* is indicative of slow population growth and low resilience, two factors that may render freshwater snails prone to extinction (Strong *et al.*, 2008).

ACKNOWLEDGEMENTS

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