BIOLOGÍA ACUÁTICA ESPECIAL XII EMEAP
ISSN 1668-4869

Effect of temperature on the freshwater gastropod *Heleobia parchappii* (d'Orbigny, 1835).

Efecto de la temperatura en el gasterópodo dulceacuícola *Heleobia parchappii* (d'Orbigny, 1835).

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ABSTRACT. Among the gastropods, important components of aquatic ecosystems, the native species *Heleobia parchappii* is particularly prevalent in the Pampas region and Argentina. This species is straightforward to maintain in aquariums and represents a promising candidate for use in ecotoxicological studies. A requisite step to confirm its status as a bioindicator species is to ascertain its response to temperature fluctuations, especially on its movement capacity. This characteristic is key to the species' survival, and its reproductive fitness. The objective of this study was: estimate the optimal movement temperature of *H. parchappii*. Made bioassays over individuals, in which temperature was increased and another in which it decreased. Thirty individuals were exposed to temperature variation, in individual containers, with dechlorinated water, in a breeding chamber. The temperature was decreased or increased at constant rate (1°C per 20 minutes) from 21°C. For each temperature, a digital recording was made for 30 seconds. These steps were repeated at 20-minute intervals until it was observed that all the individuals were immobile (8 – 44.5 °C). A generalized additive model was employed to evaluate the selected endpoint. The results indicate that studies evaluating the effect of different parameters on *H. parchappii* should be conducted within the temperature range of 20 to 25 °C. It is also important to note that the developed protocol is economical and allows the evaluation of variables effect on the gastropod's movement.

Keywords: Bioassays, *Heleobia parchappii*, change of temperature.

RESUMEN. Entre los gasterópodos, componentes importantes de los ecosistemas acuáticos, la especie nativa *Heleobia parchappii* (d'Orbigny, 1835) es particularmente abundante en la región pampeana y en Argentina. Esta especie, de fácil mantenimiento en acuarios, posee características que permitirían su uso en estudios ecotoxicológicos. Para confirmar su estatus como especie bioindicadora, es necesario determinar su respuesta a las fluctuaciones de temperatura, especialmente en su capacidad de movimiento. Esta característica es fundamental para la supervivencia de la especie y su capacidad reproductiva. El objetivo de este estudio fue estimar la temperatura óptima de movimiento de *H. parchappii*. Para ello, se realizaron dos bioensayos,

https://doi.org/10.24215/16684869e050
Recibido: 20/12/24
Aceptado: 13/06/25

uno en el que se disminuyó la temperatura y otro en el que se aumentó. Treinta individuos fueron expuestos a una variación de temperatura en recipientes individuales con agua declorada en una cámara de cría. La temperatura se aumentó o disminuyó a una tasa constante (1 °C cada 20 minutos) desde los 21°C. Para cada temperatura, se realizó un registro digital de 30 segundos. Este proceso se repitió en intervalos de 20 minutos hasta que se observó que todos los individuos estaban inmóviles (8 - 44.5 °C). Se empleó un modelo aditivo generalizado para evaluar el punto final seleccionado. Los resultados obtenidos, utilizando un protocolo económico que permite evaluar el efecto de variables sobre el movimiento del gasterópodo, indican que los estudios que evalúen el efecto de diferentes parámetros sobre *H. parchappii* deben realizarse dentro del rango de temperatura de 20 a 25 °C.

Palabras clave: Bioensayos, *Heleobia parchappii*, cambio de temperatura.

INTRODUCTION

Gastropods are important components of freshwater ecosystems, in the aquatic food webs, serving to balance the ecological niche by providing nutrients to both land and water ecosystems (Pyron & Brown, 2015; Martin & Cabrera, 2018; Dvorak *et al*., 2020; Dhara *et al*., 2022). Between gastropods, the genus *Heleobia* Stimpson, 1865 widely distributed in southern South America, is dominant in many lentic communities, and plays an important role in the life cycles of parasites (Merlo *et al*., 2016, 2022). The most common species in the Pampean Region in Buenos Aires province, Argentina, is the benthic snail *Heleobia parchappii* (d'Orbigny, 1835) (Tietze & De Francesco, 2010; Tietze *et al*., 2018) (Figure 1). This species is found in

Figure 1. Specimens of *Heleobia parchappii* under laboratory maintenance.
Figura 1. Ejemplares de *Heleobia parchappii* mantenidos bajo condiciones de laboratorio.

ISSN 1668-4869

all freshwater habitats, except under anoxic conditions, and lives on different substrata, including submersed vegetation, pebbles, boulders and mud. The species exhibits a small size (up to 9.5 mm in total length), for which an annual reproductive cycle has been suggested. This cycle is characterised by direct development to a comparatively diminutive benthic juvenile, a high reproductive rate in late spring, and a minor peak in winter (De Francesco & Isla, 2004; Merlo *et al*., 2016). Merlo *et al*. (2016) estimated a life cycle of approximately 20.33 months in their study. Then, the life cycles of *H. parchappii* ranging from annual to 20 months.

Furthermore, it plays an important role in natural habitats, and in the food web, where it acts as a detritivore/grazer and prey for several fish species, including the commercially important *Odontesthes bonariensis* (Valenciennes, 1835), and are important intermediate hosts for some groups of parasites. This snail has been observed to display a range of behaviors in a field and laboratory setting, which include floating, crawling and burrowing (Drago, 2004; Cazzaniga & Fiori, 2006; Merlo *et al*., 2016). Despite *H. parchappii* being one of the most prevalent freshwater gastropods in Argentina, our understanding of its biological characteristics remains somewhat limited (Cazzaniga & Fiori, 2006; Merlo *et al*., 2016).

This species is exposed to a variety of variables. One of them is the temperature, that is among the most important abiotic variables affecting aquatic organisms in freshwater bodies, may fluctuate daily, yearly and/or seasonally, and variations in diurnal temperature are more pronounced in shallow than in deep waters (Domenici *et al*., 2007; Bonacina *et al*., 2023). Temperature is one of the major drivers of the changes in the organism's metabolism, influencing therefore the responses of the species, and then, in the ecosystem structure and function (Rabalais *et al*., 2009; Magalhaes *et al*., 2014). The prediction of the consequences of temperature change on species physiology, distribution and survival has become a significant area of research in recent years, particularly in the context of climate change (Ravaux *et al*., 2016).

This study constitutes part of a wider investigation, the objective of which is to examine the impact of different parameters, like temperature, on the life history of the snail *H. parchappii*, with a view to maintaining multi-generation populations of this species in laboratory conditions, to facilitate ecotoxicological testing.

This species was postulated as a bioindicator (Achiorno *et al*., 2023), but then, a crucial step in confirming the species status as an effective bioindicator is to ascertain its behavioural response to temperature fluctuations, particularly in relation to its capacity for movement. This ability is significant for its survival and, for a dioic species consequently, for its reproductive fitness, which is contingent upon the frequency of encounters, which, in turn, is contingent upon locomotion (Cazzaniga & Fiori, 2006). In fact, locomotor behaviour — including both intensity and direction — is frequently used in ecotoxicological studies as it can be measured non-invasively, responds rapidly to environmental stressors, and provides ecologically relevant information (Gerhardt, 2007). Therefore, locomotor activity represents a crucial factor that can be utilized as an end point for assessment in bioassays.

The objective of this study was to conduct an exploratory study to determine the optimal movement temperature of *H. parchappii*.

Materials and Methods

Test organisms

Specimens of *H. parchappii* were collected in December of 2024 from a sampling site on the Martín stream (34°51'39"S, 58°3'56"W) in the Pampean Region, La Plata, Buenos Aires province, Argentina (Figure 2). Snails were randomly collected by hand and placed in plastic bags containing stream water. In the laboratory, they were transferred to 2-L glass containers filled with dechlorinated tap water at an ambient temperature of 21±1 °C. Snails of similar size (in mm) with a mean of 4.40, a median of 4.60, a minimum of 3.70 and a maximum of 6.13, that showed active movement were selected for the experiment. They were starved and kept in 1-L glass containers for at least 24 h before

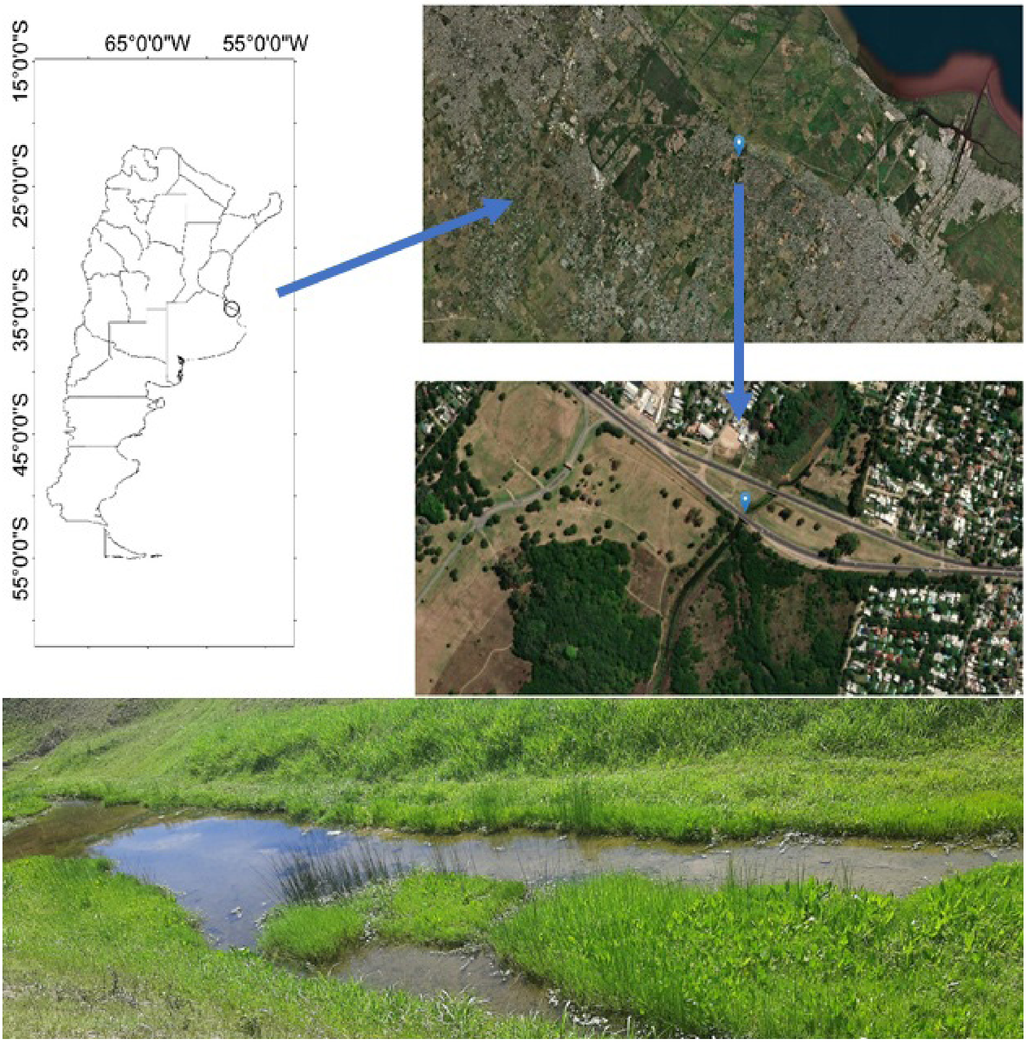

Figure 2. Sampling site in the peri-urban Martín stream in the La Plata district, Buenos Aires, Argentina.
Figura 2. Sitio de muestreo en el arroyo periurbano Martín del partido de La Plata.

the experiment at a breeding chamber at a base temperature of 21°C (the temperature of the laboratory in which the gastropods were located), with 16:8 light:dark photoperiod.

Bioassays

In a breeding chamber at the base temperature of 21°C, 30 specimens of *H. parchappii* were placed in individual containers with 2 mL of dechlorinated tap water. At the outset of the investigation (denoted as t0), a 30-second video was recorded. This procedure was repeated at 20-minute intervals, immediately following the raising or lowering of the chamber temperature by 1°C, depending on whether the experiment was designed to evaluate the effect of an increase or decrease in temperature. The bioassay was continued until all the individuals were observed to be immobile, at which point the experiment was concluded.

Data analysis

The endpoint variable is the number of mobile snails and is compatible with a normal distribution. Three possible distributions were compared: Poisson, Negative Binomial, and Normal. The cumulative distribution function

ISSN 1668-4869

(CDF) and quantile - quantile plot (Q-Q plots) indicated that the Poisson distribution did not provide a good fit to the data, whereas the other distributions showed a substantially better agreement and were considered more appropriate models. During the modeling process, the residuals produced with the best model for the negative binomial response were unsatisfactory compared to those obtained with the normal response, which is why the final selection was a Gaussian response. A preliminary graphical analysis indicated a non-linear relationship between the predictor variable (Temperature) and the number of mobile individuals as a response. This approach required the use of generalized additive models (GAM; see Wood, 2017) for the analysis. All analyses, including graphical representations and models, were conducted utilizing the R software environment (R Core Team, 2023). The mgcv package (Wood, 2017) was utilized for the GAM models (R Core Team, 2023).

RESULTS

The results of the bioassays, which were conducted to evaluate the impact of temperature on *Heleobia parchappii*, indicated a discernible trend towards decreased mobility. This trend was observed in both tests, one evaluating an increase in temperature from 21°C to 44.5°C, and the other evaluating a decrease in temperature from 21°C to 8°C. Finally, the model applied was "number of mobile individuals" = s (temperature), where s represented a smooth function. The smoother is significant (p-value <0,001, effective degrees of freedom=8.03, reference df = 9) as well as the interceptor of the parametric part (p-value <0.001). The model, which showed an adjusted R^2 of 0.945 and explained 95.5% of the deviance, predicted maximum mobility at a temperature of 23°C, and estimated that 80% or more of the individuals would exhibit mobility within the temperature range of 20° to 25.5°C. In order to facilitate clearer visual interpretation of the results, the data from both bioassays are presented in Figure 3. The scatter plot (Figure 3) demonstrates a non-linear relationship between temperature and the number of mobile individuals. Applying the model at this temperature, the predicted number of mobile individuals is 26.7, with a 95% confidence interval of [24.9 -28.4]. We observe that the difference between the initial exposure temperature and the temperature at which the individuals became immobilized was greater in the increasing temperature assay than in the decreasing one, with a difference of 23.5°C compared to 12°C.

Discussion and Conclusion

The results obtained in this investigation indicate that studies evaluating the effect of different parameters on the gastropod *H. parchappii* should be conducted within the temperature range of 20 to 25.5 ºC. Although numerous studies have investigated the effects of temperature on freshwater gastropods worldwide, this taxonomic group remains underrepresented in comparison to other macroinvertebrates, such as insects and malacostracans. For instance, Bonacina *et al*. (2023) conducted a review of the effects of water temperature on freshwater macroinvertebrates, finding that the majority of studies focused on insects, followed by malacostracans and clitellates, with gastropods being the least studied group. However, within the diverse group of gastropods, notable contributions have been made to our understanding of the relationship between temperature and activity, as exemplified by *Pomacea canaliculata* (Lamarck, 1822) a species endemic in the Southern Pampas, and recorded in the same environments where *H. parchappii* is present. Heiler *et al*. (2008) demonstrated that the activity of *P. canaliculata* increased threefold with rising temperature, from 18% active behaviour at 20°C to 54% at 32°C, and that crawling velocity increased 3.6-fold. Seuffert *et al*. (2010) also discovered a substantial correlation between

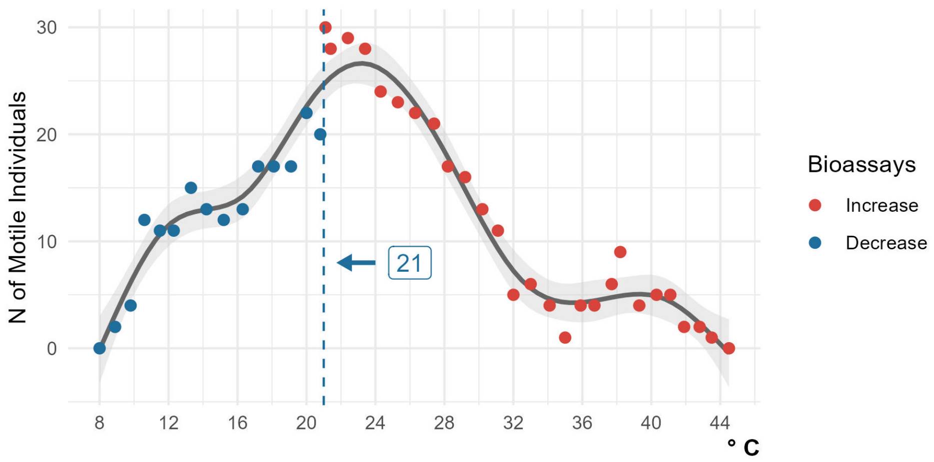

Figure 3. Graphical representation of the number of mobile individuals at the various temperatures evaluated. The black line in the graph represents the results of both bioassays. The initial temperature of both bioassays (21 °C) is indicated by a blue dotted line indicating that it corresponds to 21°C. The red points correspond to the experiment in which the effect of increasing the temperature was evaluated, whereas the blue ones correspond to the experiment in which the effect of decreasing the temperature was evaluated. The 95% confidence band for the mean mobility is shown in grey. Y-axis: Number of mobile individuals, X-axis: Temperature in °C.

Figura 3. Representación gráfica del número de individuos móviles a las temperaturas evaluadas. La línea negra muestra los resultados de ambos bioensayos. La temperatura inicial de ambos bioensayos (21 °C) se indica con una línea punteada azul en la que se indica que corresponde a 21°C. En color rojo se observa el efecto del incremento de temperatura, en color azul el efecto de la disminución de temperatura. En gris se observa la banda de confianza del 95% para la media de movilidad. Eje Y: Número de individuos móviles, Eje X: temperatura en °C.

temperature and activity levels of *P. canaliculata*, with activity reaching 100% at temperatures ranging from 25 to 30°C. Studies conducted by Bae and Park (2015) and Bae *et al*. (2015, 2021), have examined the behavioural responses of *P. canaliculata* across temperature gradients. Despite the evident differences in numerous biological traits between *P. canaliculata* and *H. parchappii*, our findings provide valuable reference points for contextualizing thermal sensitivity. In *H. parchappii*, increased mobility began around 20°C, which corresponds with the lower limits of activity documented for *P. canaliculata*. These comparisons, while interspecific, contribute to a more comprehensive understanding of the thermal ecology of *H. parchappii* within the broader context of regional freshwater gastropods. In regard to the utilization of mollusks in environmental risk assessments, de Freitas Tallarico (2015) proposes that a number of factors are of paramount importance when developing testing guidelines. Primarily, the animals in question should be straightforward to maintain and cultivate in a laboratory setting. Furthermore, it may be necessary to characterize annual reproductive or developmental cycles under defined conditions, as well as food, temperature, oxygen, conductivity, and mate selection. Furthermore, the use of short-lived organisms, such as certain gastropod species, enhances the practicality of the process. Therefore, the present study provides information that may be used to apply the gastropod *H. parchappii* as a bioindicator of effect. Considering the endpoint evaluated in this study, it is important to recognize that the effect on movement could be classified as a sublethal effect. In their review, Dallas & Ross-Gillespi (2015) posits that sublethal effects (which necessitate adaptations for survival) are frequently cumulative. These effects may manifest as a gradual process of attrition on populations and communities, whereby differential effects on individuals of different species contribute to the overall impact. It is therefore imperative to study the effects on movement, a crucial variable in gastropods, to gain insight into the dynamics of these species and their role within their environment. In this context, the experimental approach implemented in this study provides valuable insights into the temperature-dependent mobility of *H. parchappii*, a trait essential for its survival and ecological functioning. It is imperative to comprehend the thermal sensitivity of this species in order to ascertain the range of environmental conditions that facilitate individual activity and, consequently, population persistence. This study is part of a comprehensive research initiative aimed at assessing the impact of various abiotic and biotic factors on the biology of *H. parchappii*. The program's primary objective is to identify optimal conditions for maintaining laboratory cultures over multiple generations, ultimately facilitating their use in ecotoxicological evaluations. The present study focused specifically on the effects of temperature on locomotor activity. It is anticipated that future studies will encompass a broader range of factors, including water quality variables such as pH, salinity and dissolved oxygen, as well as feeding frequency, photoperiod and density. These investigations will be conducted under both constant and variable thermal conditions to examine a broader range of traits in the juvenile and adult life stages. Comparative evidence from *P. canaliculata*, a co-occurring freshwater species in the Pampas region (Heiler *et al.*, 2008; Seuffert *et al.*, 2010), suggests that *H. parchappii* may be more vulnerable to elevated temperatures. These findings emphasise the necessity for ongoing research into the potential impacts of climate change on native invertebrates, particularly those with narrower or lower thermal tolerances. In summary, this study proposes a cost-effective and reproducible experimental design that facilitates the evaluation of key functional traits in *H. parchappii*. The results obtained lay the foundation for future research and lend support to the hypothesis that this species could serve as a sensitive bioindicator in freshwater environments.

ACKNOWLEDGMENTS

We thank the anonymous reviewers for their contributions that improved this work.

CRediT Contribution Statement

C.A.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **V.A.:** Data curation, Validation, Visualization, Writing – original draft, Writing – review & editing. **G.M.:** Formal analysis, Software, Validation, Visualization, Writing – original draft. **D.G.G.:** Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

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