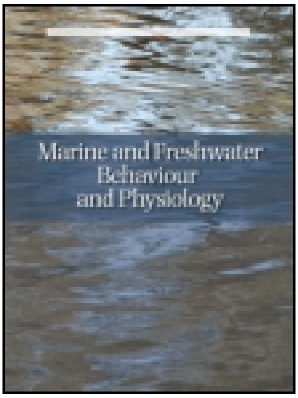

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The diel feeding rhythm of the freshwater crab *Trichodactylus borellianus* (Decapoda: Brachyura) in mesocosm and natural conditions

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Biorhythm studies have rarely used feeding rhythms of freshwater crustaceans to assess the internal clock. Even less often, they have compared the diel rhythms of juveniles and adults. In this study, the 24-h cyclic feeding behavior of the freshwater crab *Trichodactylus borellianus* was investigated in mesocosm and natural field conditions during summer. Sampling occurred throughout the day and included the dawn and dusk periods. The fullness of the stomach was used to characterize the feeding cycle and was analyzed with MESOR and a three-day time series plot. The rhythms of juveniles and adults were analyzed in the field. A bimodal feeding rhythm with midday and midnight peaks was observed in adults in the field, while juveniles had an asynchronous behavior. Mesocosm animals also showed no cyclic behavior. Rhythmic responses to nonphotic cues may result from a trade-off between foraging at an optimum time and shifting the diel rhythm to avoid competition and predation risk. Juvenile feeding asynchrony could be a strategy that allows them to be active in the same habitat as adults.

Keywords: diel feeding rhythm; freshwater crab; *Trichodactylus borellianus*; field and mesocosm

Introduction

The study of the biological rhythms of decapod crustaceans, and their synchronization with environmental factors, has advanced steadily during the past half century (Brown 1961; Webb & Brown 1965; Barnwell 1966, 1968; Naylor & Williams 1968). Solar- and lunar-based cycles have been of particular interest in the subsequent behavioral and physiological studies of rhythmicity (De Coursey 1983; Palmer & Williams 1986; Palmer 1989; Aréchiga & Rodríguez-Sosa 1997; Stillman & Barwell 2004; Aguzzi et al. 2005). Many studies have used locomotor activity as a measure of the biological clock (Abelló et al. 1991; Aguzzi et al. 2004, Stillman & Barnwell 2004; Chabot et al. 2010). These studies have been largely restricted to marine species, and the activity rhythms of freshwater decapods have only recently been examined. Feeding rhythm has rarely been used as an indicator of the internal clock for either marine or freshwater decapods. Another poorly explored issue is the effect of age on rhythmic behavior. In

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Latin America, advances in understanding the rhythmic behavior of freshwater decapods have been made in Mexico (Miranda-Anaya 2004), Argentina (Collins 1997; Renzulli & Collins 2001; Williner & Collins 2002), and Brazil (Sokolowicz et al. 2007; Zimmermann et al. 2009).

Freshwater decapods in South America are represented by true crabs (Pseudothelphusidae, Trichodactylidae), pseudocrabs (Aeglidae), prawns (Atyidae, Palaemonidae, Sergestidae), and crayfishes (Parastacidae) (Melo GAS 2003). These organisms occur in many habitats having a variety of environmental characteristics, and they inhabit both lentic and lotic water bodies (Collins et al. 2007). In the floodplain of the Middle Paraná River, several species of true crabs and prawns coexist in the littoral-benthic zone. Among these species, *Trichodactylus borellianus* Nobili, 1896 is the most common and most widely distributed crab of the family Trichodactylidae in this system (Melo GAS 2003). It is frequently associated with the roots of macrophytes (primarily the floating *Eichhornia crassipes*, the common water hyacinth) (Collins et al. 2006), where it can find food and refuge from predators. It consumes plant and animal items, and is preyed upon by fish, amphibians, birds, and mammals (Bonetto et al. 1963; Massoia 1976; Oliva et al. 1981; Beltzer 1983a, 1983b, 1984; Beltzer & Paporello 1984; Bianchini & Delupi 1992; Lajmanovich & Beltzer 1993; López et al. 2005). These trophic relationships indicate the important role of the species in the transfer of energy and matter among littoral, lotic, and non-aquatic communities (Williner & Collins Forthcoming).

The ecological feeding profile of a given population results from the coevolutionary history of the species in the presence of environmental oscillations and ecological interactions (Collins et al. 2012). The temporal organization of feeding behavior may be modulated by multiple factors, among which sunlight may be the most important in influencing the activity pattern. Freshwater decapods that are frequently found far from the sea can be expected to exhibit an activity pattern based on light–dark intervals rather than tidal rhythms (Palmer 1973). These species will then adjust both the frequency and the phase of rhythms to the cycle of the earth's rotation (Brown 1961; Daan & Aschoff 2001). That is, their circadian rhythms will be entrained by the daily light cycle. The light–dark phase is the most constant external stimulus. However, environmental variables have direct effects on the expression of circadian systems and can mask the entrainment process (Connel 1980; Daan & Aschoff 2001).

The daily feeding rhythm can be studied over a 24-h cycle by observing stomach filling and emptying over time (Collins 1997; Aguzzi et al. 2004, 2005). There have been some field studies of the rhythmic patterns of *T. borellianus* in the Pilcomayo River system. Renzulli and Collins (2001) studied the locomotor activity rhythms of this species in the autumn, taking 4-h samples in the littoral area over three days. They found greater locomotor activity during the afternoon and suggested a link to feeding behavior. Williner and Collins (Forthcoming), studying the feeding ecology of *T. borellianus*, found age-related variation in the pattern of feeding activity. During the afternoon, adults had greater stomach fullness than the juveniles. In addition, the stomach fullness of the adults differed significantly between the morning and afternoon. These reports provided the first information about the activity patterns of this crab species and evidence for different rhythms at different ages. The relationship between the feeding rhythm of *T. borellianus* and the light–dark cycle of juveniles and adults is yet unstudied.

The present study investigated the diel rhythmic feeding behavior of the freshwater crab *T. borellianus* under two conditions of natural photoperiod during the summer.

The first experimental study was made in mesocosms. This study simulated natural physical conditions but lacked predators (visually hunting fishes), other co-occurring decapods (e.g. prawns and other crab species), and had a known abundance of crabs. A second study took place in the field under the same photoperiod but in the presence of natural predators and co-occurring decapods. In this latter study, a comparison was made between juvenile and adult crabs to elucidate the feeding behavior at different ages. The diel rhythm under these two conditions (natural and mesocosm) was compared to identify a possible temporal shift in activity due to different nonphotic cues.

Materials and methods

Mesocosm study

Crabs were collected in the field with a 1-mm mesh size hand net and manually separated from the vegetation. Due to the low number of juveniles captured in the sample, they were not included in this work. The experiment was performed during the summer in two circular pools (designated *R1* and *R2*). The pools, 2.44 m in diameter, were filled with 1000 L of well water and provided with floating aquatic vegetation: mainly the Common Water Hyacinth, but also Water Lettuce (*Pistia stratiotes*), American Spongeplant (*Limnobium spongia*), and Giant Salvinia (*Salvinia biloba*). Approximately 100 crabs were added to each pool in equal proportion of sexes.

To simulate actual field conditions and the normal feeding and locomotion behavior of crabs among the roots of the vegetation, on the bottom and in the water column, the pools were stocked with pleustonic, benthonic, and zooplanktonic organisms. The pleustonic fauna was collected from aquatic vegetation in a net with a 50-cm mouth opening and a mesh size of 200 μm. To maintain a constant amount of vegetation, 4 nets with the above characteristics were used to stock each pool. The zooplankton was sampled and filtered from the field with a Schindler-Patalas trap with a mesh size of 30 μm. A total of 200 L of lake water was filtered and diluted in each pool, simulating the densities reported for floodplain lakes in this system (José de Paggi & Paggi 2007, 2008). A total of 20 L of sand was placed in the bottom of each pool. Each one also received 0.3 L of sediment containing an oligochaete species (*Limnodrilus udekemianus*), obtained from cultures at the Instituto Nacional de Limnología. To acclimate the fauna, the pools were placed in the shade of an Earpod Tree (*Enterolobium contortisiliquum*) for one week before the trial started. No additional food was added.

Study site

The study was conducted in the floodplain of the Paraná River during the late summer and in the period of low water levels (2.25 ± 0.01 m at the "Puerto Santa Fe"). The sampling site was a lateral expansion pond (Drago 1976), directly and permanently connected with the Ubajay stream (31° 33′33 70″S–60°30′52 88″W) (Figure 1). The pond showed lentic characteristics during this phase of the water cycle. The aquatic vegetation was stagnant in the littoral zone and was composed of floating and rooted species: Common Water Hyacinth, Creeping Water Hyacinth (*E. azurea*), Water Lettuce, Water Fern (*Azolla filiculoides*), Giant Salvinia, and Elephant Panicgrass (*Panicum elephantipes*). Abiotic parameters, physical, and chemical characteristics (pH, temperature, conductivity, and dissolved oxygen) were measured at each sampling with an electronic sensor (Hanna HI 98130/9146).

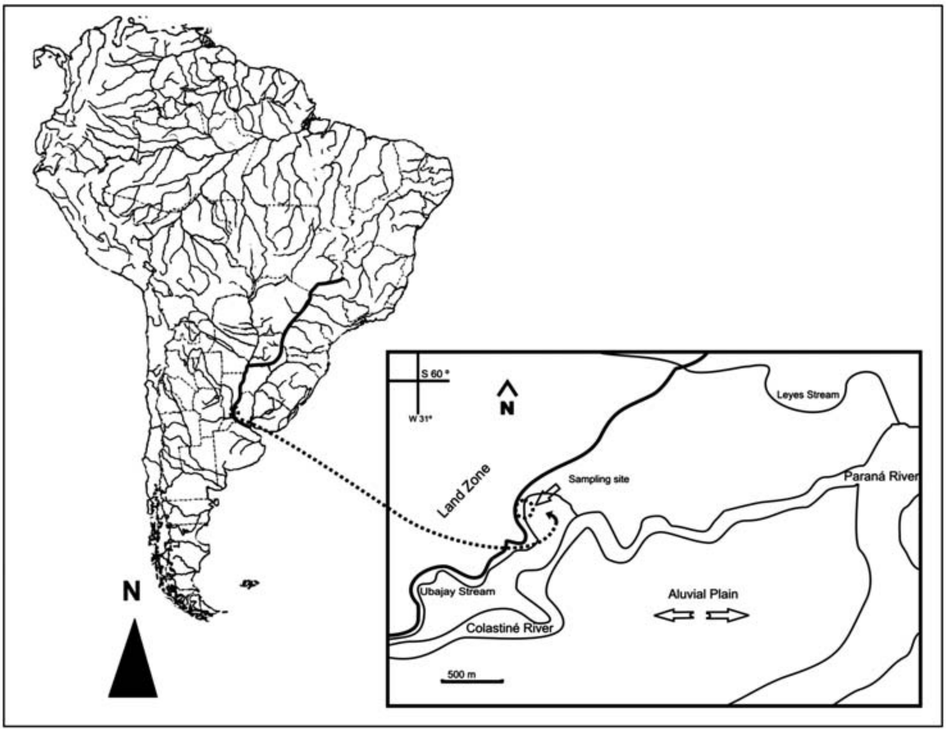

Figure 1. Schematic map of the alluvial plain where the fields study was performed (Carvalho et al. 2013).

Sample design

The same sampling design was applied to field and mesocosm studies. Samples of *T. borellianus* were collected in the aquatic vegetation using a "D-like" (1230 cm^2) and a circular hand net (615 cm^2) with a 500-μm mesh size in the field and mesocosm work, respectively. Preference was given to areas with the highest proportion of Common Water Hyacinth in the field. The sampling effort was constant throughout the study, with 2 nets placed among the vegetation. The roots of the macrophytes were manually examined. The crabs were separated and placed in containers with cold water and ice to reduce their metabolism, to inhibit maceration by the gastric mill, and to avoid regurgitation. After a few minutes in the containers, the crabs were sacrificed by adding 96% alcohol to the cold water. This humane way of sacrificing these animals was used in the absence of an approved ethical procedure in the jurisdiction where this study was carried out. The same procedure was repeated every 4 h for 3 consecutive days. Sampling occurred immediately after sunrise (6:25) and immediately before sunset (19:40 – last days of February of 2012).

Laboratory analysis

For each crab, the maximum width of the cephalothorax (CW) was measured with a caliper under a stereomicroscope to the nearest 0.01 mm, and the sex was determined by the presence of the masculine appendix. The presence of females with eggs or hatched juveniles was recorded. Only intermolt crabs were considered for analysis.

Individuals less than 6 mm in CW were considered juveniles based on pleopod development and sexual maturity (Williner et al. Unpublished). The crabs were dissected, the stomach was removed and the muscular remains were cleaned to facilitate the visualization of the gut contents. A subjective scale from 0 to 3 (0: empty, 1: 1/3 full, 2: 2/3 full, 3: full) was used to categorize the total stomach repletion (SR) (i.e. the repletion of the cardiac (CR) and pyloric (PR) chambers together). In addition, the same scale was applied in the field study to categorize the CR and PR separately (Figure 2). The presence or absence of intestinal contents (IC) was observed. To reduce the bias due to visual classification, the same person was responsible for all data acquisition.

Data analysis

In an exploratory analysis, the relative proportions of all fullness degrees for each time series were computed and plotted to visualize the feeding rhythm for the SR, CR, and PR indexes for each size category (adults and juveniles). The same procedure was applied for the presence and absence of IC. In the field study, the analysis was made considering juveniles and adult crabs separately. The visual scale used in this study is subjective but the extremes cannot be misjudged. Hence, the indexes 0 and 3 were used to assess the feeding rhythm. The data for the 3-day time series were plotted using the percentage of empty stomachs along with the sampling times. A 24-h analysis was then performed to show the time of peaks in percentage of empty and full stomachs plotted against the sampling times to display and compare the phases during which the feeding activity was reduced and increased, respectively. The percentages were assessed by calculating the vacuity (V) (Albertini-Berhaut 1979) and fullness coefficients, which are expressed as $V = \text{ES}/\text{TS} \times 100$ or $F = \text{FS}/\text{TS}$, where ES and FS are the number of empty

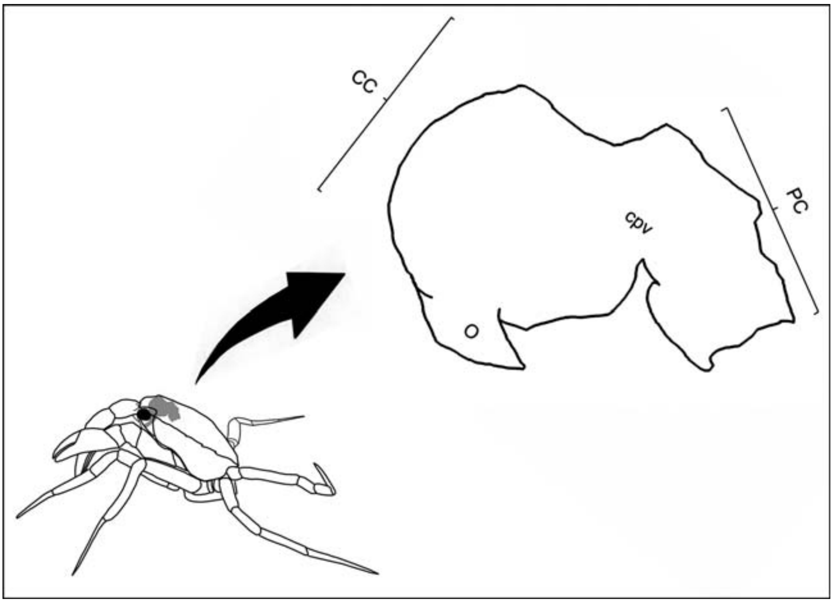

Figure 2. Schematic representation of a crab's stomach in lateral view. O: oesophagus, CC: cardiac chamber, PC: pyloric chamber, and cpv: cardio-pyloric valve. PR and CR are pyloric repletion and cardiac repletion, respectively.

and full stomachs, respectively, and TS is the total number of stomachs examined for the group. The midline-estimating statistic of rhythm (MESOR) was plotted with to the curves of vacuity and fullness to identify peaks in relation to the times of the day. MESOR can be defined as the mean value of a rhythmic variable (e.g. vacuity or fullness coefficients) for equidistant data covering a 24-h period (Aguzzi et al. 2005). Values above the mesor are indicative of a peak in the rhythmic cycle. This data analysis was applied only for the SR.

Differences between the sexes in the fullness degree at each observation time were first analyzed with a Kruskal–Wallis test. In the field study, differences between adults and juveniles were also examined. To determine the feeding rhythm and corroborate the significance of the peaks found with the MESOR, comparisons were made between size categories at the same group of hours on the three days and among the sampling hours for each size category. The SR, CR, PR, and IC observations were tested with a Kruskal-Wallis (KW) test and, if necessary, with a Mann-Whitney (MW) post-test. These analyses were made with the free statistical package PAST (version 2.169) (Hammer et al. 2001).

Results

Mesocosm study

A total of 153 crabs were analyzed, of which 44.4% were males (8.03 ± 1.7 mm of CW) and 55.6% were females (9.09 ± 1.7 mm of CW). Of the females, 18.3% carried eggs and 8.5% carried post-hatch juveniles, due to a large number of females in these reproductive stages during the season of the study. Despite the large number of ovigerous females, there were no significant differences in the SR between males and females at any sampling time ($p > 0.05$). In general, the vacuity coefficient was high in this study, reaching nearly 50% of the total stomachs analyzed. The feeding rhythm recorded over a 3-day cycle showed no clear pattern in the mesocosm. Nevertheless, a high peak of empty stomachs occurred primarily during the night at 23:00 of the third day (Figure 3 (a)). The average values of empty and full stomachs in the 24-h analysis with MESOR demonstrate the feeding rhythm. The proportion of empty stomachs increased over the evening, exceeded the midline at 19:00 and reached a maximum during the night (23:00–03:00) (Figure 4 (a)). Likewise, the proportion of full stomachs showed a trend toward more intense feeding activity at 11:00, which decreased during the evening and reached a minor peak trough the night (23:00–03:00) (Figure 4). None of these trends was statistically significant for SR (KW: 2.091, p: 0.791). The presence of IC was observed in a high percentage of crabs (62.6%) on all sampling days, with a tendency for higher proportions during the night (Figure 5(a)). This tendency was also not statistically significant (KW: 6.876, p: 0.07736).

Field study

A total of 269 crabs were analyzed, of which 53.96% were juveniles, 32% males and 14.1% females. Only a few females (2.2% of the total) with post-hatch juveniles were captured. More than 90% of the juveniles were between 3 and 5 mm in CW, whereas almost 50% of the adults measured between 9 and 11 mm in CW (Figure 6). Physical and chemical parameters remained stable over the study period (mean values ± SD; temperature: 26 ± 2.55 °C, conductivity: 0.14 mS ± 0.02, pH: 7.8 ± 0.23, and dissolved oxygen: 18 ± 0.68 ppm).

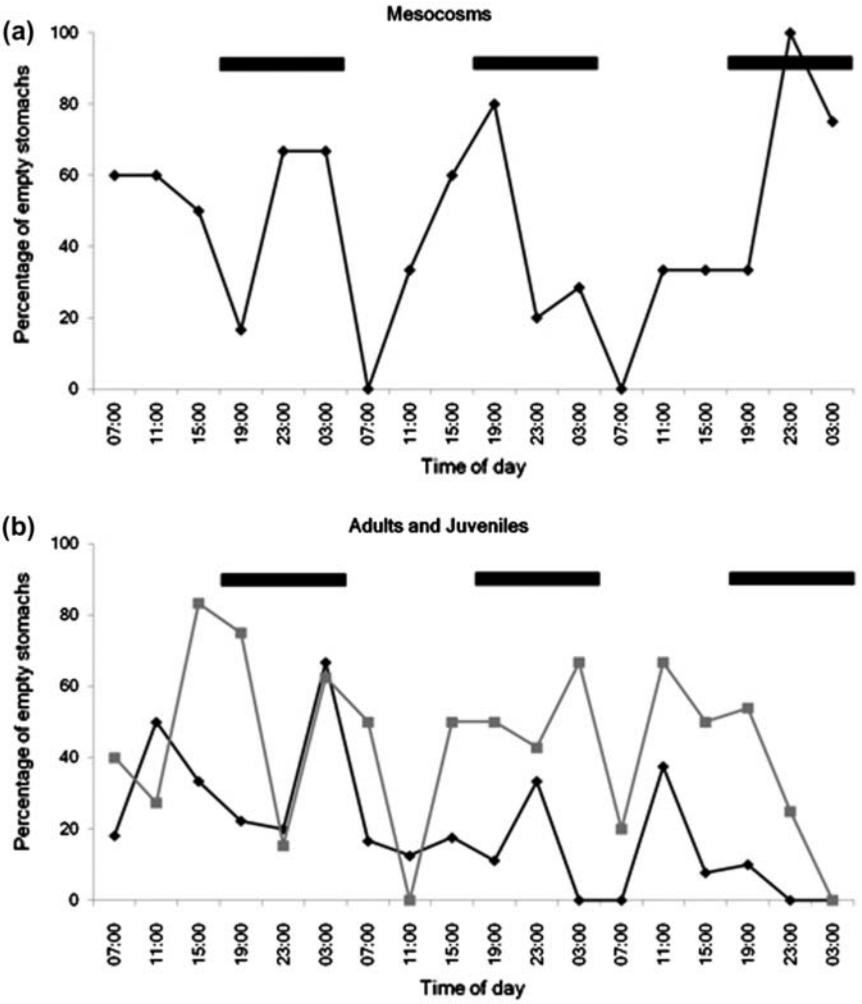

Figure 3. Percentage of empty stomachs along with the sample times (catches every 4h) to mesocosm (a) and field study (b). Grey line: adults, black line: juveniles.

The feeding rhythms varied over time and between crabs of different sizes. Regardless of this variability, some significant peaks were identified. For SR, CR, PR, and IC, the SR index values did not differ significantly over time (KW test, $p > 0.05$ for all indexes), when the adult males and females were analyzed together. However, juveniles and adults had different diel feeding rhythms. A significant difference in SR among the day hours was found for SR, CR, and PR only in adults (KW test; SR: $p = 0.0259$, CR: $p = 0.0155$, and PR: $p = 0.027$) (Figure 4(b) and (c)). The proportion of empty stomachs also varied between juveniles and adults. In general, 29.1% of all stomachs (both adults and juveniles) were empty, corresponding to 43.7% of adults and 14.9% of juveniles. Indeed, the 3 day cycle analysis showed most points exceeding 40% of empty stomachs in adults, whereas in juveniles, most points remained below this level (Figure 3(b)).

The feeding rhythm recorded over the 3-day cycle showed more marked fluctuations in adult crabs than in juveniles. This pattern is better visualized in the 24-h plot. The proportion of empty stomachs indicated some minor feeding activity with a peak during

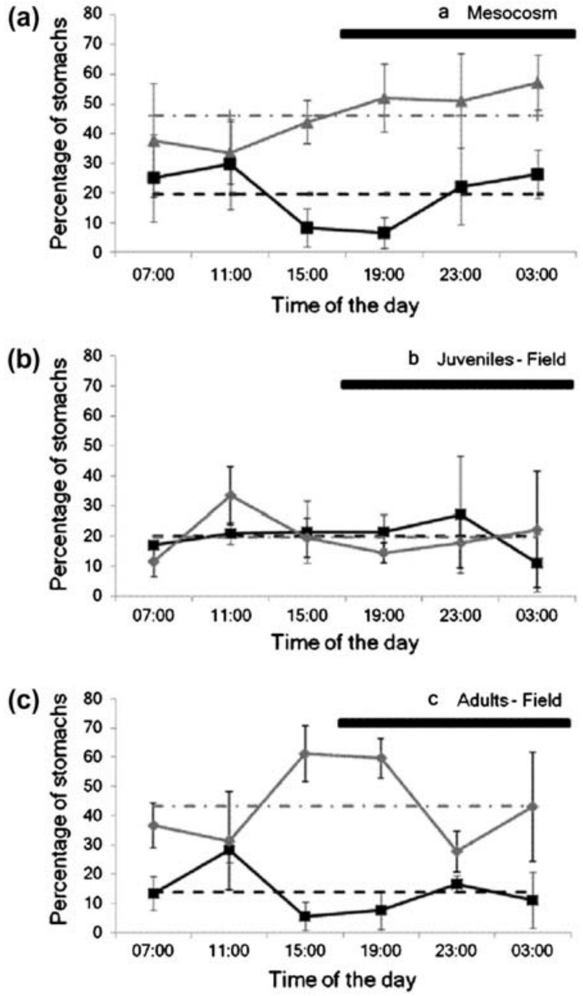

Figure 4. Proportion of vacuity (grey line) and fullness (black line) determined every 4 hours in the mesocosm (a) and in the field study for juveniles (b) and for adults (c). Grey dashed line: MESOR of vacuity coefficient, black dashed line: MESOR of fullness coefficient.

the afternoon (15:00–19:00) and a lower proportion during the hours after sunrise until midday (7:00–11:00), and during the night (23:00). Similarly, the proportion of full stomachs increased after sunrise and reached a peak at 11:00, decreased below the midline during the afternoon hours (15:00–19:00), and showed a minor peak at 23:00 (Figure 4(b)). The importance of these peaks is supported by the significant differences in the repletion index found among these hours (MW test; 11:00 vs. 15:00: $p = 0.0355$, 11:00 vs. 19:00: $p = 0.04218$, 23:00 vs. 15:00: $p = 0.0179$, 23:00 vs. 19:00: $p = 0.01711$, 23:00 vs. 3:00: $p = 0.03301$). In contrast, the feeding rhythm of the juveniles was apparently constant over time, with an overlap of fullness and emptiness percentages. Only a point at 11:00 above the midline showed a trend toward lower feeding activity (Figure 4 (c)). However, there were no significant differences found among hours (KW test; $p = 0.4085$).

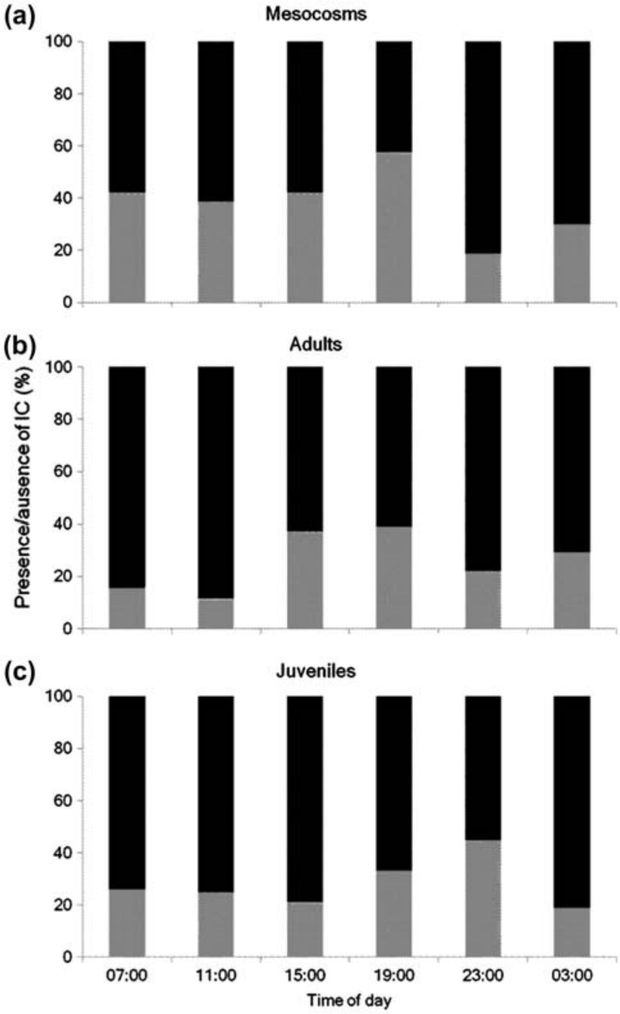

Figure 5. Proportion of presence and absence of IC of *T. borellianus* in the mesocosm (a) and in the field study to adults (b) and juveniles (c).

Examining each stomach compartment, only the cardiac chamber of the adults had a greater fullness index at 23:00 than at the other hours (MW test; 23:00 vs. 15:00: $p = 0.02848$, 23:00 vs. 19:00: $p = 0.02947$) (Figure 7(a)). In the pyloric chamber, a high proportion of fullness was observed in both size categories. However, the adults showed significantly less pyloric chamber fullness at 15:00 than at the morning and night sampling times (MW test; 15:00 vs. 7:00: $p = 0.01512$, 15:00 vs. 11:00: $p = 0.006873$, 15:00 vs. 23:00: $p = 0.01529$) (Figure 7(d)). IC were present in a high proportion of both juvenile (71.7%) and adult (74%) crabs. The percentage of crabs with IC appeared to be lower at 15:00 and 19:00 in the adults and at 23:00 in the juveniles (Figure 5(b) and (c)), but these differences were not statistically significant (KW test; $p = 0.251$).

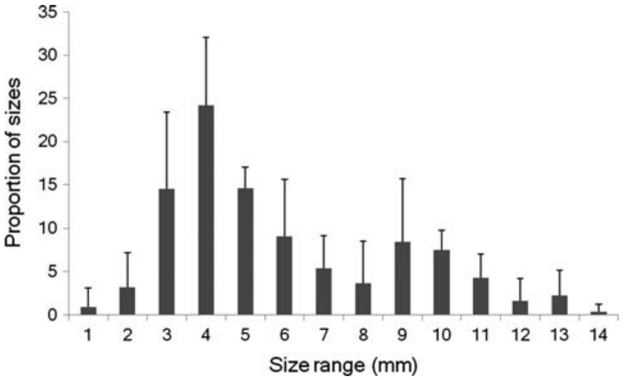

Figure 6. Mean values and standard deviation of the percentage of sizes of *T. borellianus* captured in the field study. Size ranges were calculated for each 1 mm.

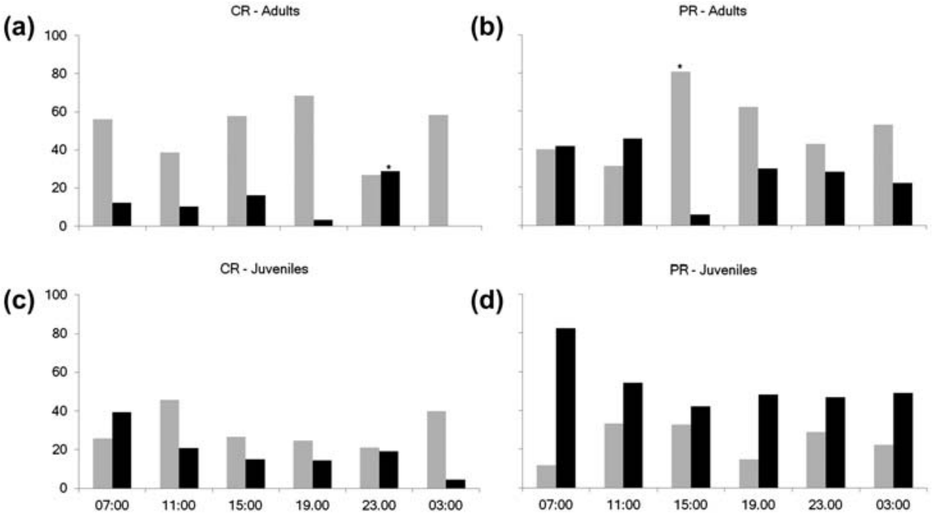

Figure 7. Relative proportion of the CR and PR repletion of adults (a, b), and juveniles (c, d) of *T. borellianus* determined every 4 hours in the field study. Light gray (0: empty) and dark (3: full). Asterisks indicate significant differences.

Discussion

The results of the present study suggest that the feeding rhythm of adults in the mesocosm and field studies are similar. However, significant peaks were only found in the field. In contrast, there was a marked difference between adults and juveniles in feeding rhythm. Under summer field conditions, adults exhibited a bimodal feeding rhythm while juveniles showed no clear rhythmicity. The peaks of the adult rhythm did not coincide with the crepuscular hours and generally occurred in the middle of the day and the middle of the night. This finding is surprising in light of previous studies of decapod species from inland waters, which indicate a crepuscular feeding rhythm (Collins 1997; Williner & Collins 2002; Aguzzi et al. 2005; Zimmermann et al. 2009). In *T. borellianus* in particular, previous studies are only partially consistent with the present results. The juvenile–adult differences in the feeding activity period demonstrated by Williner and Collins (Forthcoming) are consistent with the findings of the present work. Although the authors based their comparisons on dusk and dawn feeding only, the stomach fullness of the adults differed significantly between the morning (9:00–10:00) and afternoon (18:00–19:00), whereas the stomach fullness of the juveniles did not. In contrast to our results, Renzulli and Collins (2001) observed a high level of locomotor activity during the afternoon (15:00), predominantly in adults. In the current study, the level of feeding activity of adults was lower during the afternoon. A discordance between feeding and locomotor rhythms was observed previously in the Norway lobster *Nephrops norvegicus* (Linnaeus 1758). Despite its crepuscular emergence behavior, feeding occurred in the daytime (see Aguzzi et al. 2004). Indeed, feeding and locomotor activity could be driven by different oscillators and may result from a dual clock system in crustaceans (Fernandez de Miguel & Aréchiga 1994). The relationship between these two rhythmic behaviors awaits elucidation in the crab *T. borellianus*. Although the current results and previous studies suggest different rhythmic activities in *T. borellianus*, differences among studies in season and habitat may have influenced the expression of rhythmic behavior.

The expression of circadian rhythmic activities (e.g. feeding rhythm) is the result of output from the endogenous circadian clock, and external stimulatory and inhibitory effects (Kronfeld-Schor & Dayan 2003; Chiesa et al. 2010). External effects can be photic and nonphotic. Oscillation of the intensity of light is the most predictable cue in the environment (Daan & Aschoff 2001). However, response to nonphotic stimuli is of adaptive value and reflects the plasticity of shifting the diel rhythm and can enhance fitness (Kronfeld-Schor & Dayan 2003). Some important nonphotic cues, which can mask the true entrainment, are the presence of competitors, prey, and predators. This crab is a frequent prey item of both diurnal and nocturnal predators including fish, birds, and amphibians (Bonetto et al. 1963; Oliva et al. 1981; Lajmanovich & Beltzer 1993; López et al. 2005; Beltzer et al. 2009). It also coexists in sympatry with other decapods such as the prawn *Macrobrachium borellii* (Nobili, 1986), which can have dense populations (Collins 2005). The natural diet of this prawn is similar to that of *T. borellianus* (Collins et al. 2007), and it has a feeding activity period in the early morning and before dawn during the winter (Collins 1997). The differences in timing of daily activity patterns may represent a strategy to coexist and inhabit the same environment (Miranda-Anaya 2004; Collins 2005). Likewise, predation risk also influences temporal activity patterns and may restrict foraging time (Kronfeld-Schor & Dayan 2003). Therefore, the timing of feeding behavior may result from a trade-off between foraging at an optimum time and shifting the diel rhythm to avoid competition and predation risk.

Despite the fact that the feeding rhythm of *T. borellianus* in the mesocosm appeared to be similar to that observed in the field, daily changes in SR in mesocosm were not statistically significant, possibly due to the lower number of crabs analyzed and to individual variability (Daan & Aschoff 2001; Refinetti 2012). Yet, it is interesting to note that the feeding behavior observed in the mesocosm could be the result of a specific masking effect of external factors that were present under natural conditions before the crabs were collected from the field (Daan & Aschoff 2001). This can be analogous to the “ghost of competition past” (Connell 1980), i.e. activity patterns become “fixed” as a response to competitive pressure and are no longer amenable to manipulation (Kronfeld-Schor & Dayan 2003). Similarly, the acclimatization period (One week) to the pools’ conditions might not have been sufficient to reset the field masking effect. The possibility of a masking effect is merely speculative; indeed, another reason for the differences between the field and mesocosm results could be that the mesocosms lacked some stimulatory or inhibitory cue that is present in the field.

In contrast to the adults, the juveniles in the field study exhibited no significant peaks in feeding activity at any hour of the day. *T. borellianus* has direct development, maternal care of the young and hatched crabs with all characteristics of the adult (von Sternberg et al. 1999). The size of the juveniles varied between 3 and 6 mm. At this size, crabs are no longer receiving maternal care and tend to live among the roots of the common water hyacinth (personal observation). Many previous studies demonstrate that age can affect the overt circadian rhythm in crustaceans (see Aguzzi & Company 2010). By shifting their activity phase, juveniles can be active in the same habitat at different times of the day. Moreover, age-specific differences in the diet (Williner & Collins Forthcoming) and the predatory capacity of the animals (Carvalho et al. Forthcoming) may influence feeding behavior. Smaller crabs are less perceptible to visual predators and might be able to safely forage at times that predation pressure is high for larger individuals. Under these circumstances, the importance of age as a factor in the analyses would be considerable.

The vacuity coefficient differed between ages. A high percentage of empty stomachs was observed in adults in the mesocosms and in the field. Aguzzi et al. (2004) also recorded high percentages of empty stomachs in the lobster *N. norvegicus*. These authors suggested that the duration of digestion might be the reason for the high level of emptiness. Although there is no information on the time of digestion in *T. borellianus*, the digestive process may involve a greater delay in adults than in juveniles. The return of the appetite, associated with the time required for digestion, could initiate a new foraging bout. In *Cherax quadricarinatus*, the average return of appetite occurred at 5–10 h post-feeding, when the residuum of the previous meal was approximately 60% or less (Loya-Javellana et al. 1995). Feeding activity in the adults was found to follow a 12-h cycle, whereas no feeding rhythm was observed in the juveniles. This difference may reflect the ingestion of prey with different characteristics (e.g. with hard parts) associated with different digestion times (Carvalho et al. 2011).

Certain aspects of the digestive process may be evident from the analyses of CR and PR repletion and the data on IC. A high percentage of individuals of both ages had a full PR chamber across all hours of observation. Lower levels of IC were found only in adults during the afternoon (15:00). Moreover, IC were present in a high proportion of both adult and juvenile crabs throughout the observation period. This result demonstrates a continuity of the digestive process over the 24-h cycle.

Many aspects of the endogenous clock must be elucidated to completely understand the response to external factors. The bimodal rhythm observed in the current study could be the result of an ability to respond to competition and predation on an ecological time scale (Hut et al. 2012; Kronfeld-Schor & Dayan 2003). However, this hypothesis requires validation. In future studies, the removal of the masking effect of nonphotic stimuli could reveal the original output from the internal clock (Waterhouse et al. 1996). For example, the crabs could be deprived of all external ecological conditioning and the results could be compared with those from the field. In this way, experimental manipulations coupled with field studies could yield a better understanding of the interaction between internal and external factors in the daily activity patterns of these organisms. Another investigation of potential interest would be to test whether, in the absence of other environmental cues, food can affect circadian rhythm activity and drive a dual clock system in this crab species, as suggested by Fernandez de Miguel and Aréchiga (1994). Moreover, coupling the study of locomotor and feeding rhythms can provide valuable information about the concordance or discordance of the phase of these two activities. More research effort is necessary in the field to allow comparisons of feeding rhythm patterns across seasons. In studies of decapods inhabiting floodplains, the water level is an additional extrinsic factor that should be considered. The adaptation of decapods to freshwater conditions makes these organisms an interesting and suitable biological model for the study of the circadian expression of behavior.

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