Long-term study of the life cycle of the freshwater snail *Heleobia parchappii* (Mollusca: Cochliopidae) in a lentic environment in Argentina

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**ABSTRACT**

**Long-term study of the life cycle of the freshwater snail *Heleobia parchappii* (Mollusca: Cochliopidae) in a lentic environment in Argentina**

The gastropod *Heleobia parchappii* is distributed in lentic and lotic environments in the Pampas region of Argentina and is a host for at least 24 species of digeneans. Because ecoparasitological investigations of snail-digenean associations frequently lack knowledge of the structure and dynamics of the snail host population, the purpose of this study is a long-term analysis of the basic aspects of the *H. parchappii* life cycle as a preliminary step of an environmental parasitology study. Monthly collections were made in Nahuel Rucá Lagoon from August 2010 to February 2013, and a total of 24,931 individuals of *H. parchappii* were measured and size-frequency distributions were constructed. The von Bertalanffy growth equation and the von Bertalanffy equation with seasonal oscillation growth equations were fitted to shell length data. The size-frequency distributions of *H. parchappii* fit a polymodal distribution, and 14 age classes were identified. The breeding periods of *H. parchappii* in 2011 were in summer (February and March), autumn (June) and spring (October). However, in 2012 the periods of recruitment were in autumn (May), winter (July) and spring (October). The von Bertalanffy models best fit the data, and the likelihood ratio test comparison showed significant differences in the estimated parameters between cohorts. This is the first study in which three cohorts of *H. parchappii* were followed from recruitment until death, and a life cycle of approximately 20.33 months was estimated. The observed differences in the recruitment periods and growth parameters could be a strategy by the snail to prevent the negative effects on reproductive success caused by digeneans. We suggest that long-term studies that include a larger number of environmental and biological factors are needed to fully understand the dynamics of the recruitment and growth of juveniles of this species across its distribution range.

**Key words:** Life cycle, shell size, von Bertalanffy growth model.

**RESUMEN**

**Estudio a largo plazo del ciclo de vida de Heleobia parchappii (Mollusco: cochliopidae) en un ambiente léntico de la República Argentina**

El gasterópodo Heleobia parchappii se distribuye en ambientes léticos y lóticos de la región pampeana de Argentina y es utilizado como hospedador por, al menos, 24 especies de digeneos. Dado que los estudios parasitológicos sobre las asociaciones molusco-digeneo carecen, con frecuencia, del conocimiento previo acerca de la estructura y la dinámica poblacional del hospedador molusco, el propósito de este trabajo es analizar, mediante un estudio a largo plazo, los aspectos básicos del ciclo de vida de H. parchappii, como paso preliminar a la realización de un estudio parasitológico integral. A tal fin, se realizaron capturas mensuales en la Laguna Nahuel Rucá desde agosto del 2010 hasta febrero del 2013. Durante este período se midieron 24,931 individuos de H. parchappii, se construyó la distribución de frecuencia de tallas y se analizó si el crecimiento de H. parchappii se ajustaba a los modelos de crecimiento con y sin oscilaciones estacionales de von Bertalanffy. Las distribuciones de frecuencia de tallas se ajustaron a una distribución polimodal y pudieron identificarse 14 clases de edad. El período de reclutamiento de H. parchappii presentó diferencia entre los años estudiados, en el año 2011 el reclutamiento ocurrió en verano (febrero y marzo), en otoño (junio) y en primavera (octubre). Mientras que, en el año 2012 los períodos de reclutamiento fueron en otoño (mayo), en invierno (julio) y en primavera (octubre). El modelo de crecimiento...
INTRODUCTION

Species of the genus *Heleobia* Stimpson, 1865 inhabit environments with very different salinities, exhibit an integrated diet of many organisms and are important intermediate hosts for some groups of parasites (De Francesco & Isla, 2004a; Rodrigues & Benvenuti, 2011; Alda & Martorelli, 2014; Merlo et al., 2014; Magnone et al., 2015). However, current knowledge about their biology is not as complete as is desirable. For example, of the 16 species belonging to this genus reported in Argentina (Rumi et al., 2008), only 5 life cycles have been elucidated. *Heleobia piscium* (d’Orbigny, 1835) and *Heleobia conexa* presented an annual life cycle (12 months) with a single reproductive period during the summer or spring, respectively (De Francesco & Isla, 2004a; Martin, 2008). The life cycle of *Heleobia australis* (d’Orbigny, 1835) varied according to the study site, with a duration between 12 and 30 months and one or two periods of recruitment (summer or spring and summer) (De Francesco & Isla, 2004b; Carcedo & Fiori, 2012). In contrast, *Heleobia hatcheri* (Pilsbry, 1911) is the only species in Argentina and South America with parthenogenetic reproduction (Martín, 2002).

Finally, *Heleobia parchappii* is the fifth species for which a life cycle is known. This species is widely distributed in lentic and lotic environments in the Pampas region of Argentina and can develop and maintain stable populations in brackish waters with salinities averaging between 17‰ and 23‰ (Gaillard & Castellanos, 1976; Castellanos & Landoni, 1995; De Francesco & Isla, 2004a). Its life cycle was studied in the lower Colorado River Valley (province of Buenos Aires) and in the Mar Chiquita coastal lagoon area. At both sites, the period of juvenile recruitment occurs from spring to autumn, suggesting an annual life cycle (Cazzaniga, 1981; De Francesco & Isla, 2004a). Additionally, *H. parchappii* is used as a host by at least 24 species of digeneans (Merlo, 2014; Merlo et al., 2014).

The complex life cycles of digeneans include different larval stages that parasitize molluscs (first intermediate hosts), invertebrates and vertebrates (second intermediate hosts) and finally mature as adults in definitive vertebrate hosts. For this reason, digeneans can be considered as potential bioindicators of environmental conditions. However, the use of larval digeneans in snail hosts as biological indicators requires certain conditions that must be present in the environment under study: i) a diverse and abundant fauna of definitive and intermediate hosts, ii) a community of at least 3 species of larval digeneans in the selected mollusc and iii) preferably previous work including descriptions of larval digeneans in the area (Huspeni et al., 2005). In addition to these requirements, investigation of the temporal variations in digenean prevalence (% of parasitized hosts) requires knowledge of the life history patterns of the snail host population because seasonal changes in the digenean prevalence in these hosts can be related to seasonal changes in the age structure of the host population, host recruitment, and host mortality (Rohde, 1981; Fernandez & Esch, 1991; Snyder & Esch, 1993; Yoder & Coggins, 1998). Because ecoparasitological investigations...
of snail-digenean associations frequently lack knowledge of the structure and dynamics of the snail host population, this long-term study aims to analyse in more detail the basic aspects of the *H. parchappii* life cycle as a preliminary step of a parasitological study.

**MATERIALS AND METHODS**

**Area of study**

The Nahuel Rucá Lagoon (37°37’S, 57°25’W), located SE of the province of Buenos Aires (Argentina), has an area of 245 ha and a mean depth of 0.14 ± 0.16 m (Federman, 2003). Its vegetation is characterized by the presence of *Schoenoplectus californicus* at its margins, making these environments the main breeding and resting areas of a large number of bird species (Ferrero, 2001; Josens, 2011). Previous investigations in this lagoon include several studies related to vegetation (Stutz, 2000; Isacch, 2001; Federman, 2003), contamination in sediments, macrophytes and fish (Chiodi, 2005; Ondarza, 2005) and birds (Ferrero, 2001; Josens, 2011).

**Sampling procedures**

This study was undertaken during a 31-month period from August 2010 to February 2013. Specimens of *H. parchappii* located among the submerged vegetation and on the substratum were collected monthly with the aid of sieves (0.5 mm) and placed into plastic cups filled with water from the lagoon for transportation. The water temperature and salinity were measured monthly using a Digital Multi-Thermometer (range −50°C to 150°C) and a Portable Refractometer FG-211 Salinity/ATC 0%e⁻100%e (CHINCAN, Beijing China), respectively. In the laboratory, snails were placed in 1 L containers with filtered water from the lagoon with an aerator. Subsequently, snails were measured under a stereoscopic microscope with an ocular micrometre (precision 0.05 mm). Total shell length (the distance from the apex to the anterior margin of the aperture) was used as an estimate of size.

Life cycle of *Heleobia parchappii*

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameters</th>
<th>Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>von Bertalanffy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$L_\infty$</td>
<td>8.24</td>
<td>9.55</td>
</tr>
<tr>
<td>$K$</td>
<td>1.43</td>
<td>0.74</td>
</tr>
<tr>
<td>$t_0$</td>
<td>−0.37</td>
<td>−0.58</td>
</tr>
</tbody>
</table>

| Seasonal oscillations | $C$       | 0.56   | 0.17   | 0.24   |
|                       | $t_s$     | 0.15   | 2.93   | 1.83   |

**Data analysis**

To recognize dominant size groups, the data obtained were used to construct size-frequency distributions that were analysed using the Bhattacharya method (FISAT II software), which separates normal distributions in a mixture of distributions in length-frequency data. This method allows estimation of the mean lengths and standard deviations for the identified age groups (Gayanilo *et al.* 1995), and these results were used to separate cohorts. To confirm each component of the normal distribution from the modal progression analysis, we used the NORMSEP method also available in the FISAT II software (Pauly & Caddy, 1985).

The von Bertalanffy growth equation and the von Bertalanffy equation with seasonal oscillations fit to the shell length data obtained from cohorts 6, 7 and 8, which were used because they were followed from their recruitment until death. These growth models were expressed as $L_t = L_\infty (1 - \exp^{-K(t-t_0)})$ and $L_t = L_\infty (1 - \exp^{-K[(t-t_0)+\pi \sin(2\pi(t-t_s)/\pi)]/\pi - \exp^{-K[(t-t_0)+\pi \sin(2\pi(t-t_s)/\pi)]/\pi}})$. $L_t$ is the predicted length at age $t$; $L_\infty$ is the asymptotic length, $K$ is the growth constant of dimension time⁻¹ (year⁻¹ in most seasonally oscillating growth curves) expressing the rate at which $L_\infty$ is approached; $t_0$ is the theoretical ‘age’ of the molluscs at length zero; $C$ expresses the relative amplitude of the seasonal oscillation, and $t_s$ is the starting point of the

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Table 1. *Heleobia parchappii*. Maximum likelihood parameter estimates corresponding to the von Bertalanffy function models. Estimación de los parámetros de crecimiento de las funciones de von Bertalanffy.
Figure 1. Size-frequency distributions histograms of *Heleobia parchappii* at Nahuel Rucá Lagoon from August 2010 to February 2013. Histogramas de distribución de frecuencia de tallas de *Heleobia parchappii* en la Laguna Nahuel Rucá desde agosto de 2010 a febrero de 2013.
Life cycle of *Heleobia parchappii*

![Histograms showing shell length distribution for different months.](image)

**Shell length (mm)**
Table 2. *Heleobia parchappii*. Results of the selection model that best fits the curve of growth (Fig. 2) for cohorts 6, 7 and 8. Number of parameters (n° par), Akaike’s information criterion (AIC), Akaike differences (ΔAIC), normalized weights of AIC (w%).

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Model</th>
<th>n° par</th>
<th>AIC</th>
<th>ΔAIC</th>
<th>w(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>von Bertalanffy</td>
<td>3</td>
<td>-55.80</td>
<td>0</td>
<td>49.20</td>
</tr>
<tr>
<td></td>
<td>von Bertalanffy seasonal oscillations</td>
<td>5</td>
<td>-53.77</td>
<td>2.03</td>
<td>17.81</td>
</tr>
<tr>
<td>7</td>
<td>von Bertalanffy</td>
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<td>32.79</td>
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<tr>
<td></td>
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<td>-68.08</td>
<td>3.05</td>
<td>7.14</td>
</tr>
<tr>
<td>8</td>
<td>von Bertalanffy</td>
<td>3</td>
<td>-61.48</td>
<td>0</td>
<td>40.34</td>
</tr>
<tr>
<td></td>
<td>von Bertalanffy seasonal oscillations</td>
<td>5</td>
<td>-59.00</td>
<td>2.48</td>
<td>11.68</td>
</tr>
</tbody>
</table>

Figure 2. Growth curves of *Heleobia parchappii* estimated by cohort separation from August 2010 to February 2013. Estimated averages (± standard deviation) of the normal curves fit to the size-frequency histograms (Figure 1) are shown. Curva de crecimiento de cada cohorte de *Heleobia parchappii* desde agosto de 2010 a febrero de 2013. Tallas medias (± desviación estándar) estimadas a partir de los histogramas de distribución de frecuencia de tallas de la Figura 1.

Results

A total of 24 931 individuals of *H. parchappii* were measured; the maximum shell size recorded was 9.36 mm, while the minimum value was 0.50 mm. Size-frequency distributions of *H. parchappii* fit a polymodal distribution (Fig. 1), and fourteen age classes were identified over the 31 months of study (Fig. 2). Cohorts 6, 7 and 8 were compared using a one-way ANOVA. Tukey tests were performed for post-hoc comparisons (Zar, 2009). Homogeneity of variances and normality were confirmed prior to all analyses with Levene and Shapiro-Wilks, respectively (Zar, 2009).
followed from the snails’ recruitment until their death. Cohort 6 was followed for 18 months, while cohorts 7 and 8 were followed for 21 and 22 months, respectively. The life cycle of *H. parchappii* in Nahuel Rucá Lagoon thus showed an average duration of 20.33 months. The breeding period of *H. parchappii* took place in all seasons of the year, but these seasons differed between the years of study. In 2010, we detected one recruitment period in summer (November or December, no data were available in November). In 2011, we detected recruitment periods in summer (February and March), autumn (June) and spring (October). However, in 2012, the periods of recruitment were autumn (May), winter (July) and spring (October) (Fig. 2).

The parameters of the von Bertalanffy models of cohorts 6, 7 and 8 are summarized in Table 1; in all cases, the von Bertalanffy model was the one that best fit the data (Table 2). The likelihood ratio test comparison showed significant differences in the estimated parameters between the cohorts. The growth constant (*K*) of cohort 6 was twice those observed in cohorts 7 and 8. However, *L_∞* was similar for the three cohorts (Table 3).

Table 3. Comparison of growth parameters estimated from the von Bertalanffy model by the likelihood ratio test. Comparación mediante la prueba de la razón de verosimilitud de los parámetros estimados del modelo de crecimiento de von Bertalanffy.

<table>
<thead>
<tr>
<th>Cohort</th>
<th><em>H_01</em></th>
<th><em>H_02</em></th>
<th><em>H_03</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>6 vs 7</td>
<td>n.s.</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6 vs 8</td>
<td>n.s.</td>
<td>&lt;0.001</td>
<td>n.s.</td>
</tr>
<tr>
<td>7 vs 8</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

*Heleobia parchappii* densities ranged from 240 ind./hr (March, 2012) to 1478 ind./hr (October, 2012) and showed fluctuations throughout the months under study (*F*_{28;116} = 14.13; *p* < 0.0001). Analysing consecutive months revealed differences between June and July (2012) and between September and October (2012) (in both cases *p* < 0.05). Despite this, the temporal dynamics of the *H. parchappii* densities were relatively constant throughout the study, increasing in spring (October-November) and decreasing towards winter (June-July). Nevertheless, in the winter of 2012 (July), a peak density associated with the reproductive event mentioned previously was observed (Fig. 3).

![Figure 3](image-url)  
**Figure 3.** Mean density (± standard deviation) of *Heleobia parchappii* at Nahuel Rucá Lagoon from August 2010 to February 2013. Densidad media (± desviación estándar) de *Heleobia parchappii* en la Laguna Nahuel Rucá desde agosto de 2010 a febrero de 2013.
Regarding the abiotic factors measured in Nahuel Rucá Lagoon, the water temperature ranged from 1.2 °C (June 2012) to 28.9 °C (December 2010). Salinity was constant throughout the year, being highest in June and August (2010) (0.68‰) and lowest in October and November (2010) and in January, March and November (2011) (0.62‰) (Fig. 4).

**DISCUSSION**

This is the first study in which three cohorts of *H. parchappii* were followed from recruitment until death, and a life cycle of approximately 20.33 months was estimated for this species. Our results contrast with those of Cazzaniga (1981) in the lower Colorado River Valley (province of Buenos Aires) and De Francesco & Islas (2004a) in the Mar Chiquita coastal lagoon. These authors suggested an annual (approximately 12 months) life cycle for *H. parchappii*.

The *H. parchappii* population in Nahuel Rucá exhibits a seasonal cycle of reproductive activity with three main spawning peaks. In 2011 and 2012, two of the three breeding seasons were observed in autumn (June 2011 and May 2012) and in spring (October 2011 and 2012); a similar reproductive pattern was found by Cazzaniga (1981) and De Francesco & Islas (2004a). The spring peak seems to be more important, based on the higher abundance of snails observed. However, the third reproductive peak varied over the years of studies; in 2011 recruitment of juveniles occurred during the summer (February and March), while in 2012 recruitment was detected in winter (July). This last winter recruitment was mentioned by Cazzaniga (1981), but not by De Francesco & Islas (2004a).

Some authors have linked the recruitment of juvenile gastropods with water temperature and salinity (De Francesco, 2002; De Francesco & Islas, 2004a; Malavé *et al.*, 2012; Yakovis *et al.*, 2013). In this study, the relationship between recruitment and water temperature was partly contradictory. For example, the spring and summer recruitments coincided with water temperatures above 15 °C, and recruitment occurring in the winter of 2012 (July) coincided with an 8 °C increase of water temperature compared with the previous month (June), reaching up to 10 °C. However, fall recruitments coincided with temperatures of approximately 5 °C. These results contrast with those obtained by De Francesco & Islas (2004a), which indicated that recruitment periods start when the temperature reaches 20 °C. Furthermore, no relationship was observed between the periods of recruitment and salinity, a result mainly due to the salinity remaining constant throughout the study. From these results, temperature and salinity could not be considered as the only decisive factors affecting the breeding season.

Parasitism is another factor that may affect recruitment and growth in snails (Lefcort *et al.*, 2002; Gilardoni *et al.*, 2012). Digeneans generally reduce reproductive success and can alter the growth rate of their hosts. However, the
molluscs can employ physiological and immunological defence mechanisms to prevent infection (Trail, 1980; Minchella, 1985; Gustafson & Bolek, 2014). One alternative strategy available for the host is to vary individual life history parameters (e.g., reproductive periods, growth rate) to prevent or at least minimize the negative effects of parasitism on reproductive success, with a lower cost in energy (Minchella, 1985). Therefore, the lower growth rates observed in cohorts 7 and 8 could be an adaptation to infection by digeneans because the energy currently used for host body growth becomes available for reproduction. Additionally, Merlo (2014) found 24 species of digeneans parasitizing *H. parchappii*. These parasites showed temporal variations in prevalence; in the warmer months (December-March) prevalence values were higher than in the colder months (June-September). Overall, previous studies have shown a positive influence of water temperature on the rate of development of the eggs of digeneans and on the time of emergence and lifetime of the miracidia. In addition, a direct relationship exists between the size of the host and the prevalence of infection by digeneans (Kuris, 1979; Vanoverschelde, 1982; Flores et al., 2010). Individuals of *H. parchappii* recruited during winter (June (2011), July and August (2012)) would have minor probabilities of being infected by digeneans and would therefore have greater possibilities of development and higher reproductive success. Thus, reproductive periods of *H. parchappii* in the colder months of the year and different growth rates between the cohorts could be seen as a strategy to increase their reproductive success. As mentioned above, the use of larval digeneans as tools to study biotic communities requires knowledge of the population dynamics of their first intermediate host. Hence, recruitment periods throughout the year and the relatively short life cycle of *H. parchappii* favours the availability of individuals as potential hosts and makes possible a quick restructuring snail-digenean system. This restructuring could allow inferences about environmental changes over short periods of time, including disturbances due to human activity and fluctuations in the diversity and abundance of vertebrate hosts (Merlo & Etchegoin, 2011; Etchegoin *et al.*, 2012).

Taking into account the different results obtained in the studies of the reproductive dynamics of *H. parchappii* in different sites of its distribution in Argentina, we suggest that long-term studies that include a larger number of environmental and biological factors (e.g., the incidence of ultraviolet radiation, periods of rainfall, pollution and food availability) are needed to fully understand the dynamics of the recruitment and growth of juveniles of this species throughout its range.

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