



The life cycle of the giant water bug of northwestern Patagonian wetlands: the effect of hydroperiod and temperature regime

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Abstract. We analyzed the effect of hydroperiod and water temperature on the life cycle of the giant water bug *Belostoma bifoveolatum* in two wetlands of northwestern Patagonia, Argentina. In each wetland, we estimated adult and nymph abundance and monitored water depth and temperature throughout the study period. We determined the age structure of the giant water bug population in each wetland, and estimated the cumulative degree-days (DD) needed for eggs to hatch and for nymphs to complete their development. Individuals of *B. bifoveolatum* colonized temporary wetlands at the beginning of spring when daylight lasts 12 h. The breeding period varied with hydroperiod length and showed both univoltine and bivoltine strategies, with a relatively constant breeding season. Egg-bearing males appeared in October, carrying between 35 and 144 eggs per individual. Hatching success was high (~80% of eggs) and cumulative temperature for the hatching event was between 250 and 300 DD (which represents 3–4 weeks in nature), while complete development occurred between 800 and 1220 DD (~7–8 weeks). Individuals were more abundant in shallow and sunny patches of the wetlands, where the temperature was comparatively high, than in deeper or shaded sites. These results showed that hydroperiod duration and temperature could be good regulators of voltinism and development in *B. bifoveolatum*, driving the population structure of this giant water bug at the southern end of its distribution range.

Additional key words: *Belostoma bifoveolatum*, development, cumulative degree-days, voltinism, thermal preference

Aquatic insects are ectotherms, meaning that their body temperature is determined primarily by ambient temperature (Schowalter 2011). Thus, temperature directly affects metabolic and developmental rate (Hersey et al. 2010) and will influence the performance of individuals and the structure of species' assemblages. Studies have shown that aquatic insects, including aquatic hemipterans and coleopterans and nymphal stages of odonates, can persist and maintain sustainable populations in temporary wetlands characterized by pronounced fluctuations in environmental parameters such as temperature and water availability (Anderson & Wallace 1995; Hampton 2004; Fotz & Dodson 2009). This kind of fluctuating environment has probably imposed selection on life history patterns of many aquatic insects (Wiggins et al. 1980). Two examples which have been cited of these life history adaptations to temporary environments are egg diapause during the dry phase

of ponds, and faster larval development to reach metamorphosis during late spring in odonates and coleopterans (Wiggins et al. 1980; Schneider & Frost 1996; de Block et al. 2008). Hemipterans are among the highly adapted colonizers of temporary ponds, and among hemipteran species, there will be strong selection to favor early migration to temporary ponds during springtime for reproduction, followed by rapid development of the next generation (Wiggins et al. 1980).

The annual number of generations (voltinism) in insects is strongly regulated by environmental temperatures (Zeuss et al. 2017), with overlapping of larval generations in species that have multiple generations per year, or in those that extend one generation over several years (semivoltine or partivoltine). Environmental temperatures can differ greatly along latitudinal gradients. At high latitudes (i.e., low temperatures) voltinism is restricted to only one generation per year (univoltism) (Corbet et al. 2006; Flenner et al. 2010), while at low latitudes (i.e., warm temperatures), the occurrence of several

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generations in 1 year is common (Corbet et al. 2006). In this sense, tropical species have larvae that grow continuously during winter and have a low response to photoperiod cues (Bradshaw & Holzapfel 2008), while species at high latitudes typically undergo diapause (growth is arrested during unfavorable winter period) and have a strong response to photoperiod cues (Bradshaw & Holzapfel 2006, 2008).

Belostomatids are a group of hemipterans that include the largest aquatic insect species, and often represent the largest invertebrate predators in temporary wetlands (Smith 1997). Species within the genus *Belostoma* (Latreille 1907), commonly known as giant water bugs (Smith 1997), are native to North and South America, with a considerable number of species occurring in the tropical region of South America. Females cement eggs to the backs of males as cohesive units (egg pads) and males subsequently provide uniparental care of the eggs (Kight et al. 2011). Giant water bugs are voracious sucking ambush predators (Peckarsky 1982) that consume benthonic, planktonic, and neustonic organisms (Runck & Blinn 1994; Armúa De Reyes and Estévez 2005; Ohba & Nakasuji 2006) and even eat prey larger than themselves, such as fish (Tobler et al. 2013) and amphibians (Toledo 2005; Toledo et al. 2007; Nakazawa et al. 2013; Ohba & Haruki 2015). They can exert strong predation pressure, shifting the structure and function of a community by directly regulating the abundance of mesopredators (Boersma 2013).

North American species of giant water bugs generally have two discrete breeding seasons per year (Kight et al. 2011), whereas in tropical and subtropical regions of South America, they have continuous breeding with several generations per year (Kehr & Schnack 1991; Armúa De Reyes & Kehr 2005; Heckman 2011). However, no information on reproductive patterns is available for species of giant water bugs inhabiting the southern extreme of their distribution in South America (Patagonia). The north Patagonian temporary wetlands begin filling with rain and snowmelt in autumn (Perotti et al. 2005). The wet season lasts from 7 to 10 months. Cold temperatures and freezing periods prevail from autumn until early spring, while warmer temperatures and drying processes are typical during the spring and summer seasons. These environmental changes may have shaped the life cycles of wetland-dwelling species of northern Patagonia, possibly restricting their breeding season.

In southern locations of South America, two giant water bug species are present, *Belostoma bifoveolatum*

SPINOLA 1852 and *Belostoma elegans* (MAYR 1871) (Schnack 1971, 1976). *Belostoma bifoveolatum* is a ubiquitous species in Patagonia (Argentina and Chile) and is the most southerly occurring belostomatid species, with the presence of populations in Chubut province (Rio Turbio locality, 42°17'52.32" S; 71°54'31.48"W; L. Epele, unpubl. data). This species has diurnal activity. Males provide parental care by carrying eggs on their backs to prevent egg predation and desiccation, by using their hind legs to clean eggs, and by approaching the water surface to aerate eggs, similar to behaviors in other, related species (Thrasher et al. 2015). The life cycle of this species is completed through five nymphal instars that range in total length (TL) from 5.8 mm (instar I) to 19.51 mm (instar V) (Schnack 1971; Jara 2016).

Our objective was to analyze how fluctuating water availability and temperature within the wetlands of northern Patagonia affect the life cycle and age structure of the giant water bug *B. bifoveolatum*. We studied breeding phenology, seasonal abundance, and spatial distribution of populations of *B. bifoveolatum* in two temporary wetlands where this species reproduces. Additionally, we studied the spatial distribution of *B. bifoveolatum* and evaluated habitat use in relation to microhabitat temperature.

Methods

Study site and sampling methods

We studied two wetlands: Llao Llao (41°2'58"S; 71°33'58"W) and Las Cartas (41°04'22.9"S; 71°32'17.5"W) located within the Llao Llao Natural Reserve in northwestern Patagonia, Argentina. The vegetation surrounding these water bodies is typical of Andean forest, with perennial trees dominated by the austral beech (*Nothofagus dombeyi*) (Mermoz et al. 2009). The climate in this area is cold temperate with markedly seasonal precipitation, which defines the dry (spring and summer) and wet seasons (fall and winter). Mean annual precipitation in the area during the sampled years (2012–2016) was 1526 mm (± 122 mm) (automated weather monitor [Davis Vantage Pro]), collected in a location close to the study sites (41°7'43.33"S; 71°25'12.03"W; at an elevation of 800 m).

Llao Llao wetland is a small, shallow water body with a surface area of ~ 0.16 ha and shallow depth ranges from 0.1 to 0.38 m (Fig. S1A), while Las Cartas wetland (~ 1.26 ha) has a depth that ranges 0.42–1.6 m (Fig. S1B). Both wetlands have profuse vegetation dominated by *Juncus balticus* and *Juncus*

invulcratus (Juncaceae), *Schoenoplectus californicus* and *Carex niderdenliana* (Cyperaceae), *Agrostis leptotricha* (Poaceae), *Potamogeton linguatus* (Potamogetonaceae), and *Chara* sp. (Charales).

Field sampling

We performed weekly sampling of *Belostoma bifoveolatum*, counting all individuals collected in the field between 12:00 and 17:00 hours, from September until the wetlands dried. We sampled wetlands during the spring, coincident with the water bug reproductive season (at Llao Llao in 2012, 2013, 2014, and 2016; at Las Cartas in 2016). Also, at each wetland we set data loggers (20-cm water depth) (i-Button temperature loggers, temperature range -40 to 80°C , accuracy 0.5°C) to take hourly temperature measurements. Temperature obtained from data loggers provided us with the data to calculate the cumulative degree-days (DD) between sample weeks (López-Rodríguez et al. 2009).

To study the seasonal abundance of *B. bifoveolatum*, we used the dip-netting procedure indicated as the most efficient method for capturing different taxa and individuals of macroinvertebrates (Florenzio et al. 2012; Jara et al. 2013). We employed a rectangular dip-net with a 1-mm mesh. Each sampling consisted of sweeping the water column and the bottom ten times at three sites in each of the wetlands. We identified different sites based on water depth and shade conditions, which usually determine differences in water temperature (Jara, unpubl. data). We then chose three different sites: site 1, located in a sunny area and low depth (20 cm in Llao Llao and 36 cm in Las Cartas); site 2, located in the deepest zone of each wetland (38 cm in Llao Llao and 68 cm in Las Cartas); site 3, located in a partially shaded area and low depth (24 cm in Llao Llao and 30 cm in Las Cartas). At each site on every sampling date we also measured water depth and water temperature. We poured the captured individuals into a white plastic tray and counted individuals of each developmental stage (adults and nymphs). After counting individuals, we placed them in plastic bags to avoid counting more than once. We registered adults by sex and size, and measured them with a digital caliper. Males bearing eggs were photographed from a dorsal view using a digital camera (Canon PowerShot S5IS, Image Stabilizer, Canon Inc.) in order to measure TL, maximum body width (BW), and the number of eggs on their backs. We used the same methodology at each wetland to compare patterns

of colonization, breeding phenology, and nymph development in *B. bifoveolatum*.

Outdoor incubations

In November and December 2013, we performed replicated incubations of eggs to study development in *B. bifoveolatum* in relation to temperature. For this purpose, in November 2013, we captured 25 males carrying eggs in the Llao Llao wetland. We selected only males with freshly laid eggs and kept them in separate plastic containers filled with pond water. We recognized recently laid eggs (~ 24 – 48 h after oviposition) by their size (2 mm long) and dark color. To prevent egg detachment or discarding behavior, we collected males with particular caution, handling, and transporting them carefully to the laboratory (Kight et al. 2011). Incubation was conducted outdoors, in 32-L tanks (diameter 0.44 m, height 0.21 m) filled with 12 L of pond water, up to 0.11 m depth, under a natural photoperiod (day length=14 h) and temperature. Each tank contained an individual male of *B. bifoveolatum* carrying eggs, a data logger, and six artificial plants. The data loggers recorded water temperature every hour to calculate cumulative DD, and plants provided perching sites for males. We checked tanks daily to maintain a constant 0.11 m water depth, and covered them with fine mesh to prevent escape of individuals or predation by birds.

Before setting up the replicates (25 tanks; one male per tank), we measured TL and maximum BW of males and fed them daily *ad libitum* mosquito larvae and amphipods, which are abundant coexisting prey in the wetlands. In addition, we checked tanks daily, removed hatched nymphs, and transferred them to new containers to feed on mosquito larvae. We repeated this procedure until incubation finished, at which point we removed the egg pads from males and recorded the final date. In order to count egg capsules and aborted eggs, we preserved the egg pads in a 70% ethanol solution.

Data analysis

We calculated the relative abundance of each stage (female, male, male bearing eggs, and nymphal stages) as the percentage of the total individuals captured on each sampling date. To calculate DD, we determined the average daily temperature from the data logger temperature profiles collected at site 1 in both wetlands. We used information from site 1 (sunny areas and low depth) because *B. bifoveolatum* was present in these sites at high abundance. We

calculated DD by applying the following formula:

$$DD = \sum_{t_0}^{t_n} D$$

where t_0 is the date on which we found the first male of *B. bifoveolatum* bearing eggs, t_n is the sampling date of new developmental stages recorded in the population, and D is the average daily water temperature. Thus, the DD parameter considers the average daily cumulative temperature that influences insect development (Tokeshi 1985).

In each wetland, we compared the monthly average water temperature among sites using one-way ANOVAs followed by Holm–Sidak *post hoc* tests. To test for differences in the distribution of *B. bifoveolatum* among all three sites in each wetland (i.e., the number of individuals captured at each site), we used repeated measures ANOVA (2012, 2013, 2014 at Llaolao and 2016 at Las Cartas), followed by two-sample t-tests using the Bonferroni correction.

To test differences in the size of egg pads (i.e., clutch size) carried by males among years (at Llaolao wetland), we used a one-way ANOVA (after testing normality and homogeneity of variance) with year as a factor, and in the case of significant differences, we used two-sample t-tests with the Bonferroni correction. In all cases where the assumptions of normality and variance homogeneity were not met, we used the Kruskal–Wallis *H*-test and the Holm–Sidak correction for multiple comparisons. To evaluate whether there is a correlation between clutch size and male morphology (TL and maximum BW), we used Pearson product–moment correlation. We assessed the correlation between each male morphological variable and egg pad size for each of the different years at Llaolao (2012, 2013, and 2014) and for the sampled year at Las Cartas wetland. We performed all analyses in R 3.2.2 (R Core Team 2016).

Results

Wetlands hydroperiod and temperature

Llaolao wetland showed a variable hydroperiod over the years studied (2012, 2013, and 2014), with continuous precipitation even during spring. As spring progressed, there was generally a pronounced decrease in water, except for December 2013, when accumulated precipitation was higher than in the other years; the shortest hydroperiod was in 2014 (Fig. S2). The thermal regime of this

wetland was similar during the three studied years, showing the highest temperatures during 2013, and an increase in daily average water temperature with the advance of the spring season (Fig. S2). Hydroperiod was long in Las Cartas wetland during the monitored year (2016). The regime of water temperature followed the same pattern as Llaolao wetland, with the average daily temperature increasing as spring progressed. Maximum water depth decreased from 80 to 48 cm during spring.

Seasonal abundance of the giant water bug

During the three sampled years, we detected overwintering adults in Llaolao wetland in the second week of October in 2012 and 2013, and during the first week of October in 2014 (Fig. 1), when day duration was ~12 h. We did not capture any individuals of *Belostoma bifoveolatum* during the sampling year 2016 in Llaolao. Breeding was synchronous, and we captured egg-bearing males from late October to the end of November in 2012 and 2013, but during a shorter period in 2014. Females were more abundant than males (between 60% and 70% of the adult population), except in November when egg-bearing males represented 100% of the population. The nymphal stages dominated the population structure from December, with population peaks varying depending on month and year (Fig. 1). Newly emerged adults appeared between December and January, coincident with the end of the hydroperiod (Fig. 1), but this new generation did not reproduce in the wetland.

The giant water bug *B. bifoveolatum* in Las Cartas wetland showed differences in breeding phenology, voltinism, and population age structure from Llaolao wetland. Colonization by overwintering adults occurred 2 weeks earlier than in Llaolao. Also, reproduction began 2 weeks earlier than in Llaolao wetland and extended for a longer period than in Llaolao (Fig. 2). Instars I, II, and III appeared during November, with abundance peaks in November and December, while instars IV and V appeared at the end of November. We also noted newly emerged adults early in December, and these were reproductive soon after (Fig. 2).

Sex ratios and egg pad size

Females dominated the Llaolao population during all three studied years (sex ratio ranged 0.6–0.78). During spring, males bearing eggs increased

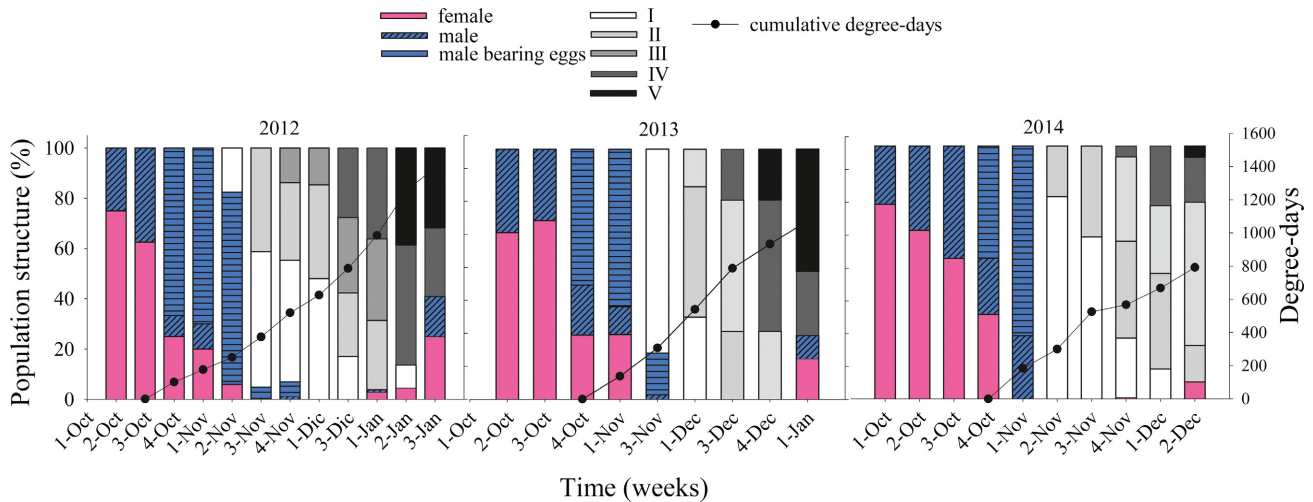


Fig. 1. Age structure of the population of *Belostoma bifoveolatum* (female, male, male bearing eggs, and five nymph instars) at the Llao Llao wetland, northwestern Patagonia, Argentina, over a period of three consecutive years (2012, 2013, and 2014). The line depicts the cumulative degree-days (DD) calculated from the moment when the first male bearing eggs was observed. X-axis indicates the sampling week (1–4) of each month.

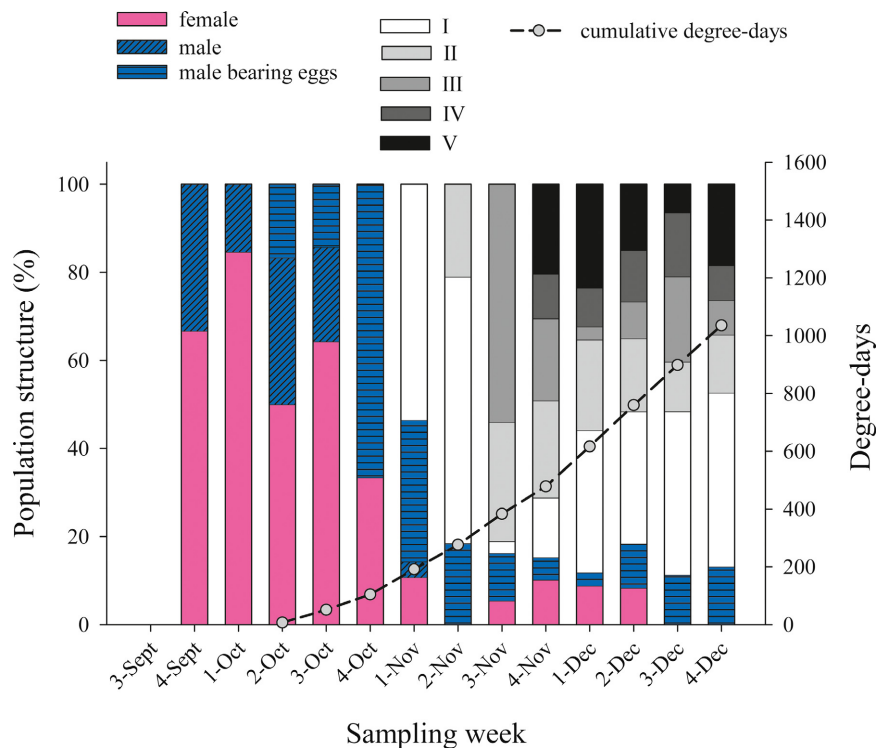


Fig. 2. Age structure of the population of *Belostoma bifoveolatum* (female, male, male bearing eggs, and five nymph instars), and cumulative degree-days (DD) in Las Cartas wetland, northwestern Patagonia, Argentina, during 2016. The dashed line depicts the cumulative DD. The DD were calculated from the moment when the first males bearing eggs were observed.

in number until they dominated the adult population. Egg pad size was significantly different among years (Kruskal–Wallis $F_{2,24}=19.4$, $p<0.001$). Egg pad size was smaller in 2014 than in 2012 and 2013 (2014 vs. 2012 $t=3.5$, $p=0.017$; 2014 vs. 2013, $t=3.2$, $p=0.025$). Only during 2013 was egg pad size correlated positively with male TL at Llao Llao

(Table 1). The Las Cartas population showed a similar sex ratio pattern to Llao Llao, with females being the dominant sex during spring (2016) but decreasing in November, when egg-bearing males increased in abundance (Fig. 2). Egg pad size at this location was large than at Llao Llao (Table 1), and we did not observe any correlation between egg pad

size and the two morphological variables measured in males (TL and BW) (Table 1).

Distribution of the giant water bug in the wetlands

Analysis of the distribution of *B. bifoveolatum* in Llao Llao wetland showed that they were more abundant at site 1 (shallow and sunny) than at sites 2 and 3 (Table 2, Fig. 3). This pattern was observed during all 3 years sampled (Table 2, Fig. 3). The three sites sampled showed differences in temperature during spring months in all years sampled (Table 3). In general, higher temperatures were observed at site 1 than at sites 2 or 3 (Table 3). The same distribution pattern was observed in Las Cartas wetland during the spring of 2016 (Table 2, Fig. 3). Adults and nymphs were captured in different numbers between sites, and individuals were more abundant at site 1 than at sites 2 and 3 (Table 2, Fig. 3). The average water temperature during October and November was significantly different between sites, with site 1 showing higher temperatures than sites 2 and 3 (Table 3).

DD and the life cycle of the giant water bug

Calculation of DD at Llao Llao showed that the length of egg incubation was ~ 288 (± 30 SD) DD until eggs hatched, this being the longest stage of the life cycle. The complete life cycle of *B. bifoveolatum* (from egg to adult) lasted from 8 to 10 weeks, and total development in field conditions varied widely, requiring between 721 and 1260 DD (Fig. 1). At Las Cartas, we found that egg incubation was ~ 200 DD, which is the equivalent of

4 weeks, and the complete development of one generation was 692 DD, or ~ 7 weeks (Fig. 2).

Outdoor incubations

Of 25 males in the controlled experimental tanks, only five discarded the egg pads with no signs of nymph hatching. Temperature was not significantly different among tanks (Kruskal–Wallis $H=5.7$, $p=0.1$). Average water temperature was 10.27°C ($\pm 0.39^\circ\text{C}$) and thermal amplitude was 9°C ($\pm 0.89^\circ\text{C}$), with an average minimum temperature of 6.16°C ($\pm 2.1^\circ\text{C}$) and average maximum temperature of 15.2°C ($\pm 2.5^\circ\text{C}$). The number of eggs per male was 74 ± 4 , and hatching success was 81% ($\pm 3\%$). Egg incubation required 309 (± 42) DD, which was $\sim 32 \pm 4$ d of incubation (Fig. 4). The hatching of nymphs was not synchronous, with hatching rates fluctuating from 2–6 nymphs d^{-1} to 20–29 nymphs d^{-1} (Fig. 4). The total elapsed time from first to last hatched nymph in an egg pad was 8 d (± 0.5 d) ($n=20$).

Discussion

Temporary wetlands in northwestern Patagonia are affected by a rainy autumn regime (which strongly influences hydrologic conditions) and seasonal changes in photoperiod and temperature. Within this environment, the giant Patagonian water bug *Belostoma bifoveolatum* generally has a univoltine life cycle. However, when environmental conditions result in a longer hydroperiod, as observed in one of the studied wetlands (Las Cartas), individuals of *B. bifoveolatum* may exhibit a bivoltine life cycle, with one generation developing

Table 1. Number of eggs per male (egg pad size) and two morphological body size variables in males of *Belostoma bifoveolatum* during 3 years at Llao Llao wetland and 1 year at Las Cartas wetland. Correlation coefficients (r), p values, and sample sizes (n) are shown for each pair of variables.

Location	Year	Number of eggs (mean \pm SD)	Male morphology (mean \pm SD, mm)	r	p	n
Llao Llao	2012	84 \pm 14	TL=25.2 \pm 1.03	0.05	0.8	15
			BW=12.06 \pm 0.88	0.5	0.08	15
	2013	83 \pm 17	TL=25.7 \pm 1.01	0.6	0.02*	12
			BW=11.83 \pm 0.67	0.5	0.09	12
	2014	57 \pm 11	TL=23.6 \pm 0.8	-0.289	0.4	10
			BW=11.23 \pm 0.29	-0.05	0.8	10
2016	nwb	–	–	–	–	
Las Cartas	2016	89 \pm 27	TL=21.6 \pm 0.95	-0.295	0.25	17
			BW=10.07 \pm 0.9	-0.12	0.64	17

BW, maximum body width; nwb, no water bugs present; TL, total length. Asterisk indicates a significant correlation.

Table 2. Repeated measures ANOVA comparison of the distribution of *Belostoma bifoveolatum* during spring season at the three sampled sites in the studied locations (Llao Llao and Las Cartas wetlands).

Wetland	Spring	F	p	Site comparison	t	p
Llao Llao	2012	15.778	<0.001	Site 1 versus 3	5.5	0.001*
				Site 1 versus 2	3.9	0.002*
				Site 2 versus 3	1.5	0.424
	2013	18.351	<0.001	Site 1 versus 3	5.9	0.001*
				Site 1 versus 2	3.9	0.001*
				Site 2 versus 3	1.9	0.06
	2014	6.399	0.008	Site 1 versus 3	3.342	0.004*
				Site 1 versus 2	0.56	0.579
				Site 2 versus 3	2.7	0.012*
Las Cartas	2016	31.645	<0.001	Site 1 versus 3	7.17	0.001*
				Site 1 versus 2	6.57	0.001*
				Site 2 versus 3	0.6	0.5

Asterisks indicate significant differences between sites.

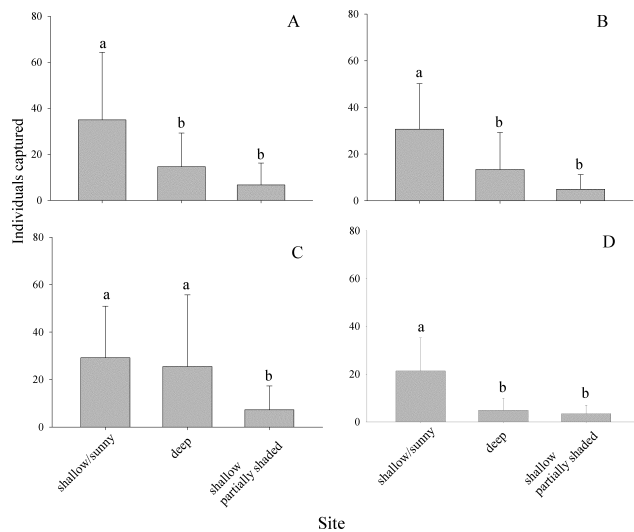


Fig. 3. Spatial distribution of individuals of *Belostoma bifoveolatum* in Llao Llao and Las Cartas wetlands. **A, B, C:** Llao Llao wetland hydroperiod 2012, 2013, and 2014, respectively, **D:** Las Cartas wetland hydroperiod 2016. The bars represent the mean number of individuals captured at each site ± 1 SD. At both wetlands, sites 1 were shallow and sunny; sites 2 were deep; and sites 3 were shallow and partially shaded. Different letters indicate significant differences ($p < 0.05$).

in early spring and a second-generation in later spring or early summer.

Belostoma bifoveolatum is the southernmost species of the genus and showed a short breeding season that is associated with a short period in which males actively carry eggs. This observation contrasts with species living in the north of Argentina, such as *Belostoma elegans*, that breed throughout the year, with peaks in the number of males carrying eggs in

different months, even during winter (Armúa De Reyes & Kehr 2005). The climate conditions in north-western Patagonia impose a limit for reproduction in this giant water bug, and these climate conditions appear to be more severe than those experienced by water bug species in North America. For example, giant water bug species in temperate populations in North America have two discrete breeding seasons, one during spring and another during autumn (Kight et al. 2011), even when climatic conditions are harsh.

The giant water bug populations studied here showed a similar pattern of breeding phenology. Soon after the end of the breeding season, abundance of females decreased drastically in the population, and all the males observed were carrying eggs. With the progression of the season, nymphs became more abundant, and adults disappeared from the water bodies, presumably because adults die after reproduction or migrate to other habitats.

Sex structure in both populations of water bugs indicated that females were more abundant than males. Thus, it is likely that males mate with more than one female during the breeding season, as was observed in other giant water bugs (Kight et al. 2011; Ohba et al. 2016). All females captured during the colonization period in both populations showed swollen abdomens, suggesting the presence of mature oocytes (preliminary results suggested that females can produce 30–40 eggs). This could indicate that females and males migrate from overwintering locations ready for reproduction in temporary ponds, as is the case at our studied sites.

We confirmed that in both wetlands only males carried the egg pad. Iglesias et al. (2012) found that in *B. bifoveolatum*, and in other belostomatid

Table 3. Water temperature ($^{\circ}\text{C}$, mean \pm SD) during the survey time (12:00–17:00 hours) at three sampling sites in each wetland.

Wetland	Spring	Month	Site 1	Site 2	Site 3	F	p
Llao Llao	2012	October	8.04 \pm 1.6 ^a	5.36 \pm 1 ^b	4.5 \pm 2 ^b	39.48	0.001*
		November	15.24 \pm 3.2 ^a	10.7 \pm 2.4 ^b	8.5 \pm 1 ^b	28.55	0.001*
		December	18.5 \pm 2	18.1 \pm 2	17.5 \pm 2	1.80	0.242
	2013	October	7 \pm 0.5 ^a	6 \pm 0.3 ^b	4 \pm 1 ^c	86.27	0.001*
		November	12.1 \pm 2 ^a	10.5 \pm 1 ^b	8 \pm 1.5 ^b	28.55	0.001*
		December	16.5 \pm 2 ^a	14 \pm 1 ^b	12 \pm 0.5 ^c	46.69	0.001*
	2014	October	11.6 \pm 0.6 ^a	8 \pm 0.3 ^b	6.8 \pm 0.7 ^c	110.55	0.001*
		November	12.6 \pm 3 ^a	9 \pm 2 ^b	7.5 \pm 2 ^b	19.11	0.001*
		December	23 \pm 4 ^a	18 \pm 3.5 ^b	15 \pm 2 ^c	27.84	0.001*
Las Cartas	2016	October	11 \pm 1 ^a	9 \pm 3 ^b	8 \pm 1 ^b	12.53	0.001*
		November	13 \pm 2 ^a	9 \pm 1 ^b	8 \pm 0.5 ^b	23.5	0.001*
		December	23 \pm 4	19 \pm 2	21 \pm 1.5	1.55	0.241

Asterisks indicate significant ANOVA results. Superscript letters indicate significant temperature differences between sites estimated by t-tests.

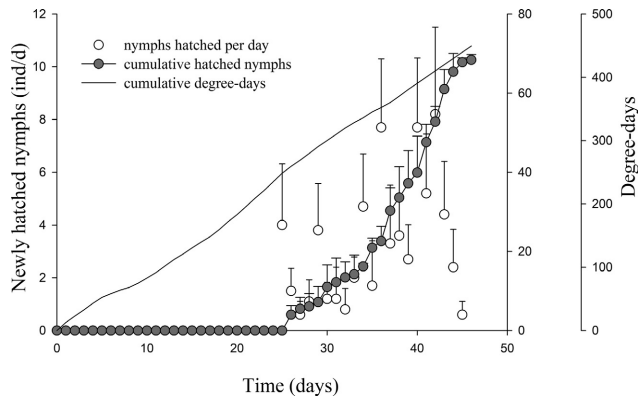


Fig. 4. Incubation of eggs by males in outdoor containers ($n=20$). The figure shows the average number of nymphs I hatched daily (open dots), the mean cumulative number hatched (gray dots), and cumulative degree-days (line). The time=0 corresponds to the first day of experiment.

species, the males have relatively longer middle and hind legs than females and provide parental care. The morphology of males allows them to maintain effective locomotion even when carrying the egg pads (Iglesias et al. 2012). We observed that egg pad size varied among years at Llao Llao, but this result was not associated with variation in male morphology in either wetland. However, several authors proposed that females choose males according to size, and that females prefer males bearing eggs over males without eggs (Ohba et al. 2016). Several factors could affect egg pad size in this species; for example, we found that males carried small egg pads during the shorter hydroperiod in 2014, and 2014 was also the year with lower temperatures compared with 2012 and 2013. Therefore, further

investigations are needed to evaluate the factors that influence interannual variation in egg pad size in this species.

Water temperature in both wetlands fluctuates markedly throughout the day, which could have a strong influence on growth and development in *B. bifoveolatum*. The number of DD required for egg hatching was around ~ 250 – 300 DD, while the total DD required to complete one generation (eggs to new adults) was 800–1220 DD. The difference between hatching time and total generation time may reflect the influence of other environmental variables at the studied wetlands that could interact with temperature and alter life cycle length, especially during nymphal or larval stages of aquatic insects (e.g., increase in nymph densities, food depletion; de Block & Stoks 2005). For example, nymphs may respond to different environmental signals that could accelerate development (Lund et al. 2016). Differences in the hydroperiod (with fast drying of ponds) and an increase in population density (crowding) could influence the behavior of larval stages of many aquatic insects (de Block & Stoks 2005; Lund et al. 2016). Several authors showed that, in some aquatic insects, the presence of cannibalism increases the rate of development in a population, in comparison to populations in which this behavior is not present (de Block & Stoks 2004; Lancaster & Briers 2008). Reduction of the wetland area at the end of the spring could lead to cannibalism opportunities in *B. bifoveolatum*.

Environmental conditions, such as temperature, are frequently considered the major abiotic factor affecting the phenology of aquatic invertebrates and

their spatial distribution (Vannote & Sweeney 1980; Sweeney et al. 1986; Cayrou & Cereghino 2005; Ohba et al. 2010). We found that *B. bifoveolatum* had selective spatial distribution in the wetlands at our study sites. Adults and nymphs, in different stages, occupied shallow and sunny regions during the day, resulting in exposure to high levels of solar radiation. At both wetlands, some locations (site 1) received more light from early morning than others (sites 2 or 3). The amount of light reaching a wetland surface affects abiotic factors such as water temperature. Considering these observations, we suspect that both conditions, light and warm temperatures, could directly or indirectly influence adult distribution, which would benefit reproduction and development of the nymphs. For example, pigmented eggs can absorb solar radiation, increasing warming rate and accelerating development. In addition, abundant food resources (invertebrates and tadpoles) in sunny, warm sites could favor faster growth of nymphs, reducing their vulnerability to predation in the early stages of their growth.

Finally, our results provide insights into the biology of the giant water bug *B. bifoveolatum* in southern populations (north Patagonia) that live under harsh climate conditions with a short growing season, in which one or two generations can successfully develop. Our investigation showed that *B. bifoveolatum* is well adapted to this region, with a short univoltine or, less commonly, bivoltine life cycle. The voltinism of this giant water bug seems to be associated with hydroperiod duration and the temperature regime in these temporary wetlands. The distribution pattern observed in warm microhabitats could promote rapid development in this cold region. Variations in local climate conditions could lead to changes in the breeding phenology and voltinism in *B. bifoveolatum*. This may have implications for the structure of wetland communities, since this species, with few competitors, is the largest predator in these systems and has the potential to exploit both invertebrate and anuran prey (Jara 2016).

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Supporting information

Additional Supporting information may be found in the online version of this article:

Fig. S1. Studied wetlands: Llao Llao wetland (A) and Las Cartas wetland (B).

Fig. S2. Accumulated precipitation, water depth, and daily average water temperature in Llao Llao (left panel) and Las Cartas wetlands (right panel). Precipitation data from January to July are shown together.