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Demographic life-history traits and diet of *Physalaemus biligonigerus* (Anura: Leptodactylidae) in central Argentina

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The Neotropical weeping frog *Physalaemus biligonigerus* (COPE, 1861) is one of the most common anurans inhabiting the grasslands, shrublands, and dry forests of Argentina, Bolivia, Brazil, Paraguay, and Uruguay (CEI 1980, LAVILLA et al. 2000). Populations often include thousands of individuals and the species is currently classified as of least concern and with a stable population trend (IUCN 2023). Due to their biomass and trophic ecology, they play an important role in the Pampean ecosystem (undisturbed and agriculturally used) feeding on herbivore arthropods (AGOSTINI et al. 2021). However, although it is a common species, there is a lack of detailed information on the life history and ecology of this frog, with available data originated from distant locations with different land use (ZARACHO et al. 2005, CAMARGO et al. 2008, BIONDA et al. 2013, NICOLINO et al. 2018). In this study, we provide an extensive data set on demographic life history traits and trophic diversity features in a large population in the Pampa of central Argentina.

The population studied inhabited a grassland area used for cattle grazing in Barreto (Juárez Celman Department, Córdoba Province, Argentina; 63°19'20"W, 33°21'42"S, altitude: 146 m (Fig. 1). The local climate was temperate with Mediterranean characteristics (average minimum temperatures during 1993 were 3.3 °C in July and 18 °C in January, and average maximum temperatures ranged from 15.6 °C in July to 31.7 °C in February. The absolute minimum and maximum temperatures recorded during 1992 and 1993 were -5 °C in July and 36 °C in January; annual rainfall is highly variable, with very wet years (1,272 mm in 1991) and

very dry years (508 mm in 1995). Annual rainfall in 1992 and 1993 was 747 mm and 934 mm, respectively). During heavy summer rainfalls (December to February) large parts of the study area were flooded, and temporary roadside ditches suitable for breeding were formed. From January 1992 to December 1993, we collected specimens in 12 pitfall traps (15 cm diameter, 23 cm height, filled up to 7 cm with 10% formaldehyde; CORN 1994), placed in line about 2 m distant from each other, and parallel to 2 m of the flooded roadside ditches of an internal dirt road (Fig. 1). The traps were checked once a week during the austral summer and once a month during winter, preserved specimens were removed and formaldehyde replaced.

In a 100 m² transect in the flooded ditches, the number of nests present was determined and four nests were collected. The volume of each nest was measured, then placed in separate containers for larval hatching and taken to the laboratory. After hatching the number of larvae was estimated. For this purpose, 10 samples were taken per nest using a 2 ml pipette. Averages were calculated for each sample, and the number of larvae per nest was determined based on the total volume of each nest.

The specimens collected were submerged in water for 24–48 h to remove excess formaldehyde, and then preserved in 70% ethanol and stored in the Herpetological collection of the Universidad Nacional de Río Cuarto (lots ECO-EA 101, 104, 107, 108). For this study, we selected 367 preserved specimens collected during the main activity periods in 1992 (January N = 64, February N = 42, December N = 80) and in 1993 (January N = 52, February N = 49, De-

ember $N = 80$). The complete size range was represented in the sample, but we gave preference to larger individuals (external features of adults: presence of vocal sac in males and of eggs in females; CEI 1980) to ensure an adult size of the femur for age determination. Each individual was dissected, snout-vent length (SVL, distance between snout tip and cloaca) measured to the nearest 0.1mm using a caliper Vernier Somet Inox Extra, body mass recorded to the nearest 0.1g using a Pesola® balance, and sex determined investigating the gonads to distinguish between immature and adult frogs.

Out of this sample, we randomly chose 27 specimens per month resulting in a total of 162 individuals, which were processed for age determination and diet analysis. For age determination, we applied the standard procedures of skeletochronology on femur cross sections (SINSCH 2015). Bone samples were embedded in Histo-resin™ (JUNG) and stained with 0.5% Cresyl violet. Diaphysis was cross sectioned at 12µm using a JUNG RM2055 rotation microtome. Cross sections were examined light microscopically for the presence of growth marks at magnifications of 400× using an OLYMPUS BX 50. We distinguished strongly stained lines of arrested growth (LAGs, annual growth marks)

in the periosteal bone, separated by faintly stained broad growth zones. We selected diaphysis sections in which the size of the medullar cavity was at its minimum and that of periosteal bone at its maximum. The number of LAGs represents the number of years completed.

For diet analysis, the stomach content of each individual was extracted and preserved in 70% ethanol until screening using a stereomicroscope ZEISS BW40. We identified prey items to the lowest possible taxonomic level, but due to the high fragmentation of prey items they were summed up by order (exception: Formicidae) (analogous to RÖDNER 2008). For each category, the absolute number of items (N) was counted, the frequency of occurrence (FO[%], number of stomachs containing that taxon divided by the total number of stomachs studied), and the relative volume (V[%]) using the formula for an ellipsoid (DUNHAM 1983, ATTADEMO et al. 2007). We calculated the Shannon-Weaver Diversity Index to describe trophic niche breadth.

Data obtained from pitfall trap sampling show that the studied population of *P. biligonigerus* was very large with 6781 individuals sampled in 1992, and 1443 in 1993 (Fig. 2). The maximum number of captures were in December and January, mostly comprising metamorphs and imma-

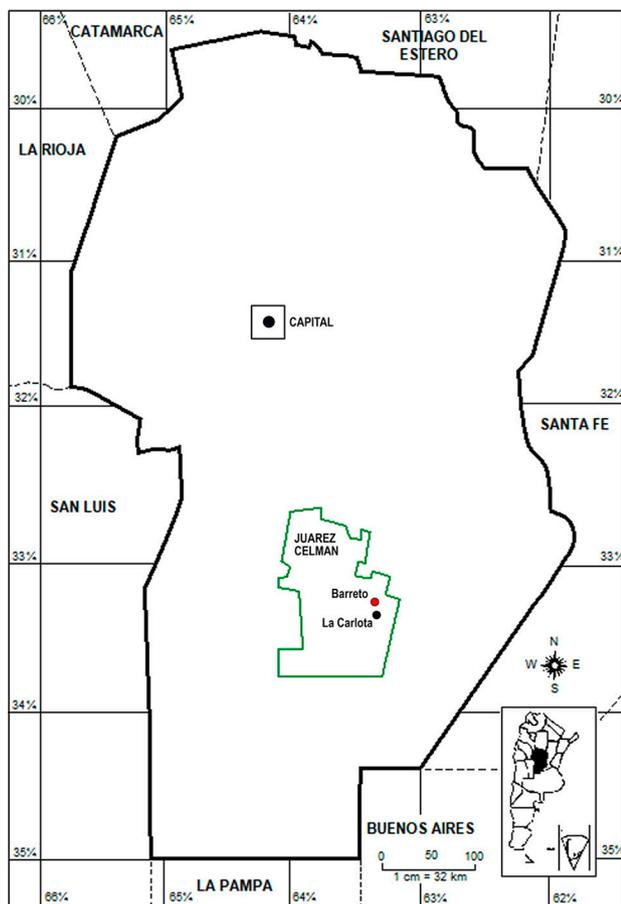


Figure 1. Location of the study area (left) and the sampling site (right), a characteristic breeding site used for *P. biligonigerus*, represented by a flooded ditch on a dirt roadside.

ture juveniles. Besides the focal species, the local anuran community comprised nine more species (*Ceratophrys ornata*, *Rhinella arenarum*, *R. fernandezae*, *Leptodactylus gracilis*, *L. latinasus*, *L. macrosternum*, *Odontophrynus americanus*, *Boana pulchella*, *Elachistocleis bicolor*). From December to February breeding males called floating on the water of shallow puddles among the aquatic vegetation. Pairs in amplexus produced foam nests during the same period. The results obtained an average of 1.57 nests of *Physalaemus biligonigerus* per square meter. The development occurred within 24–48 hours of capture and the average number of larvae per nest was 2753.5 individuals. In the 100 m² transect, 157 nests were counted, allowing for a total estimate of 432299.5 larvae. Based on these results and field information, it was also possible to evaluate the total number of individuals born during a year in a 100 m² breeding site. If we assume a minimum of two clutches per year, the estimated number was 864,599 individuals. Fecundity shows a wide geographical variation from 200–400 tadpoles per foam nest in Uruguay to 1051–1688 in Corrientes, and 1193 ± 683 in Córdoba, Argentina, partially due to variation in female size (ZARACHO et al. 2005, CAMARGO et al. 2008, BIONDA et al. 2013, NICOLINO et al. 2018). Aquatic development from fertilized egg to metamorphosis lasts 20–24 days, promoting fast recruitment of terrestrial metamorphs from the mostly ephemeral breeding puddles (CHULIVER & FABREZI 2019).

Accordingly, the first metamorphs were collected in late December with 13.7–14.9 mm SVL and a corresponding body mass of 0.3–0.6g. Roughly half of the studied specimens (N = 179) were immature, with an average SVL of 20.8 mm (range: 13.7–25.9 mm) and an average body mass of 1.2 g (0.3–3.0 g). The subadults comprised 75 individuals with immature testes and 104 with immature ovaries. Adults did not show sexual size dimorphism (aver-

age SVL ± standard deviation): 30.2 ± 2.6mm (106 males) vs. 30.0 ± 2.9mm (81 females) (t-test, t = 0.66, p = 0.5091; Fig. 3A). Average body mass did not differ significantly either: 4.1 ± 1.1g vs. 3.9 ± 1.2g (t-test, t = 1.07, P = 0.2851; Fig. 3B). Fecundity varies geographically, but central Argentinean populations like the one studied seem to produce more viable tadpoles than those in the northern and southern limits of the geographic range. Female size is probably not the key driver of fecundity because at an average SVL of 30 mm the females of the study population had a five times greater fecundity than those in Uruguay, whereas the larger females (35.3 mm SVL) of the garden population had about the same fecundity (CAMARGO et al. 2008, NICOLINO et al. 2018). As recruitment of offspring is a key factor of population dynamics, variation of fecundity requires more attention in future studies.

Age determination was successful in all individuals. LAGs were resolved as dark stained, sharp lines contrasting from the faint violet background colouration of the periosteal bone (Fig. 4). All immature individuals (N = 81) lacked LAGs, i.e., they originated from nests produced during the same activity period. The medullar cavity was completely filled with tissue and the line of metamorphosis visible, separating the larval bone from the fainter-stained post-metamorphic bone (Fig. 4A). Sexual maturation occurred usually during the second year of life (1 LAG present) and required a minimum SVL from 23–25 mm. The line of metamorphosis was absorbed at least partially in most individuals (Fig. 4B). Occasionally, early metamorphs of a summer reached sexual maturity (9.1% of males, 5.4% of females) before winter, i.e., during their first year of life (0 LAG; Fig. 5). Longevity was low, the oldest males (22.7%) and females (5.4%) were captured in their third year of life (2 LAGs; Fig. 4C). Existing demographic data are anecdotal referring to a single isolated popula-

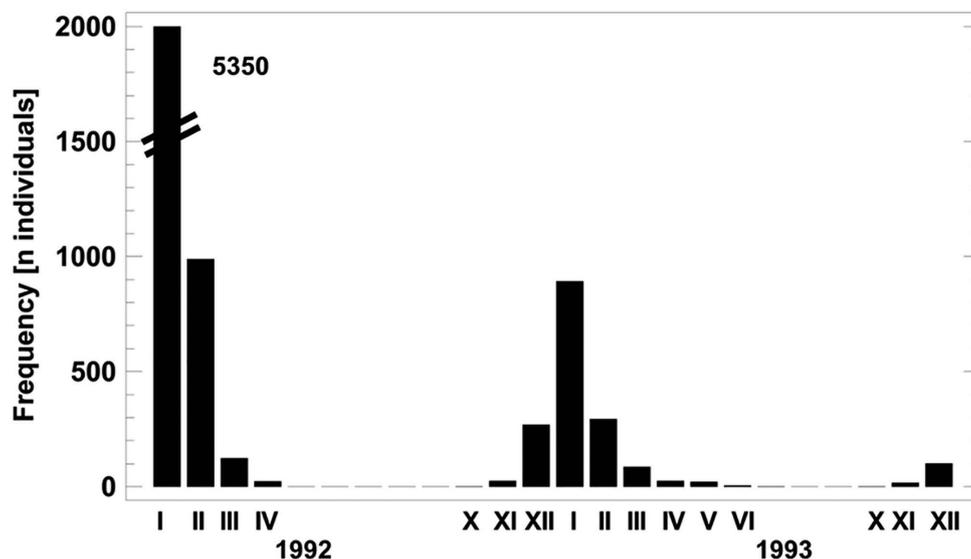


Figure 2. Phenology of pitfall trap captures of *P. biligonigerus* in 1992 and 1993. The months with captures are highlighted with roman numbers.

tion at the campus of the National University of Rio Cuarto, Argentina (NICOLINO et al. 2018). The youngest males matured in the 3rd year of life and females in the 4th year of life. Longevity extended to six years in males and five in females, together with late maturation unusual features for a r-strategist in the lowlands of the temperate zone. The

deviating data obtained from members of a garden population of *P. biligonigerus* may result from a combination of low local predation pressure and low numbers of individuals studied (NICOLINO et al. 2018).

The stomachs of 159 individuals contained prey items, only three were empty. We counted 2553 prey items yield-

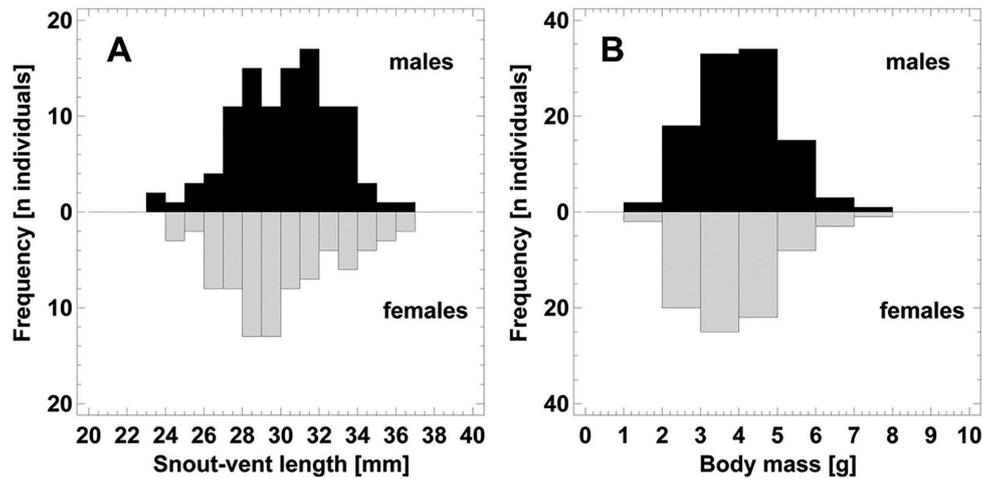


Figure 3. Size (A) and body mass distributions (B) of adult *P. biligonigerus*. There is no sexual dimorphism (for statistical details see text).

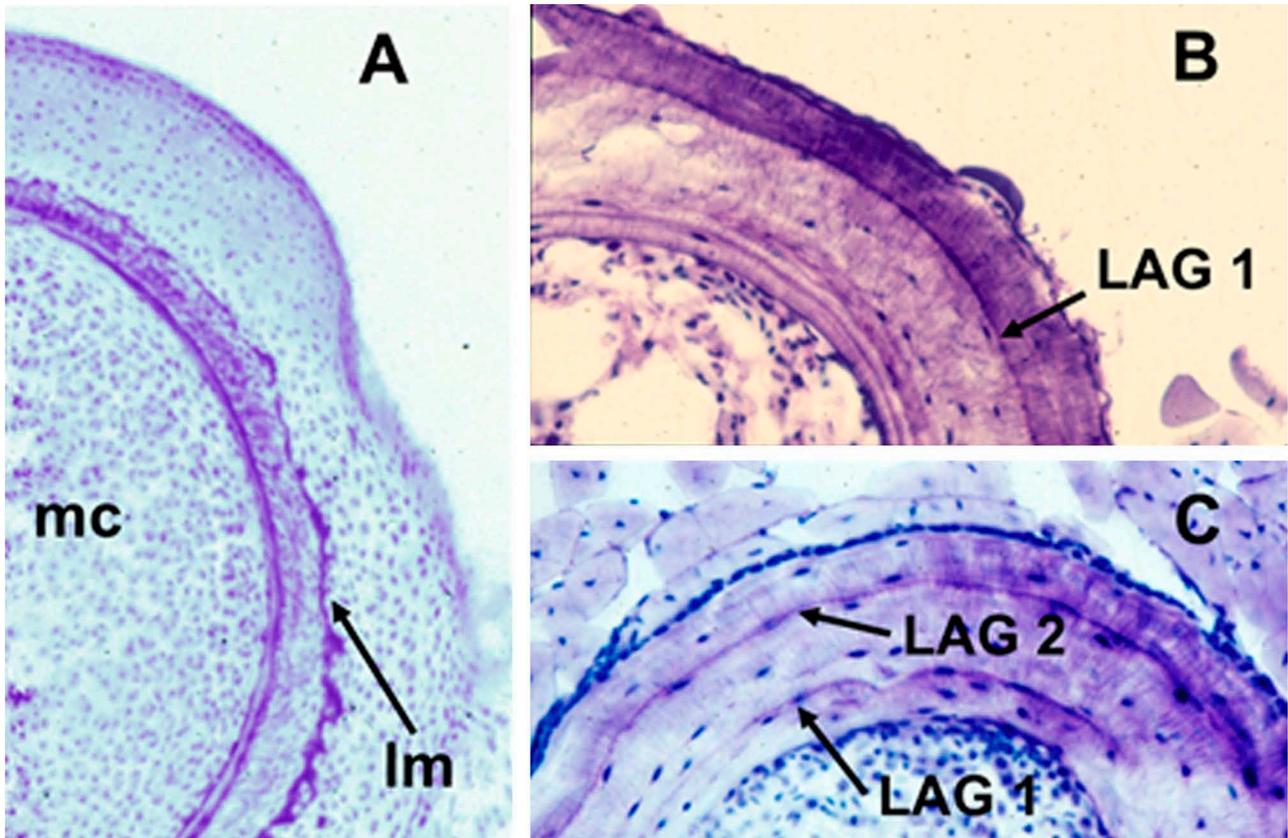


Figure 4. Stained femur cross sections of *P. biligonigerus*. (A) Juvenile without LAG; (B) male with 1 LAG; (C) female with 2 LAGs. Abbreviations: mc = medullar cavity; lm = line of metamorphosis.

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Table 1. Prey categories found in the stomach contents of. Report of prey taxa includes N = number of individuals of a given prey category in all stomachs examined, FO [%] = frequency of occurrence of prey category per stomach, V [%] = relative volume occupied by a prey category in all stomachs. We contrast our results with those of four diet analyses published before.

Locality	Córdoba, Argentina (this study)	Córdoba, Argentina (ATTADEMO et al. 2007)	Rio Grande, Brazil (DE OLIVEIRA et al. 2015)	Porto Alegre, Brazil (FARINA et al. 2023)	Chaco, Paraguay (RÖDDER 2008)
Number of stomachs analysed	162	27	49	8	58
Prey taxa	N FO [%]/V [%]	N FO [%]/V [%]	N FO [%]/V [%]	N FO [%]/V [%]	N FO [%]/V [%]
Arachnida					
Acariformes	88 12.0 / 0.1	–	8 10.9 / 1.8	2 12.5 / 0.2	–
Aranea	38 17.6 / 1.0	1 3.7 / 0.1	6 6.5 / 1.8	3 37.5 / 1.0	1 2.9 / 0.04
Opiliones	1 0.63 / 0	–	–	–	–
Pseudoscorpiones	–	–	1 2.2 / 0.1	–	–
Unidentified	–	–	2 2.2 / 0.2	–	–
Crustacea					
Amphipoda	–	–	–	2 25.0 / 0.7	–
Isopoda	158 23.9 / 10.0	142 66.7 / 46.5	–	2 25.0 / 2.2	2 5.9 / 9.8
Myriapoda	5 1.9 / 0.3	1 3.7 / 0.3	–	–	–
Insecta					
Coleoptera	280 49.7 / 22.4	6 11.1 / 1.3	79 39.1 / 13.2	4 25.0 / 3.0	10 14.7 / 1.4
Collembola	667 9.4 / 0.2	–	4 4.3 / 0.2	–	1 2.9 / 0
Dermaptera	–	–	–	1 12.5 / 0.1	–
Diptera	76 28.9 / 4.8	1 3.7 / 0.2	4 6.5 / 0.7	3 25.0 / 1.5	3 2.9 / 0.3
Formicidae	872 61.0 / 21.2	185 44.4 / 11.0	848 76.1 / 50.8	159 100 / 52.1	7 8.8 / 0.3
Hemiptera	169 28.9 / 7.2	4 7.4 / 11.8	–	–	–
Heteroptera	–	–	–	–	1 2.9 / 0.3
Hymenoptera (non-Formicidae)	70 16.4 / 15.9	–	–	1 12.5 / 1.0	7 11.8 / 2.7
Isoptera	48 1.9 / 9.3	40 11.1 / 2.1	57 10.9 / 1.8	–	248 29.4 / 82.2
Odonata	1 0.63 / 0.9	–	–	–	–
Orthoptera	2 1.3 / 1.1	2 3.7 / 7.8	–	4 37.5 / 2.3	13 8.8 / 12.0
Terr. larvae	78 23.9 / 6.8	5 7.4 / 15.3	–	9 37.5 / 24.5	3 5.9 / 0.5
unidentified	–	–	? 50.0 / 19.3	–	1 2.9 / 0
Plant material	present	present	present	present	present
Total (prey items)	2553	418	1027	190	297
Shannon-Weaver Diversity Index H'	1.90	1.54	0.63 (recalculated)	0.78 (recalculated)	0.34

ing an average stomach content with 16 prey specimens. We identified 16 prey categories (Table 1). Ants and collembolae were the most frequent prey items accounting for 60% of all prey and of 21.4 of total prey volume. Small beetles, homopteran and crustaceans, all herbivores, summed up to another 23.7% of all prey, but represented 39.6% of total prey volume. The trophic niche diversity was $H' = 1.90$ (Shannon index) being considerably greater than those obtained for Brazilian and Paraguayan populations (Table. 1). However, diet composition of adults does not show significant geographical variation consisting of a variety of arthropods with ants being the most frequent prey in Argentina and Brazil, and Isopterans in Paraguay (ATTADEMO et al. 2007, RÖDDER 2008, DE OLIVEIRA et al. 2015, FARINA et al. 2023). The trophic niche breadth is thought to be narrow due to the preference of colonial terrestrial arthropods (RÖDDER 2008). The width of the trophic niche has probably been underestimated due to an analysis on a population from Paraguay (RÖDDER 2008), whereas our study in agreement with those from Brazil and Argentina indicated a considerably greater diversity of prey items (ATTADEMO et al. 2007, DE OLIVEIRA et al. 2015, FARINA et al. 2023). We speculate that broader local diversity of prey availability may partially override the otherwise notable preference for ants and termites. Since diet is always dominated by herbivore arthropod and crustacean species, *P. biligonigerus* may provide previously unrecognized ecosystem services in large regions of the Pampa used soybean plantation and cattle grazing.

In conclusion, our study on a *P. biligonigerus* population inhabiting a grazed grassland demonstrates that the life history is the result of r-selection with high fecundity, fast larval development occurred within 24–48 hours of capture, early sexual maturity, and short longevity. *Physalaemus biligonigerus* shares the demographic traits (early maturity, short lifespan) observed in the study population with many other small- and medium-sized anuran species under r-selection, as for example hyperolids (SINSCH & DEHLING 2017) and ceratophryds (SZÉKELY et al. 2018).

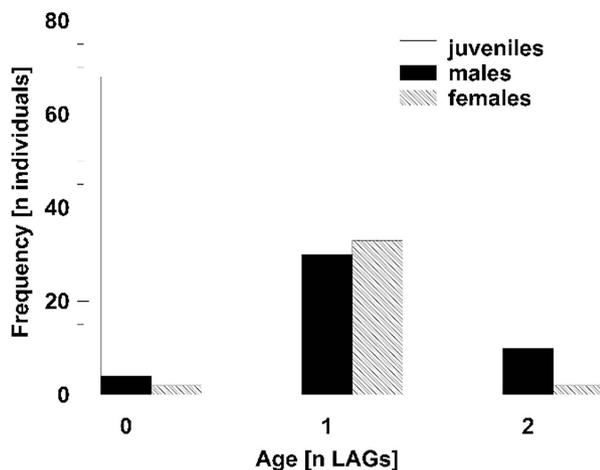


Figure 5. Age distribution of juvenile, male, and female *P. biligonigerus*.

Acknowledgements

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