GUT EVACUATION TIME OF *MACROBRACHIUM BORELLII* (CARIDEA: PALAEMONIDAE) FEEDING ON THREE TYPES OF PREY FROM THE LITTORAL-BENTHIC COMMUNITY

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ABSTRACT

Gut evacuation time is a very useful parameter that allows us to understand the daily trophic cycle of a predator. *Macrobrachium borellii* is an inhabitant of the littoral-benthic community of the Paraná River system that preys on various organisms. In this work, three types of prey (cladocerans, dipteran larvae, and oligochaetes), which are different in shape and mobility, were offered to *M. borellii* to investigate the gut evacuation time of each one. All prey represent the natural diet of this prawn based on previous work. Results showed a faster digestion of cladocerans than mosquito larvae and oligochaete. These last two types of prey are cylindrical and may interfere with the maceration process in a stomach that lacks a gastric mill. In addition, the high mobility of cladocerans and mosquito larvae implies greater energy expenditure in their capture. However, cladocerans are the prey with the lowest caloric value per individual and therefore offers the lowest net energy to the prawn. This implies that a cladoceran diet would require higher daily consumption and more time foraging, and thus potentially exposing the prawn to greater predation risk. All of these factors direct selection toward the more profitable prey (oligochaetes and dipteran larvae), in agreement with the natural diet observation, suggesting that the time of digestion may have little importance in prey selection.

KEY WORDS: Caridea, digestion, gut evacuation, *Macrobrachium borellii*, Paraná River, prawn

INTRODUCTION

Macrobrachium borellii (Nobili, 1896) is an endemic decapod prawn of the Paraná River system in Argentina that lives primarily in vegetated areas of the littoral zone (Collins et al., 2004) and is a frequent prey of fishes and birds (Bonetto et al., 1963; Beltzer and Oliveros, 1982; Beltzer 1983a, b, c, 1991; Oliveros and Rossi, 1991). A previous study of foregut contents (Collins and Paggi, 1998) revealed that the natural diet of this crustacean is composed preferentially of oligochaetes and dipteran larvae and, more sporadically, of cladocerans. Therefore, its omnivorous diet plays an important role in the matter-energy exchange between bottom and top trophic levels in the freshwater environment (Collins et al., 2006).

When we consider other ecological interactions, such as competition for space or trophic resources, *M. borellii* is an aggressive competitor. Collins (2005), who studied the coexistence mechanism between prawns, observed that *M. borellii* displace a heterospecific prawn, *Palaemonetes argentinus* Nobili, 1901, to other niches. This aggressiveness was also verified in a laboratory evaluation of *M. borellii* as a predator of mosquitoe larvae and shown that this species is even more voracious than other malacostracan (Collins, 1998). One way to verify the predator capacity is to estimate the predation rate through the study of gut evacuation time, i.e., the amount of food that passes out of the gut per unit time. This permits infer the transfer of energy and matter from low to high trophic levels in a freshwater system using decapods as intermediate link. Gut evacuation time is also a very useful parameter for assessing information regarding food consumption (Loya-Javellana et al., 1995). Once the digestive process has emptied the stomach, the appetite returns and a new feeding may occur. Temperature, size, amount and quality of prey, the molt cycle, and methods of capture by crustaceans can influence this process (Karjalainen et al., 1991).

The aim of this work was to verify the gut (stomach and intestine) evacuation time of *M. borellii* fed with three bioform types of prey from the littoral-benthic community with distinct characteristics of mobility and shape.

MATERIALS AND METHODS

Macrobrachium borellii was sampled in ponds of floodplain environments of the Paraná River using a bottom net (1 mm mesh size) among the vegetation. Individuals were transferred to the laboratory, maintained in aquaria for acclimation during one week and fed with fish muscle. Prawns used for feeding trials were placed individually in 500 ml containers at 21°C (room temperature). Only adult prawns (mean cephalotorax length LC 16.4 ± 0.4 mm) and those in the intermolt period were used. A total of eighteen individuals were tested in each trial. One trial was performed for each prey, with a total of three essays no simultaneous. Experiments were carried out after 24 hours of fast to prevent presence of food in the digestive system and to maintain the same level of starvation for all prawns. When was observed that no gut content could be seen through the crustacean body, trials were initiated. The prawn's transparent body permits the correct discrimination between stomach and hepatopancreas, allowing an accurate date taken.

The types of prey used represented bioforms of natural potential food resources for *M. borellii* and were chosen based on previous literature (Collins and Paggi, 1998). They were supplied in an amount greater than
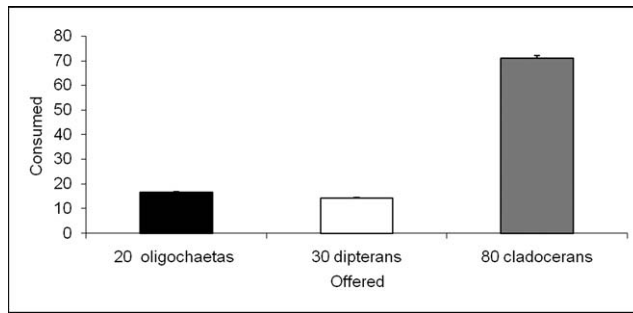
Fig. 1.   Average of preys consumed in the first hour of trials. Horizontal axis shows the number and type of prey offered.

its observed capacity of predation in one hour. The cladoceran *Daphnia magna* Straus, 1820 (spherical form and active movement), the oligochaete *Limnodrilus udekemianus* (long, cylindrical form and scarce movement), and the dipterean larvae *Culex pipiens* (cylindrical form and active movement) belong to cultures at Instituto Nacional de Limnología and were offered as prey in these trials. All prey were alive when offered to the prawns. The volume of prey items were determined by approximation to regular geometric shapes or water displacement (Edmondson and Winberg, 1971; Dumont et al., 1975; Collins, 2000).

Each prey was offered in a different amount to stomach repletion, and fullness was confirmed through visual inspection of the crustacean body. In the first experiment, eighty (80) cladocerans were offered to each prawn over 1 hour. Uneaten prey were removed and counted. The transit of food in the stomach and intestine was observed only through the prawn's transparent body. Bulk-gut passage time was examined each hour along 10 hours of ligth to make possible the visualization of the digestive tract regardless the trophic circadian rhythm (Collins, 1997). A non-metric scale ranging from 0 (empty) to 3 (full) was used to measure the degree of repletion of the foregut (stomach) and midgut (intestine) separately. The hour of first feces production was also recorded for each individual. The same procedure was applied to the other prey types, although twenty (20) oligochaete and thirty (30) dipterean larvae were used.

Possible differences between sexes were identified with a *t* test. A Mantel test was used to identify correlation between time of stomach and intestine bulk-passage of each predator's group in all types of prey. The groups (a data matrix – time vs. degree of repletion of stomach or intestine of each individual) were analyzed in pairs. Differences among the same hour of digestion between groups were evaluated with a Kruskal-Wallis non-parametric test and a Mann-Whitney post test (Zar, 1996). All statistical tests were conducted with the PAST statistical package (Hammer et al., 2001).

RESULTS

All prawns actively captured their prey after placement and ate between 50% and 90% of offered prey within 1 hour (Fig. 1). This amount was transformed into volume (Table 1) and demonstrates the capacity of storage for each type of prey in the cardiac chamber of adult prawns. The mean volume stored for the three types of prey was 29.7 ± 4.3 mm^3; the cladocerans and oligochaetes had the highest mean volume values (35.5 ± 1.2 mm^3 y 32.5 ± 1.7 mm^3; respectively). The *t* test found no significant differences between males and females in the volume of

Table 1.   Mean values of number and volume (mm^3) of ingested prey by *M. borellii* over one hour.

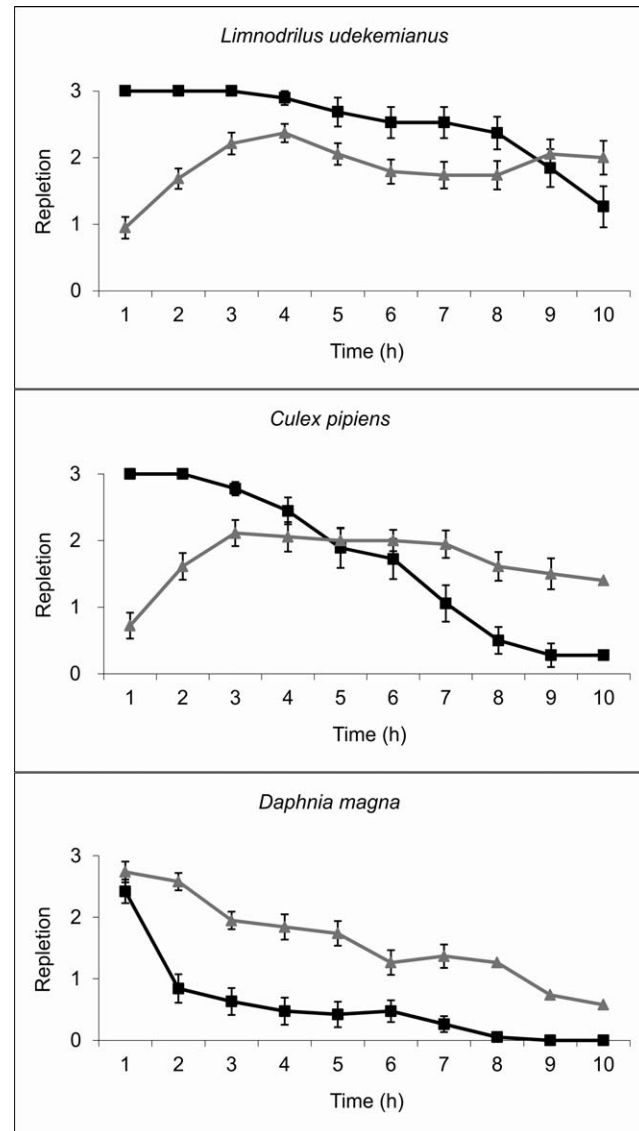|	Preys		
	D. magna	*C. pipiens*	*L. udekeminus*
Volume of 1 indivividual	0.5	1.5	1.96
Mean number	70.9 ± 2.4	14.2 ± 0.8	16.6 ± 0.8
Mean volume	35.5 ± 1.2	21.3 ± 1.2	32.5 ± 1.7

Fig. 2.   Mean and standard values of stomach (■) and intestine (▲) repletion degree of *M. borellii* over time for three prey species.

prey consumed (cladocernas: T $= -0.3071$, $P = 0.7781$; dipteran larvaes: T $= 0.7696$, $P = 0.4576$; oligochaetes: T $= 1.8179$, $P = 0.2530$); therefore, all individuals were grouped for further analysis.

Results of the gut evacuation trial (Fig. 2) show a faster digestion of cladocerans than mosquito larvae and oligochaete. The stomach clearance of this prey occurred primarily during the first hour of the trial, and by the third hour all prawns had begun to evacuate (Fig. 3). This result demonstrates that time of intestine evacuation only differed in cladocerans relative to the two others prey types, while time to stomach digestion differed for all experimental groups. Oligochaetes and dipteran larvaes have a slower digestion process (Fig. 2) and both showed two peaks of feces production (Fig. 3). It was confirmed by the Mantel test that no significant correlation ($P > 0.05$) of stomach digestion time existed among trials; however, there was a positive and significant correlation for the intestine
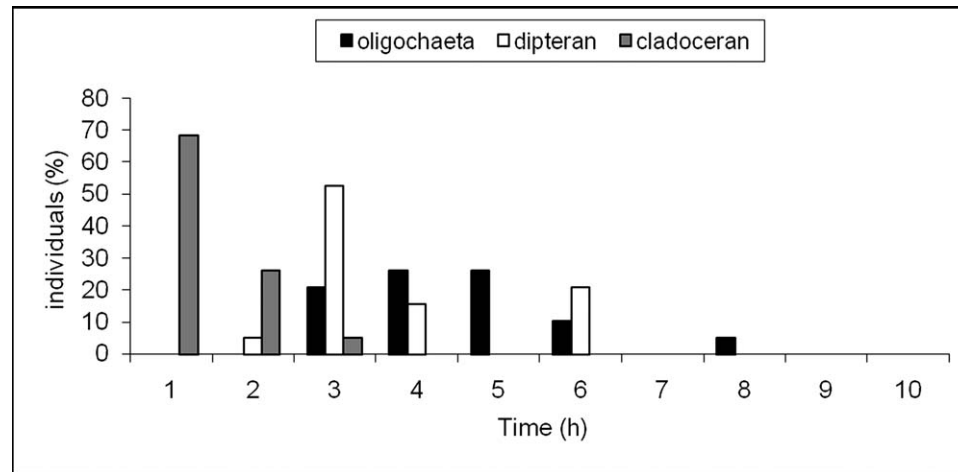
Fig. 3. Percent of individuals of *M. borellii* that evacuated over time for each type of prey.

evacuation time of cladocerans prey with respect to the other two prey types (Table 2).

These results can be supplemented by a Kruskal-Wallis test. The first hour of stomach digestion was statistically equal for all trials (KW, $H_{(0.05) \ 18, \ 9} = 5.345$, $P = 0.069$). However, if was rejected H_0 by the Kruskal-Wallis test, was impossible to know wich group could differ from which other group, and the result, as shown by the Mantel test, had not only estatistical importance but also biological inference. Therefore, the post test was performed and revealed that the first hour of stomach digestion of cladocerans was statistically faster than other prey types ($P = 0.048$). On the other hand, for intestine evacuation time, only the second (KW, $H_{(0.05) \ 18, \ 9} = 31.53$, $P = 1.42 \times 10^{-7}$), ninth (KW, $H_{(0.,05) \ 18, \ 9} = 18.88$, $P = 7.96 \times 10^{-5}$) and tenth (KW, $H_{(0.05) \ 18, \ 9} = 11.03$, $P = 4.03 \times 10^{-3}$) hours presented significant differences among groups. The Mann-Whitney post test showed differences only among cladocerans and the two other prey types (Table 3). These differences could be related to the number of prawns that began to release feces per hour (Fig. 3). More than 60% of individuals that ate cladocerans liberated the first feces in the first hour of the study. This percentage was only reached at the third and fifth hours for diptera and oligochaete prey, respectively. Furthermore, forty percent (40%) of prawns that ate oligochaetes regurgitated from the fourth hour of observation until the tenth (Table 4).

DISCUSSION

Macrobrachium borellii was shown to be a predator with a high predation capacity due to its ability of catch a large number of prey of different bioforms in only one hour (Fig. 1). However the intensity of predation may depend on, first, the time taken to digest the gut content and, second, the capacity to storage food in the cardiac chamber. On the one hand, the maximum number of consumed prey, when is converted into volume, allow to infer the stomach storage capacity. On the other hand, the absolut number of caught prey could help to infer the predation capacity in the environment. Both of them (digestion time and storage capacity) will depend on some prey characteristic like shape (form and length) and presence of rigid structures.

Macrobrachium borellii can caugth and storage a different amount of individulas depending on the type of prey (Fig. 1). *Daphnia magna* is an spherical microcrustacean with a large swimming antennae and a chitinous exoskeleton. Its shape besides the small size enabled the high number of individuals consumed and volume (35.5 ± 1.2 mm³) storaged in the adult prawns' stomach (LC 16.4 ± 0.4 mm). Larvae of *C. pipiens* are a cylinder-like prey with a sclerotized head capsule and scattered hairs covering the chitinous exoskeleton (Courtney et al., 1996). This last characteristics could impede the storage of more larvae and therefore underestimating the mean volume (21.2 ± 1.2 mm³). Additionally, although *L. udekemianus* is also cylindrical, it has a more elongated body that could roll in the stomach's prawn. Its outer cuticle is composed by scleroprotein cuticle or mucopolysaccharide fibers and chaetes (Brusca and Brusca, 1990). This last structure is often found intact in foregut contents, indicating a resistance to digestion (unpublished data).

Other characteristic that help to explain the differences observed in the three trial groups with respect to time of stomach clearance is related to the internal morphology of

Table 2. Values of Mantel test comparing each group of predator preying on a type of prey. Correlation between matrices: (R), uncorrected significance level: *P* (uncorr).

	Stomach		Intestine	
	R	*P* (uncorr)	R	*P* (uncorr)
C. pipiens x D. magna	0.166	0.094	0.404	0.006
C. pipiens x L. udekeminus	−0.032	0.518	0.404	0.003
D. magna x L. udekeminus	−0.078	0.635	0.168	0.378

Table 3. Significant *P* values for the time of intestine digestion of *M. borellii*.

	Time		
	2 h	9 h	10 h
D. magna vs. *C. pipiens*	0.0014	0.0218	0.0075
D. magna vs. *L. udekeminus*	0.0003	0.0003	0.0007

Table 4. Amount of individuals fed with *L. udekemianus* that regurgitate per hour. n° = absolute number of individuals that regurgitate, %/hour = percentage of individuals that regurgitate per hour, % total = cumulative percentage of individuals that regurgitate until a determined hour.

| | Time trial | | | | | | |
	4 h	5 h	6 h	7 h	8 h	9 h	10 h
n°	1	1	1	0	1	1	2
%/h	0.05	0.05	0.05	0	0.05	0.05	0.11
% total	5	11	17	17	22	28	40

the cardiac chamber. Caridea exhibit part of the trend of gastric armature within the suborder Pleocyemata as pointed out by Icely and Nott (1992), who report that *M. borellii* has a type III foregut. "The absence of gastric armature, other than setae, together with complete fusion of the dorsal and lateral ossicles and no calcification" implies a less efficient stomach food trituration process than other decapods, depending on the type of prey. However, the manipulation of prey is more complex.

The characteristics of prey and the internal morphology of the prawn stomach could be determining the time of bulk stomach passage. The rigid structures such as the sclerotized capsule of mosquitoe larvae and the type of cuticle and presence of chaetes of oligochaetes, resist digestion and may prolong the time of gut clearance. Additionally, the chitinolytic enzymes of crustaceans are not only involved in the resorption of parts of old cuticle during the molt cycle but also work as digestive enzymes (Spindler-Barth et al., 1990), thereby acting in the dipteran's and cladoceran's chitinous exoskeleton.

The shape of each prey is also an important factor in the digestion process. The absence of a gastric mill and the presence of only setaes in the cardiac chamber impede the maceration of large prey. If food particles are not small enough to pass through the cardiopyloric valve, the stomach content can be regurgitated, which happened with some of the prawns that ate oligochaetes (Table 4). Indeed, the mesh size of the cardiac setal screen (a structure that selects the particle size that passes to the superior cardiac chamber and remains in the digestive process) has been measured in a number of species and particles greater than 1 µm are retained and cannot enter the pyloric chamber (Icely and Nott, 1992). Furthermore, this study excludes other factors that could be aiding in digestion, such as sand grains. In the natural environment, sediment particles are available to be ingested by prawns.

All these items discussed suggest that once the bolus has returned from the hepatopancreas to the stomach, the time required to clear the intestine is similar regardless of the prey type; therefore, the time of the total evacuation is not dependent to the intestine clearance. Differences in time of intestine bulk-passage observed in the second, ninth and tenth hours (Table 3) for *D. magna* may be explained by its quick digestion (Fig. 2). The total stomach content passed to the hepatopancreas could be not enough to fill it blind ends tubules, which may permits the ingestion of more cladocerans. The enzimatic digestion of *D. magna* is not so complex due to the low protein and lipid compounds, therefore, the rapid passage of digested contents to the

intestine ends in an earlier emptying time and results, in this case, in repeated feeding cycles throughout the day.

As suggested in previous works (Loya-Javellana et al., 1995; Simon and Jeffs, 2008), the return of appetite is related, among others factors, to the rates of foregut evacuation. The time required to digest different prey types will determine the circadian rhythm of feeding. *D. magna* was the prey with the shortest retention time in the prawn's gut, allowing a new forage behavior and greater food consumption. However, this prey has a lower caloric value per individual (Riccardi and Mangoni, 1999; Eggleton and Schramm, 2004; Ciancio and Pascual, 2006) and also has a high mobility, like mosquito larvae. The swimming capacity of this prey requires more energy expenditure in their search and capture, once this prawn actively seek its prey. Therefore, although dipterans and oligochaetes have a longer period of digestion, allowing a lower daily consumption, they contain a high proportion of protein (Hepher, 1989) that bring more net energy and decrease the vulnerability to predators, which makes them more advantageous in terms of cost-benefit.

Positive selection to the dipterans larvae and oligochaetes and a negative selection to microcrustaceans had already been observed in an earlier study of the natural diet of *M. borellii* (Collins and Paggi, 1998). This finding suggests that the long digestion time, as observed in oligochaetes and mosquito larvae, is not an important factor influencing selection in this case. The availability, mobility, shape, caloric value, and vulnerability to predators prevail and direct selection toward the more profitable prey according to the optimum forage theory (MacArthur and Pianka, 1966). The ingestion of more advantageous prey, in this case, have a slower digestion time and will result in fewer foraging events in a day. However, the daily trophic cycle could vary with the diet and the prey availability.

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