



## Do the changes in temperature and light affect the functional response of the benthic mud snail *Heleobia australis* (Mollusca: Gastropoda)?

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### ABSTRACT

The aim of this study was to determine the influence of temperature increase combined to conditions of light incidence on functional response of *Heleobia australis*. Experiments were conducted using nine to ten food concentrations for each treatment: 20°C without light; 30°C without light and, 30°C under low light intensity. For each experiment, the functional response type III (sigmoidal) was fitted and equation parameters were determined. Results suggest that, if the sediment temperature increases, *H. australis* will not have its ingestion rates affected negatively, whilst its feeding behavior seems to be negatively affected by light. Ingestion rates estimated for organic content in the Guanabara Bay were: 0.34  $\mu\text{gC ind}^{-1}\text{h}^{-1}$  at 20°C without light, 1.44  $\mu\text{gC ind}^{-1}\text{h}^{-1}$  at 30°C without light and 0.64  $\mu\text{gC ind}^{-1}\text{h}^{-1}$  at 30°C under light incidence. Higher ingestion rates were estimated at the high temperature, even under light incidence, and temperature seems to have outweighed the light effect. In contrast, if higher carbon content is considered, despite high temperature, the experiment conducted with light incidence showed lower ingestion rates than those from the experiment at 20°C without light. This study provides the first quantification of *H. australis* ingestion rates and the effects that changes in temperature and light have on its feeding behavior.

**Key words:** Hydrobiid, ingestion rates, estuarine system, gastropod.

### INTRODUCTION

*Heleobia australis* is distributed from Rio de Janeiro, Brazil, to western San Antonio, Argentina and occurs in estuarine systems and coastal lagoons, generally, associated to shallow areas (0.4 - 3.5 m) with anoxic-mud sediment (Gonçalves Jr et al. 1998, Albertoni et al. 2003, De Francesco and Isla 2003, 2004, Figueiredo-Barros et al. 2006, Alda et al. 2010). In the Guanabara Bay, Brazil, it is the

dominant benthic species in shallow waters (Neves et al. 2013). *Heleobia australis* compose the diet of some commercially important estuarine fishes and crustaceans such as: whitemouth croaker *Micropogonias furnieri* (Figueiredo and Vieira 1998), shrimps *Macrobrachium acanthurus*, *Farfantepenaeus brasiliensis*, *F. paulensis* (Albertoni et al. 2003, Jorgensen et al. 2009) and the swimming crab *Callinectes sapidus* (Kapusta and Bemvenuti 1998, Oliveira et al. 2006). *Heleobia australis* feeds on the surface sediment (Bemvenuti et al. 1992),

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microphytobenthos (Alvarez et al. 2013), and likely its food sources are similar to other hydrobioids that feeds on organic matter, diatoms, dinoflagellates, ciliates and bacteria (Kofoed 1975, Bianchi and Levinton 1984, Blanchard et al. 2000, Sokolowski et al. 2005, Pascal et al. 2009). Thus, *H. australis* plays a role as a link between the benthic microbial food web and the upper consumers. Estimates of *H. australis* ingestion rates may contribute to the understanding of the estuarine food web.

Ingestion rates may be affected, within other factors, by changes on the estuarine sediment in terms of organic matter content, temperature and light incidence, which are consequence of natural and anthropogenic impacts, such as eutrophication, global warming and siltation as explained below.

Coastal areas and estuaries are constantly receiving inputs of domestic and/or industrial waste (Smith et al. 1999), which increases the availability of organic matter, a potential food source for the deposit feeders (Nixon 1995, Espinosa et al. 2007, Rao and Charette 2012). The increase in ingestion rates in response to increasing food density was defined as a 'functional response' by Holling (1959), who recognized three major types of responses described as a functional response: i) type I - the consumption rate rises linearly with prey density, ii) type II - consumption rate rises with prey density, but gradually decelerates until a plateau is reached (hyperbola) and iii) type III - similar to a type 2, but at low prey densities the type 3 response has an accelerating phase where an increase in density leads to a more than linear increase in consumption (sigmoidal) (Begon et al. 2006).

In addition, global warming has been strongly related to the increase of water temperature (Rabalais et al. 2009), that affects the organism's metabolism and, therefore, influence species feeding rates and functional responses (Hylleberg 1975, Abdel-Hamid et al. 1992, Bougrier et al. 1995, Gitonga et al. 2002, Pascal et al. 2008, Rico-Villa et al. 2009). Higher temperature tend to

increase ingestion rates up to a maximum, above which any temperature increase would inhibit the ingestion rates, as demonstrated for *Hydrobia ulvae* (Hylleberg 1975, Pascal et al. 2008), *H. neglecta* and *H. ventrosa* (Hylleberg 1975).

Finally, the marginal areas of estuaries are becoming worldwide shallower by a process of siltation; i.e. Bahia Blanca estuary – Argentina (Cuadrado et al. 2006), Guanabara Bay – Brazil (Godoy et al. 2012), Jiaozhou Bay - China (Liu et al. 2012), Keu River Estuary- Korea (Kim et al. 2006), Mandovi and Zuari estuaries – India (Rao et al. 2011), Ord River estuary – Australia (Wolanski et al. 2001). This process is either originated by natural causes over large temporal scale (circulation processes) or by rapid changes in response to environmental and man-made modifications to the hydrologic regimes (Wolanski et al. 2001). Siltation is characterized by an increase in sedimentation rate (Bach et al. 1998), that may reduce the water column making the sediment subject to higher influence of temperature and light incidence or increase in suspended sediment particles thereby increasing turbidity. Light intensity potentially affects species behavior (Russel 1926) and, therefore, it may affect the feeding of benthic species. In contrast, the raise of sea level may increase the water column depth and decrease the incidence of light on the sediment (Kennedy et al. 2002). Thus, habitat changes may affect the organisms that live or feed on the sediment and increase the relevance of experimental studies as a first approach to understanding how organisms respond to environmental changes.

The aim of the current study was to determine the effect of temperature and light increase on the functional responses of *H. australis*, thus three experimental conditions were described: i) the averages of temperature and light incidence from where *H. australis* occurs in the Guanabara Bay (20°C without light), ii) higher temperature without light and iii) higher temperature under low light incidence.

The latter two conditions attempt to represent possible scenarios that *H. australis* may be susceptible to changes on the coastal estuarine habitats.

## MATERIALS AND METHODS

### STUDY AREA

The Guanabara Bay is one of the largest bays in Brazil, with drainage that covers 4.080 km<sup>2</sup> with 45 rivers and streams (JICA 1994). The bay is a semi-enclosed water body surrounded by urban areas and is one of the most degraded estuarine systems along the Brazilian coast (Valentin et al. 1999) exposed to constant human impacts, i.e. river canalisation and landfills, inputs of untreated domestic sewage and industrial residues, ship-source oil pollution, and urban wastes. The Bay has a semi-diurnal tidal regime with tide amplitude ranging from 0 to 1.4 m (Amador 1997), and the sedimentation rates are approximately 1 cm year<sup>-1</sup>, representing an increment of five-fold compared to the sedimentation rates described in the 1950s (Godoy et al. 2012). Temperature and light intensity on the sediment of shallow areas of the Guanabara Bay ranges, respectively, from 16 to 26°C and from dark to 40 μmol m<sup>-2</sup> s<sup>-1</sup> (personal observation).

### SAMPLING AND EXPERIMENT DESIGN

Specimens of *H. australis* were collected in May and September 2010, using a Van Veen grab (0.05 m<sup>2</sup>) at a fixed sampling station (3-5 m deep) located in one of the most organic polluted areas of the Guanabara Bay. Sediment samples were sieved through 0.05 mm mesh, and individuals were transported to the laboratory in local water. Temperature and salinity of bottom water were measured using a thermosalinometer. Five sediment samples were collected and the upper layer (~10 cm) of the sediment was dried for 48 h at 60°C (APHA 1998). Sediment organic matter was determined from the weight loss after ignition at 500°C during 4 h, and carbon content was estimated according to Schumacher (2002).

The individuals of *H. australis* (~2 mm; ~0.30 mg dry weight) were transferred to Petri dishes (9 cm in diameter) with 30 ml of filtered (GF/F) water from the Guanabara Bay. The density in each Petri dish was 17 individuals, a number described as appropriate to avoid density-dependent effects for a larger species (3 – 4 mm), e.g. *Hydrobia ulvae* (Blanchard et al. 2000). Functional response estimates were conducted measuring ingestion rates at nine to ten different food concentrations, for each treatment: 1) temperature of 20°C without light; 2) temperature of 30°C without light; and 3) temperature of 30°C under light incidence of 16 μmol m<sup>-2</sup> s<sup>-1</sup>. Individuals were acclimated to each experiment condition and food concentration for 24 h, and kept in filtered water during 2 h for egestion, as proposed for *H. ulvae* (Calow 1975). Simultaneously, controls were done for each food concentration in the same conditions as those for the treatments, but without the snails. Treatments and controls were incubated for 2 h and were conducted in triplicate for each food concentration.

Water and food were filtered through GF/F membranes, dried in an oven at 60°C for 48 h, and then weighed on analytical balance to determine food concentration in terms of carbon dry weight. Artificial food for invertebrates (fine powder, SeraMicron®), containing 50.2 % crude protein, was used in the experiments. The carbon content of the food was determined assuming a conversion factor of 0.86 Carbon: protein (Simon and Azam 1989).

Food concentrations at the beginning and end of experiment, both in treatments and controls, were compared using a t-test. At each concentration of food, the variation in food quantities over the experiment duration (2 h) were compared between treatments and controls, also using a t-test. In the controls there were no differences between food concentrations at the beginning and end of experiments ( $p > 0.05$ ). Thus, the controls were not used to estimate ingestion rates. Ingestion rates (μgC ind<sup>-1</sup> h<sup>-1</sup>) were estimated

by the variation of food concentration at the beginning and end of experiment and divided by the number of individuals in the Petri dish ( $n = 17$ ) and the experiment duration (2 h). The ingestion rates were plotted against food concentration (Functional Response) and a non-linear regression was determined using the software Sigma Plot 10.0. Using a t-test, the curve parameters were compared between experiments with different lighting condition and same temperature and between experiments with different temperatures and absence of light.

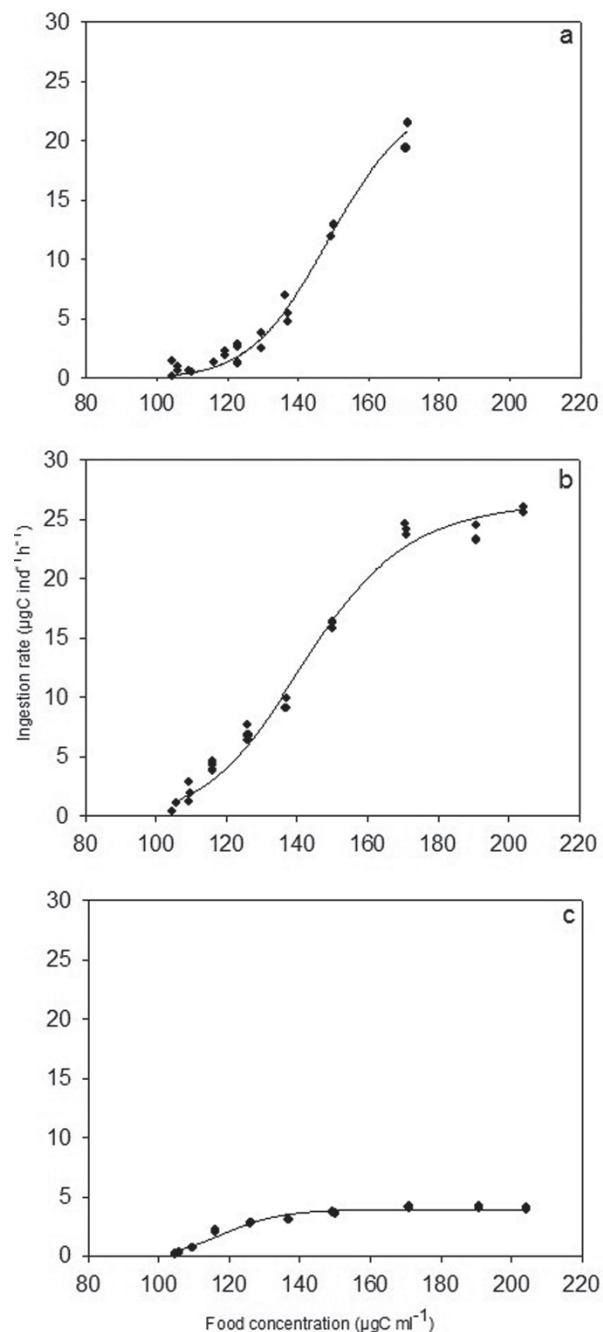
### RESULTS

During the samplings the water temperature in the field ranged between 20 and 23°C at the bottom and surface, respectively. The mean organic-matter content in the sediment was 203  $\mu\text{g ml}^{-1}$  sediment ( $\pm 0.03$ ), corresponding to 107  $\mu\text{gC ml}^{-1}$  sediment.

The decrease of food during the experiment was significantly higher ( $p < 0.01$ ) in the replicates with *H. australis* than in the control, for all food concentrations and treatments, showing that there was ingestion of the artificial food offered to snails. Functional responses (Fig. 1) showed the best fit to the non-linear regression Type III (Hill equation,  $I = (I_{\max} \cdot x^b) / (k^b + x^b)$ ), where  $I$  is Ingestion Rate,  $I_{\max}$  is the Maximum Ingestion Rate,  $k$  is the Coefficient of Half-Saturation,  $b$  is the parameter of Hill Equation and  $x$  is the Food Concentration. The parameters of functional response equation are shown in Table I.

Experiments performed in the dark at 20 and 30°C showed a trend to reach the asymptote in food concentration  $\sim 170 \mu\text{gC ml}^{-1}$  (Fig. 1). Comparisons of equation parameters did not show significant differences, except for parameter  $k$ , which was significantly higher at 20°C (t-test,  $p < 0.05$ ). Thus, *H. australis* reached half-saturation in lower food concentrations at 30°C.

Comparisons between the experiments conducted at 30°C, with and without light incidence,



**Fig. 1** - *Heleobia australis* functional responses estimated for (a) 20°C in darkness, (b) 30°C in darkness, and (c) 30°C in light intensity of  $16 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

showed differences between all parameters (t-test,  $p < 0.05$ ). The experiment carried out with light showed a significantly higher  $b$  than the experiments conducted in the dark ( $p < 0.05$ ), and the  $I_{\max}$  was  $\sim 7$ -fold lower under the light incidence.

**TABLE I**  
**Parameters, coefficient of determination and p values for the statistically significant functional responses determined for the experiments conducted with *Heleobia australis*. Different letters indicate that parameters were significantly different (t-test,  $p < 0.05$ ).**

| Experiments       | Parameters   |                   |  |       |         |
|-------------------|--|-------------------|--|-------|---------|
|                   | $I_{\max}$<br>( $\mu\text{gC}\cdot\text{Ind}^{-1}\cdot\text{h}^{-1}$ ) | b                 | K<br>( $\mu\text{gC}\cdot\text{ml}^{-1}$ ) | $R^2$ | p       |
| 20°C in the dark  | 24.9 <sup>a</sup>  | 12.7 <sup>a</sup> | 150 <sup>a</sup>                           | 0.98  | <0.0001 |
| 30°C in the dark  | 26.6 <sup>a</sup>  | 9.9 <sup>a</sup>  | 143 <sup>b</sup>                           | 0.98  | <0.0001 |
| 30°C in the light | 3.96 <sup>c</sup>  | 15.2 <sup>c</sup> | 119 <sup>c</sup>                           | 0.96  | <0.0001 |

$I_{\max}$ , maximum ingestion; K, half-saturation coefficient; b, Hill coefficient;  $R^2$ , correlation coefficient.

### DISCUSSION

Few studies describe ingestion rates and functional responses of benthic deposit feeders. Functional responses of *H. australis* showed a progressive increase with food concentration tending to reach an asymptote, this pattern is similar to that of some invertebrates as the bivalve *Abra ovata* (Grémare et al. 2004), the polychaete *Abarenicola pacifica* (Hymel and Plante 2000, Linton and Taghon 2000) and also the hemichordate *Saccoglossus kowalevskii* (Karrh and Miller 1994). Moreover, the ingestion rates of *H. australis* in low food concentrations were in the range of those found for other gastropods; *Hydrobia truncate*, *H. ulvae* and hydrobiid feeding on diatoms and bacteria (Haubois et al. 2005, Pascal et al. 2008, 2013). Based on previous studies, in which the functional response did not reach the asymptote, we provided larger amount of food attempting to better describe the functional responses. As a result, *H. australis* presented high ingestion rates at high food concentrations that have not been examined before. Despite the use of artificial food, the results suggest that the snails did not avoid this type of food, but on the contrary they ingested large amounts of it. In the current study, the food was not mixed to the sediment and considering that the mixture of food and sediment may increase the time of food manipulation, our estimates of ingestion rates may have been overestimated. Although the extrapolations from our results to natural environment require caution (see below),

our study was representative to describe the effects of temperature and light incidence on the feeding behavior of *H. australis*.

Functional responses for all experiments had the best fit to Type III curve. The difference between Type II and III responses is the concavity (slowdown) in the early part of the curve, which is determined by several factors that influence the time predators spend searching and handling food (Holling 1965, 1966), i.e. predator efficiency. Generally, this efficiency tends to increase with the number of encounters between predator and prey that is described by parameter “b” in the equation of functional response Type III. Thus, predators with higher efficiency require lower encounter rates to ingest their prey and, therefore, the values of parameter “b” in their functional response are lower. In fact, when  $b = 1$ , the equation of functional responses Type II and III are equal, and the difference between the responses is interpreted as a slow learning behavior of predator at the initial portion of the response type III (Real 1977).

Experiments performed with no light incidence at 20 and 30°C did not show stabilization of functional responses, only a trend to reach the asymptote. The half-saturation coefficient (k) was significantly higher at 30°C, indicating higher ingestion rates in lower food concentrations than at 20°C. It is possible that individuals were more active and potentially more efficient in their food search at higher temperatures. Although comparisons

between temperate and tropical organisms should be carefully conducted, it was also demonstrated that the polychaete *Abarenicola pacifica* (Hymel and Plante 2000) and hydrobiids (Pascal et al. 2008) showed higher ingestion rates at 30°C, when experiments were conducted in a range of temperatures. In the present study, we did not determine the temperature which resulted in the maximum ingestion rate for *H. australis*, but noted that if the sediment temperature increases (i.e. 30°C) *H. australis* will not have its ingestion rates affected negatively. However, further studies concerning the gastropod metabolism are necessary to assess possible changes in food requirement due to temperature increase.

Comparisons between the experiments conducted with and without light at 30°C indicated differences in all curve parameters. When exposed to light, even low incidence, *H. australis* showed significantly lower  $k$  and  $I_{\max}$  and higher “ $b$ ”, which means that it was less efficient in finding its food and, in order to reach a given ingestion rate, it will require higher food densities than those snails from the experiments conducted with no light incidence. Moreover, the maximum ingestion rate of *H. australis* was ~7-fold lower under light incidence. Studies have shown that feeding rates may be positively (Buikema 1973, Orvain and Sauriau 2002, Barnes 2003), negatively (Barnes 1986) or non-affected by light (Bianchi and Levinton 1984, Pascal et al. 2008). Here, feeding rates of *H. australis* were affected by lower light incidence than the maximum value we recorded for the Guanabara Bay (i.e. 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Thus, it is possible that light might already be influencing feeding behavior and, consequently, the growth of this species in the bay.

Applying the organic carbon content measured at the sampling site to the functional responses equation (see parameters in Table I), the following ingestion rates were calculated of: 0.34  $\mu\text{gC ind}^{-1}\text{h}^{-1}$  at 20°C without light incidence, 1.44  $\mu\text{gC ind}^{-1}\text{h}^{-1}$  at 30°C without light incidence and 0.64  $\mu\text{gC ind}^{-1}\text{h}^{-1}$  at 30°C under light incidence. Although the ingestion rates

may have been overestimated, as *H. australis* was fed only on pure food and not on food combined to the sediment (see above), this study provided the first quantification of ingestion rates of *H. australis* and it serves as a basis for future estimates of carbon transfer to the upper trophic levels. Moreover, this study enabled us to make predictions of the effects of environmental conditions on the ingestion rates. It is important to note that, in food concentrations at the initial portion of the curve, corresponding to organic carbon content from ~100 to 128  $\mu\text{gC ml}^{-1}$ , which includes the carbon content at the sampling site, the lowest ingestion rates occurred at the experimental condition of 20°C with no light incidence, which is the one often found at shallow waters of the Guanabara Bay. At these food concentrations, the ingestion rates of *H. australis* were at the beginning part of the type III curve i.e. before the concavity (slowdown) (Fig. 1). Thus, considering ingestion rates at the specific organic carbon content of the Guanabara Bay sediment, at high temperature (30°C) *H. australis* showed higher ingestion rates even when exposed to light incidence, showing that the effect of temperature seems to have outweighed the light effects. In contrast, if higher carbon content is considered, despite the high temperature, the experiment with light incidence showed lower ingestion rates for *H. australis* than that conducted at 20°C without light incidence. Temperature does not seem to be the only factor determining feeding behavior. Barnes (2006) pointed out the importance of other factor, i.e. tidal cycle, influencing the feeding behavior of *Hydrobia ulvae*. Here, it is shown that opposing results can be obtained if ingestion rates are analyzed at one specific food concentration instead of at a range of food concentrations. More studies approaching functional response experiments are necessary as they provide more consistent predictions about the effects of environmental changes on feeding behaviors.

In this study, we evaluated the influence of temperature increase combined to changes in light on the functional response of *H. australis*. There is

no prediction of how temperature and light covariate in the bottom of shallow areas of the Guanabara Bay and other estuaries, and this topic is beyond the aim of the current study. However, the process of siltation, which decreases the water column depth, was already recorded for the Guanabara Bay (Godoy et al. 2012) and, therefore, two scenarios may be expected: i) light incidence on the sediment decreases due to high water turbidity by suspended sediment particles; or ii) light incidence on the sediment increases as water column depth decreases enough that, despite particles in suspension, the light reaches the sediment. In the specific case of the Guanabara Bay, there is a frequent entrance of coastal waters, including the South Atlantic Central Waters, which reaches shallow areas and increases the water column transparency. Thus, applying our results to the scenario where temperature, light and food content (organic carbon) increase, which is a possible situation to occur in the Guanabara Bay, ingestion rate would decrease in relation to current conditions of this bay. On the other hand, if temperature increases, light might either increase or not, but organic content on the sediment would remain as it is today, then the ingestion rates of *H. australis* would increase. Further studies approaching a wide temperature range and light incidence on feeding and growth of *H. australis* and other benthic species will be useful to make other predictions about the effects of environmental changes on coastal habitats.

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complied with ethical standards in the treatment of our animals, adhering to national guidelines.

#### RESUMO

O objetivo deste estudo foi determinar a influência do aumento de temperatura associado à incidência luminosa na resposta funcional de *Heleobia australis*. Experimentos foram conduzidos utilizando nove a dez concentrações de alimento para cada tratamento: 20°C sem luz, 30°C sem luz e 30°C sob baixa incidência luminosa. Para cada experimento, a resposta funcional tipo III (sigmoidal) apresentou o melhor ajuste e os parâmetros das equações foram determinados. Os resultados sugerem que com o aumento da temperatura no sedimento, *H. australis* não terá sua taxa de ingestão afetada negativamente. Por outro lado, seu comportamento alimentar parece ser negativamente afetado pela incidência luminosa. As taxas de ingestão estimadas para o conteúdo orgânico na Baía de Guanabara foram: 0,34  $\mu\text{gC ind}^{-1}\text{h}^{-1}$  a 20°C em ausência de luz, 1,44  $\mu\text{gC ind}^{-1}\text{h}^{-1}$  a 30°C em ausência de luz e 0,64  $\mu\text{gC ind}^{-1}\text{h}^{-1}$  a 30°C sob incidência luminosa. Nesta situação, as maiores taxas de ingestão foram estimadas em condições de alta temperatura, mesmo com incidência luminosa, sugerindo que a temperatura parece ter um efeito que predomina sobre o efeito da luz. Porém, considerando maiores concentrações de carbono, mesmo em temperatura alta, o experimento realizado com incidência luminosa apresentou taxas de ingestão mais baixas que aquelas do experimento conduzido a 20°C em ausência de luz. Este estudo fornece a primeira quantificação das taxas de ingestão de *H. australis* e os efeitos das modificações na temperatura e incidência luminosa no seu comportamento alimentar.

**Palavras-chave:** hidrobióide, taxas de ingestão, sistema estuarino, gastrópode.

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