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Comportamento, ecologia e reprodução de caranguejos ermitões (Crustacea, Decapoda, Anomura) no sudeste brasileiro

Alexander Turra

Orientador: Profa. Dra. Fosca Pedini Pereira Leite

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Banca examinadora

Profa. Dra. Fosca Pedini Pereira Leite

Profa. Dra. Ana Maria Setubal Pires Vanin

Ana M SP Vanin

Prof. Dr. Roberto Munehisa Shimizu

Roberto Munehisa Shimizu

Prof. Dr. Thomas Michael Lewinsohn

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Prof. Dr. José Roberto Trigo

Profa. Dra. Antônia Cecília Zacagnini Amaral

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Índice

Resumo

O presente estudo foi realizado com o objetivo de ampliar o conhecimento sobre a história natural de espécies de caranguejos ermitões (Crustacea, Decapoda, Anomura) com ocorrência no sudeste brasileiro. Para tanto, experimentos foram realizados enfocando aspectos comportamentais, reprodutivos e da ecologia destes animais. Um estudo inicial sobre o período de atividade de algumas espécies (*Clibanarius antillensis*, *C. sclopetarius*, *C. vittatus* e *Pagurus criniticornis*) mostrou que o padrão de atividade varia entre espécies e pode ser regido por estímulos circadianos e circamereais, embora todas as espécies tenham apresentado alta atividade no período noturno. O comportamento reprodutivo destas espécies também foi avaliado e não revelou alterações marcantes frente a descrições prévias para as espécies, gêneros ou famílias estudados. Entretanto, demonstrou-se a existência de novos comportamentos bem como a possibilidade de indivíduos intersexo das três espécies de *Clibanarius*, com característica tanto de machos como de fêmeas, copularem com sucesso como machos. Estes indivíduos foram mantidos em laboratório para acompanhar o destino dos poros genitais e, com base em informações morfológicas, comportamentais e populacionais, foi elaborada uma discussão sobre uma eventual possibilidade de hermafroditismo sequencial protogínico em ermitões. Um estudo descritivo sobre o desenvolvimento embrionário destas espécies de ermitão revelou que o tamanho dos embriões e o tempo de duração do desenvolvimento varia entre espécies, bem como algumas características morfológicas como clivagem e momento do surgimento da mancha ocelar. No entanto, sete estágios puderam ser identificados com base em características morfológicas e três em função da duração relativa desses. A relação entre o tamanho de ermitões e conchas selecionadas revelou que eles utilizam conchas sub-ótimas na natureza e que nem o tipo de concha (arquitetura) nem a espécie de ermitão têm influência sobre as relações entre suas dimenções (exceto para medidas da abertura das conchas). A hipótese do modelamento, ou seja, do efeito da experiência prévia dos ermitões com determinados tipos de concha na forma e padrões futuros de seleção de concha, foi comprovada. Ermitões criados em conchas com abertura estreita tornam-se dorso-ventralmente achados e tendem a selecionar estas conchas com mais frequência que indivíduos mantidos em conchas com abertura arredondada. Também foi demonstrado o efeito da partilha de recursos nos padrões de utilização de conchas por duas espécies simpátricas de ermitões, *C. antillensis* e *P. criniticornis*, bem como a influência de diferentes estratégias competitivas (exploração e interferência) na dinâmica de troca de conchas entre elas. Demonstrou-se ainda que *P. criniticornis* é um bom explorador, apresentando grande capacidade de resposta a eventos simulados de predação de gastrópodes. Entretanto, esta espécie perde as conchas recentemente obtidas para *C. antillensis*, o qual o domina em brigas por conchas. Por fim, verificou-se que a predação de caramujos por caranguejos quebradores de conchas pode influenciar positivamente a disponibilidade de conchas e sua futura utilização por ermitões, mas que este efeito depende da espécie de concha (diferentes graus de proteção) e de predador (diferentes estratégias e habilidades de predação), do tamanho do predador e do tamanho da presa em relação ao predador.

Abstract

This study was conducted to furnish information on the natural history of hermit crab species (Crustacea, Decapoda, Anomura) from South-eastern Brazil. Some experiments were designed to focus behavioral, reproductive and ecological aspects of the biology of these organisms. A study on the periods of activity of some hermit crab species (*C. antillensis*, *C. sclopetarius*, *C. vittatus* and *Pagurus criniticornis*) showed that the activity patterns vary among species and may be related to circadian and circatidal cues, although all species presented high nocturnal activity. The reproductive behavior of these species was also evaluated and did not reveal marked differences in relation to previous descriptions for the studied species, genera and families. However, new behaviors were described as well as the possibility of intersex individuals of the three species of *Clibanarius*, with both male and female external characteristics, copulate successfully as males. Such intersex individuals were maintained in the laboratory to follow the fate of the gonopores and, based on morphological, behavioral and populational information, a discussion about an eventual possibility of sequential protogynic hermaphroditism in hermit crabs was elaborated. A descriptive study on the embryonary development of these species revealed that egg size and developmental time varied among species as well as some morphological characteristics as cleavage and the time the eye pigment appeared. However, seven stages were identified based on morphological characteristics and three on their relative duration time. The relationship between the sizes of hermit crabs and selected shells revealed that the crabs are using sub-optimal shells in nature and that neither shell (architecture) nor hermit crab species influence the size relationships between crabs and selected shells (except for measures of shell aperture). The molding hypothesis, i.e., the effect of previous utilization of certain shell types on form and future patterns of shell selection in hermit crabs, was corroborated. Hermit crabs maintained in narrow-aperture shells become dorso-ventrally compressed and tend to select such shells in a higher frequency than individuals maintained in rounded-aperture shells. The effect of resource partitioning on the shell utilization patterns of two sympatric hermit crab species (*C. antillensis* and *P. criniticornis*) was also demonstrated, as well as the influence of different competitive strategies (exploitation and interference) in shell exchange dynamics between them. It was shown that *P. criniticornis* is a good exploiter, presenting high ability to attend gastropod simulated predation events, but looses such newly acquired shells to *C. antillensis*, which dominates the former species in shell fights. Finally, the positive influence of shell-breaking crabs on shell

availability and its future utilization by hermit crabs was demonstrated to be dependent on shell (variable protective degrees) and predator species (different predatory strategies and abilities), predator size and relative prey-predator size.

Apresentação

Os caranguejos ermitões ou paguros apresentam uma íntima relação com conchas de gastrópodes, as quais lhes servem de abrigo. Há cerca de 900 espécies descritas no mundo vivendo desde áreas marinhas profundas até o ambiente terrestre. Dentre os crustáceos são os únicos cujo abdômen é assimétrico e não esclerotizado, motivo pelo qual devem mantê-lo resguardado no interior das conchas. Por outro lado, estas conchas atuam como armaduras que restringem o seu crescimento forçando o ermitão a uma busca incessante por conchas maiores. Essas informações iniciais já indicam a complexa inter-relação existente entre ermitões, conchas e, num escala mais ampla, seu ambiente. Entretanto, a complexidade comportamental existente entre os ermitões não se restringe apenas às relações com as conchas. Eles possuem elaborados comportamentos utilizados durante a corte e cópula e durante encontros agonísticos. Como há inúmeras questões relativas aos ermitões, uma série de estudos, ora relacionados ora não, foram planejados para ampliar o conhecimento da história natural de algumas espécies (*Clibanarius antillensis, C. sclopetarius*, *C. vittatus* e *Pagurus criniticornis*) que são comuns no litoral sudeste brasileiro.

Dado o efeito limitante que as conchas impõem aos ermitões e a constante necessidade de obterem conchas novas e maiores para poderem crescer, estes animais procuram conchas ativamente. Entretanto, nem sempre há condições adequadas para que a busca desse ou de outros recursos ocorra, principalmente para as espécies que vivem nas áreas costeiras expostas ao regime das marés. Além disso, a luminosidade pode tornar os ermitões presas fáceis para predadores visualmente orientados como peixes e aves. Neste sentido, seria de se esperar que a atividade dos ermitões fosse regulada por variáveis ambientais tais como os ciclos de maré e de luminosidade. Sabe-se que ermitões terrestres restringem sua atividade ao período noturno, quando há um menor risco de desidratação. Outras espécies escondem-se em fendas ou sob pedras quando a maré está cheia ou baixa procurando evitar, respectivamente, serem predadas ou ressecadas pelo calor do sol. Entretanto, não se sabe até que ponto estes padrões comportamentais estão estabelecidos nas diferentes espécies como uma característica inata. Este foi o contexto a partir do qual o Capítulo 1 foi planejado, o qual visou descrever a atividade diária de quatro espécies de ermitões da região entremarés (*Clibanarius antillensis*, *C. sclopetarius*, *C. vittatus* e *Pagurus criniticornis*).

Além de procurar conchas, ermitões machos buscam ativamente parceiras para se reproduzir. A concha pode parecer um empecilho para a cópula uma vez que a fecundação interna é uma regra nos crustáceos. No entanto, esta dificuldade aparente é facilmente superada pois os ermitões, ao saírem parcialmente da concha, conseguem realizar com sucesso a transferência de gametas masculinos. Este simples procedimento muitas vezes é o final de uma longa e elaborada corte empreendida pelos machos para avaliar o grau de maturação das fêmeas e para demonstrar suas habilidades em manipulá-las e defendê-las frente a outros machos. Sabe-se que o comportamento reprodutivo pré e pós-copulatório é muito variável entre espécies e, à despeito das cerca de 50 espécies de ermitões presentes no litoral brasileiro, registros fragmentados do comportamento reprodutivo existem apenas para algumas delas baseados em observações de populações caribenhas. Por isso, o Capítulo 2 foi planejado de forma a fornecer descrições do comportamento reprodutivo de quatro espécies de ermitões ocorrentes no Brasil (*Clibanarius antillensis*, *C. sclopetarius*, *C. vittatus* e *Pagurus criniticornis*) com base em populações situadas no litoral norte do Estado de São Paulo. Durante observações do comportamento reprodutivo dos ermitões verificou-se que indivíduos intersexuados de três espécies (*Clibanarius antillensis*, *C. sclopetarius* e *C. vittatus*) com características de machos e fêmeas, realizaram cópulas bem sucedidas atuando como machos. Estes indivíduos foram mantidos em laboratório para verificar se ocorreriam alterações morfológicas, ou seja, se características de um sexo predominariam sobre as características do outro. Com base em informações morfológicas, comportamentais e populacionais foi elaborada uma discussão sobre uma eventual possibilidade de hermafroditismo sequencial protogínico nestas espécies de ermitão (Capítulo 3).

Partindo-se da análise do comportamento reprodutivo buscou-se levantar informações sobre o desenvolvimento embrionário destas espécies. Dentre as várias estratégias de vida possíveis para organismos com desenvolvimento larval planctônico, há os extremos em que as larvas permanecem muito tempo se alimentando no plâncton, as planctotróficas, e as que consomem reservas vitelínicas durante o curto período que passam na coluna de água, as lecitotróficas. Para uma compreensão dessas estratégias de vida é fundamental obter informações sobre o tamanho relativo de embriões, larvas e juvenis e sobre o tempo de desenvolvimento embrionário e larval. Embora o desenvolvimento larval e juvenil de ermitões seja bem conhecido, há poucas informações disponíveis sobre o desenvolvimento embrionário. Foi com o intuito de ampliar a quantidade de informações referentes ao desenvolvimento embrionário dos ermitões que o estudo apresentado no Capítulo 4 foi realizado. Nele há informações sobre seis espécies de ermitão (*Clibanarius antillensis*, *C. sclopetarius*, *C. vittatus*, *Pagurus criniticornis*, *P. brevidactylus* e *Paguristes tortugae*) muito comuns no litoral brasileiro. Espera-se que com esses dados e com futuros estudos com outras espécies haja a possibilidade de se descrever e compreender a variação na história de vida deste grupo.

Os Capítulos 5 e 6 enfocam um aspecto muito importante na vida dos ermitões: a seleção de tipos e tamanhos de concha. Como dito anteriormente, as conchas de gastrópodes são um recurso fundamental para esses animais, protegendo-os contra predadores e contra a desidratação provocada pela exposição prolongada ao sol durante as marés baixas. Entretanto, além destes benefícios, as conchas podem limitar o crescimento e a fecundidade dos ermitões e, quando em péssimas condições ou em tamanhos inadequados, reduzir sua capacidade protetora. Isto é mais comum do que se poderia imaginar uma vez que a quantidade de conchas vazias na natureza geralmente é muito baixa. Assim, o modo como os ermitões selecionam as conchas vem intrigando cientistas, principalmente a partir da década de 60. De fato, até hoje não se avaliou criteriosamente o efeito do tipo ou arquitetura das conchas nas relações entre o tamanho dos ermitões e as dimensões das conchas selecionadas. O Capítulo 5 apresenta os resultados de um estudo planejado para enfocar essa questão bem como outras informações importantes relativas à seleção de tamanho de conchas em ermitões.

Outra questão recorrente relacionada à seleção de conchas diz respeito ao efeito da experiência prévia nos padrões futuros de seleção de concha. Acredita-se que recrutas de ermitões tenham preferência não por determinada espécie de gastrópode mas sim por uma arquitetura típica. Isto é até esperado, uma vez que as espécies de concha disponíveis variam enormemente dentro da amplitude de distribuição geográfica das espécies de ermitão assim como entre diferentes ambientes geograficamente próximos. De fato, foi demonstrado que conchas com espira alta são preferidas por ermitões jovens que não tiveram qualquer experiência prévia. Também observou-se que os ermitões aprendem, acumulando conhecimento baseados em experiências prévias. Entretanto, este aprendizado só mostrou influência no tamanho e não no tipo de concha selecionada. Indivíduos mantidos em conchas inadequadas e relativamente pequenas tendem a selecionar conchas de uma determinada espécie de gastrópode proporcionalmente maiores que aqueles submetidos a um regime de conchas adequado aos seus tamanhos. Entretanto, alguns estudos sugeriram a possibilidade de que o uso continuado de um determinado tipo de concha pudesse aumentar a possibilidade dessas conchas serem selecionadas no futuro. Além do aprendizado, a hipótese do modelamento da forma do corpo pela concha foi sugerida como uma possível explicação para isto. De fato, as conchas podem afetar o crescimento e a forma dos ermitões, mas nenhum estudo testou adequadamente o efeito da experiência prévia nos padrões futuros de seleção por tipos de concha, muito menos a hipótese do modelamento. Os resultados referentes a esse tema estão apresentados e discutidos no Capítulo 6.

Como as conchas são consideradas um fator limitante para os ermitões, sua baixa disponibilidade pode levar indivíduos da mesma espécie ou mesmo de espécies diferentes a competir por elas. A obtenção de conchas por intermédio de disputas, as quais são altamente ritualizadas, é um dos meios pelo qual os ermitões podem conseguir conchas novas. Entretanto, há adaptações sensoriais que permitem aos ermitões localizar conchas vazias, guiados por moléculas de carbonato de cálcio emitidas pelas conchas ou orgânicas originárias da hemolinfa das presas (gastrópodes ou coespecíficos) ou das glândulas salivares dos predadores. Neste sentido, há ermitões que têm uma grande habilidade em perceber e localizar conchas antes destas serem utilizadas por outras espécies ou serem perdidas no ambiente, constituindo-se excelentes exploradores. Há outras espécies que conseguem novas conchas subjugando espécies competitivamente inferiores, interferindo diretamente no seu uso de conchas. Estas duas estratégias competitivas, exploração e interferência, respectivamente, parecem ser importantes na ordenação do uso das conchas pelos ermitões. O capítulo 7 reafirma que a coexistência de espécies de ermitão está associada à partilha de conchas (recursos) e à diferentes estratégias competitivas empregadas por elas. Além disso, são apresentados resultados que evidenciam a importância dessas diferentes estratégias competitivas na dinâmica de troca de conchas entre os ermitões.

Dentre os vários fatores que influenciam a vida dos ermitões, como a disponibilidade de conchas e alimento e a competição intra- e interespecífica, a predação também é altamente relevante. O suprimento de conchas novas para os ermitões depende da taxa de mortalidade natural dos gastrópodes, a qual é muitas vezes consequência da exposição desses a fatores físicos ambientais extremos. A predação sobre gastrópodes também pode levar a um aumento na disponibilidade de conchas. Entretanto, alguns caranguejos quebradores de conchas destroem totalmente os caramujos para consumi-los, inviabilizando a futura utilização dessas pelos ermitões. Dentre os vários organismos que consomem gastrópodes temos outros gastrópodes, caranguejos, siris, lagostas, peixes, polvos e aves, cada um com modos específicos para consumir as presas. Assim, o tipo de predador e de estratégia empregada por eles para consumir os caramujos irá determinar a quantidade e o tamanho de conchas disponíveis para os ermitões. Por outro lado, alguns desses predadores também consomem ermitões e acabam tendo uma influência negativa em suas densidades populacionais. Com base neste panorama, o estudo apresentado no Capítulo 8 procurou entender o papel que caranguejos quebradores de conchas têm sobre ermitões, realizando tanto amostragens na natureza quanto experimentos em laboratório. Algumas das conchas de gastrópodes mais utilizadas pelos ermitões nas áreas costeiras brasileiras foram comparadas quanto às defesas estruturais contra predação que podem oferecer aos seus ocupantes.

Capítulo 1

Daily activity of four tropical intertidal hermit crabs from South-eastern Brazil

Abstract

This study describes the daily activity under a simulated high tide situation of four species of hermit crabs (*Pagurus criniticornis*, *Clibanarius antillensis*, *C. sclopetarius* and *C. vittatus*) that coexist in an intertidal flat in South-eastern Brazil. Observations were done in two-hour intervals during two subsequent days (48h) in three replicate pools with thirty crabs each. There was an evident variation in the patterns of activity among species (between and within genera). Three patterns could be distinguished. The circadian patterns of activity of *C. antillensis* and *C. vittatus* could be characterized as evening and nocturnal, with resting peaks during the morning and afternoon. The circadian pattern of activity of *C. sclopetarius* was characterized by two marked peaks of inactivity, corresponding to dawn and evening, which could represent an intrinsic association to the semi-lunar tidal cycles of the study area. *Pagurus criniticornis* showed high activity during the entire observed period, which was not influenced by day/night conditions. This variation in the patterns of activity of the studied hermit crabs should be taken into account in the design of further experiments. More precise and accurate interspecific behavioral comparisons among species could be achieved in nocturnal experiments, the period of high activity of all species.

Keywords: Rhythms, activity patterns, behavior, *Clibanarius*, *Pagurus*, diogenidae, paguridae.

Introduction

Most crustacean species are subjected to cyclic light/dark environments associated to day and night periods. Diurnal and semi-diurnal tides are also periodic events that profoundly influence intertidal species. In fact, as DeCoursey (1983) and Webb (1983) pointed out, crustaceans have biological rhythms that match some of the cyclic variations of their environments. Behavioral periodicities may have tidal, diurnal, semi-lunar, lunar, and annual components although circadian and circatidal patterns of activity are specially common to crustaceans (DeCoursey 1983, Webb 1983).

Hermit crabs are very abundant in coastal areas (Reese 1969) and have elaborated reproductive and shell-related behaviors (see Hazlett 1981 for a review). In addition, they present behavioral rhythms that may be influenced by cyclic environmental conditions. *Pagurus longicarpus* (Rebach 1978, 1981), *Clibanarius vittatus* (Fotheringham 1975) and *Diogenes nitidimanus* (Asakura and Kikuchi 1984, Asakura 1987) may have a seasonal vertical migratory behavior associated to drastic variation in water temperature in intertidal and shallow subtidal areas through the year. Intertidal hermit crabs may have patterns of dispersal and clustering associated to the tidal cycle (Snyder-Conn 1980, Gherardi and Vannini 1989, Gherardi *et al.* 1989). They are generally inactive in clusters or in refuges during low and high tides and present high activity during flood and ebb tides when they disperse to foraging areas. Tides also dictate the frequency of tree climbing behavior in the terrestrial hermit crabs *Coenobita caviceps* and *C. rugosus* in mangroves (Barnes 1997a). Activity in the terrestrial hermit crab *Coenobita clypeatus* is nocturnal and is associated to lower temperature and higher air humidity at night (De Wilde 1973). Other terrestrial hermit crabs show nocturnal peaks of activity but also have low frequency of particular behaviors during daylight (Barnes 1997b). Ball (1968) showed that the activity patterns of the intertidal hermit crabs *Pagurus samuelis* and *P. granosimanus* follow the normal day-night conditions but are not persistent under constant illumination or darkness. The periods of activity of eleven Caribbean intertidal and subtidal hermit crabs were also examined under high tide conditions (Hazlett 1966) and revealed patterns that varied between subsequent days and among and within genera. Similarly, Mitchell (1973) recorded opposed patterns of activity for two coexisting populations of *Pagurus bernhardus* (daylight activity) and *P. prideauxi* (nocturnal activity). Underwater field observations using time-lapse cameras showed that *Paguristes oculatus* has a reduced mobility in the hours of darkness and is largely active during daylight (Stachowitsch 1979). Activity in hermit crabs is habitat related (Barnes 1997b), i.e., the frequency of active crabs varies according to the constraints imposed to them by different habitats. Thus, it is important to investigate if coexisting hermit crab species have intrinsic patterns of activity dictated by tides. In this way, it is important to evaluate if such individuals, even when submersed and then not exposed to the influence of tides, still have a circatidal and even a circadian component in their pattern of activity as shown for pagurid and diogenid hermit crabs by Hazlett (1966).

Four intertidal hermit crabs species (*Pagurus criniticornis*, *Clibanarius antillensis*, *C. sclopetarius*, and *C. vittatus*) coexist in the Araçá region, São Sebastião Channel, South-eastern Brazil. The patterns of activity of these species in nature are influenced by tides (Turra and Leite 2000, Turra *et al.* 2000). *Clibanarius sclopetarius* and *C. antillensis* generally remain in clusters (exposed or in refuges) during low tides. *Clibanarius vittatus* withdraw into its shell and remain exposed to the air, on or under cobbles, or bury itself in the mud, and may undergo both vertical and horizontal dispersion at flood tides (Turra *et al.* 2000). *Pagurus criniticornis* is less tolerant to emersion than the three coexisting *Clibanarius* species (Turra and Denadai 2001) and is active during low tides only in tide pools; otherwise the individuals of this species are buried in the muddy substrate. All these species may display some degree of activity in cloudy days or at nocturnal low tides (A. Turra unpublished data). This study aims to describe the daily activity under a simulated high tide situation for these four intertidal hermit crabs and was designed to test the hypothesis that these hermit crab species have intrinsic rhythms independent from tidal cycles.

Material and methods

Individuals of *Pagurus criniticornis*, *Clibanarius antillensis*, *C. sclopetarius*, and *C. vittatus* were collected in the tidal flat of the Araçá region, São Sebastião Channel, São Paulo State, Brazil (23°49'S, 45°24'W). Despite these species partitionate microhabitats in nature they may occupy all substrate types available, from mud to fragmented rocky shore (Turra *et al.* 2000). The experiments were conducted in the Centro de Biologia Marinha of Universidade de São Paulo (CEBIMar-USP). A total of ninety individuals of each species was observed in three plastic swimming pools (1.8 x 1.3 m) with circulating seawater, comprising three replicates with 30 individuals each. Despite this value of density was superior to that recorded in the field for the studied species (see Turra *et al.* 2000), the use of a smaller number of individuals in the experiment would cause inaccuracy in the estimates of mean frequency of active crabs. Moreover, the effect of crowding on the behavior of hermit crabs can be considered negligible after periods of adjustment (Hazlett 1968), justifying the three-day acclimatation period in tanks before the experiments. Experimentation with single crabs was not considered once isolation has a persistent positive effect on crab aggressiveness and a negative one on locomotory behavior

(Courchesne and Barlow 1971). The pools were located outdoors but in a shady place, under a tree with a wide canopy, to simulate natural illumination without the direct influence of the sun light. This caused a small fluctuation in the water temperature during the experiment (27.4 ± 0.7°C). The bottom of the pools was filled with sediment from the collection site.

The individuals were observed in 2 h intervals during uninterrupted 48 h and the most conspicuous behaviors were described and recorded. The nocturnal observations were done using a red light in order to avoid influence on crab behavior (Hazlett 1966). The patterns of activity were classified according to Hazlett (1966). The frequency of each behavior was calculated for each replicate (pool) and then averaged for each species through the time intervals.

Preliminary observations revealed that the most conspicuous behaviors displayed by the studied hermit crabs were: 1. Locomotory; 2. Reproductive; 3. Agonistic; 4. Feeding; 5. Stopped in the sediment surface; 6. Burying; 7. Retracted into the shell. The locomotory behavior was identified when the crabs were continually moving in the sediment surface using ambulatory legs and/or chelipeds. Reproductive and agonistic behaviors were observed and characterized following the descriptions of Hazlett (1966) for both diogenid and pagurid hermit crabs. Movements of the chelipeds toward the mouth region characterized feeding. Some hermit crabs were active but stopped in the sediment surface as in a 'stand by' manner without moving any appendix as also described for *Paguristes oculatus* by Stachowitsch (1979); they could undergo any of the above-mentioned behaviors as long as they wish, thus enabling the classification of such individuals as active. Burying was characterized by inactive crabs with their shells partially or totally covered by sand. Other inactive individuals were completely retracted into their shells so that pereopods could not be seen through shell aperture. Hypothesis concerning the relationship between particular behaviors were tested using the Pearson correlation analysis (Zar 1999).

Results

Clibanarius antillensis was frequently recorded walking or stopped in the sediment surface. Locomotory behavior was predominantly nocturnal and showed a positive relationship with feeding (Pearson correlation: n=24, r=0.42; p=0.039), while 'stand by' did not show any clear circadian pattern (Fig. 1). Some of the individuals of *C. antillensis* were completely retracted into their shells, with shell apertures positioned either up or downward. Burying was rare in this species and was generally associated with clusters of inactive individuals. Agonistic and reproductive behaviors were seldom recorded. The pattern of activity of *C. antillensis* could be characterized as evening and nocturnal, with subtle resting periods during the morning and afternoon (Fig. 1). This pattern was less evident in the second day of observation. No relationship could be established between the activity pattern of this species with the tidal cycle during the observation period.

The most conspicuous behaviors presented by *Clibanarius sclopetarius* and *C. vittatus* were: burying, locomotory and feeding (Fig. 1). Peaks of feeding were positively associated with peaks of locomotion for *C. sclopetarius* (n=24; r=0.52, p=0.009) and negatively for *C. vittatus* (n=24; r=-0.46; p=0.024). This means that feeding in *C. vittatus* is independent from the frequency of locomotion, i.e., the crabs are displacing for other reasons than foraging. Peaks of activity of these two species were alternated with peaks of inactivity evidenced by relatively high frequency of buried individuals. Clusters of inactive individuals were also recorded for these two species (mainly for *C. sclopetarius*). Few reproductive and agonistic behaviors were recorded as well as individuals completely retracted into their shells. The pattern of activity of *C. vittatus* could be characterized as evening and nocturnal, with resting peaks during the morning (Fig. 1). The pattern of activity of *C. sclopetarius* presented two marked peaks of inactivity in a period of 24h, corresponding to dawn and evening. High activity of this species was recorded both in the middle of the day and of the night and was associated with flooding tides. The relationship between activity and tidal cycles was not apparent for *C. vittatus*.

High activity was recorded for *Pagurus criniticornis* during the whole 24h period without evident resting times (Fig. 1). This continuous activity pattern of *P. criniticornis* was associated to high frequency of feeding (Fig. 1). This species differs from the others because feeding events were interspersed by short-distance displacements. *Pagurus criniticornis* used the ambulatory legs to move over the substrate and the chelipeds to pick particles into the mouth while stopping briefly. The other species clearly stopped in the sediment surface for longer periods than *P. criniticornis* while manipulating the food, which characterizes a different foraging strategy. The individuals of *P. criniticornis* displaying locomotory behavior, such as recorded for the other studied species, were only those that were displacing for longer distances without stopping for feeding. This particularity caused a non-significant relationship between feeding and locomotory behavior in *P. criniticornis* (n=24; r=0.10; p=0.653). Clusters of inactive individuals as well as buried crabs were seldom recorded during the experiment. Reproductive displays and agonistic encounters were also rarely observed in this species.

Discussion

The four studied hermit crabs showed consistent patterns of activity in the two days of observation. However, there was an evident variation in the activity among these species and three distinct patterns could be distinguished. The results evidenced that the three species of *Clibanarius* have circadian rhythms of activity during a high tide simulated situation while *P. criniticornis* does not. There were no evidences of intrinsic circatidal rhythm for these species, although the bimodal semidiurnal pattern of activity of *C. sclopetarius* could support this assumption once the tides are also semidiurnal in the study area. In fact, there was a relationship between the periods of high activity of *C. sclopetarius* with the periods of flooding tide in São Sebastião Channel during the experiment as also recorded for *C. digueti* (Snyder-Conn 1980) and *C. laevimanus* (Gherardi and Vannini 1993) in nature.

As exposed above, patterns of activity may vary markedly between and within genera. Such variation was also verified by Hazlett (1966) for five genera of Caribbean hermit crabs. Hazlett (1966) also recorded the patterns of activity of *C. antillensis* and *C. cubensis* (synonym of *C. sclopetarius*, see Forest and Saint Laurent 1967). In this study he demonstrated that *C. antillensis* have high activity at night and a peak of inactivity in the middle of the day. These results are very similar to those presented for this species in the present study. However, the description of the periods of activity of *C. sclopetarius* made by Hazlett evidenced an evening peak of feeding and a nocturnal pattern of aggressive behavior. This is quite different from the bimodal semidiurnal activity/resting peaks presented by the population studied here. These results may indicate a geographical variation in the pattern of activity for some species, although the comparisons with the findings of Hazlett (1966) are not conclusive because the activity pattern was not evaluated from 0 to 6 h in his study.

The continuous activity of *P. criniticornis* was very different from the highly variable patterns of activity recorded for other pagurid species by Hazlett (1966): *P. miamensis* (night peak), *P. pygmaeus* (night peak), *P. bonairensis* (morning peak) and *P. marshi* (morning peak). In addition, *Pagurus samuelis* and *P. granosimanus* showed a marked nocturnal pattern of activity when submitted to normal day-night photoperiod without controlling the temperature

(Ball 1968). As the temperature was well correlated to variation in illumination it becomes difficult to separate the effects of temperature from those associated to a circadian day-night variation in field and outdoor experiments. However, the shady conditions employed in the present study prevented the direct access of the sun rays and, as a consequence, maintained the water temperature constant in the experimental pools. Excepting *Pagurus criniticornis*, continuous patterns of activity were only recorded for *P. samuelis* and *P. granosimanus* under constant temperature and under both constant illumination or darkness conditions (Ball 1968), which also indicate the absence of an intrinsic circadian rhythm in these species. In addition, *Pagurus bernhardus* was more active during day while the coexisting *P. prideauxi* presented higher activity at night (Mitchell 1973), while *Paguristes oculatus* showed a diurnal activity pattern (Stachowitsch 1979).

Each hermit crab species may be characterized by a particular behavioral pattern. In this way, the pattern of activity of *C. sclopetarius* and *C. vittatus* may be evidenced not only by the frequency of active individuals but also by the frequency of buried crabs. The burying behavior was characteristic of these two species although some individuals of *C. antillensis* and *P. criniticornis* also buried themselves in the sediment. The low frequency of burying in *C. antillensis* may be associated to the fact that this species is typical from consolidated substrates. In contrast, the low frequency of burying in *P. criniticornis* does not support field observations during low tides, when almost all individuals of this species are buried in mud (A. Turra unpublished data). This is evidence that burying in this species may be a behavioral response to a tidal cycle and not to a resting behavior governed by a daily light/dark variation. Inactive individuals completely retracted into the shells may also characterize the resting time of *C. antillensis*. As this behavior was previously recorded in the field for individuals of this species during low tides (Turra and Leite 2000, Turra *et al.* 2000), it seems to follow a circadian rhythm, although a circatidal rhythm may be also involved.

The three species of *Clibanarius* formed clusters of inactive individuals. Clustering was not recorded for *Pagurus criniticornis*. These results corroborate field observations on the patterns of dispersion and clustering of these four hermit crab species in the Araçá region (Turra *et al.* 2000). Clustering behavior in hermit crabs is well documented in the literature (Snyder-Conn 1980, Gherardi and Vannini 1993, Turra and Leite 2000, Barnes and Arnold 2001) and is associated to the risks of predation and desiccation during the periods of high and low tides, respectively. As clustering (or resting) occurred periodically under a constant high tide situation, it is supposed that the occurrence this behavior is independent from tidal cycles. This rationale can be also applied to other behaviors such as burying and retraction into the shells. Once these behaviors are associated to crab inactivity and are influenced by tides (as exposed above), it becomes evident the higher frequency of these behaviors during low tides in nature. Although clustering has been supposed to reduce their risks of desiccation (Snyder-Conn 1980, Turra and Leite 2000) and that clusters may have other ultimate causes (Gherardi and Vannini 1993), the present data suggest that clustering is not a direct response of hermit crabs to emersion. Although the occurrence of buried individuals and individuals retracted into the shells was not a direct consequence of tidal cycles, these behaviors may enhance survivorship at low tides as pointed out by Reese (1969) and Taylor (1981).

Despite the experiments were conducted in the reproductive period of the studied species (Turra and Leite 2000), reproductive and agonistic behaviors were recorded in low frequencies for all species. This prevented the identification of any pattern for their variation. Hazlett (1966) verified that agonistic/aggressive behavior is positively related to the frequency of locomotory behavior for some species. Hazlett (1968) also demonstrated the effect of crowding in the aggressive behavior of *Pagurus bernhardus*, although aggressiveness may be markedly reduced in some few days when crabs are adjusted to the new density levels (Grant and Ulmer 1974). Thus, the lower density of individuals in the experimental pools and the higher acclimatizing period in the present study in relation to that used by Hazlett (1966) may be responsible for the low frequency of aggressive behaviors in the present study. However, the apparent increase in the frequency of active crabs from the first to the second day of observation (see Fig. 1) may suggest that the crabs are still adapting to the experimental density even after three days of acclimatation. This does not invalidate the results once the activity pattern of the studied species were generally consistent during the two subsequent days of observation. Finally, as the experimental density of the studied species was higher than in nature (Turra *et al.* 2000), one would expect a low level of agonistic encounters in the field for these species. In the same way, experimental densities may have lead to few encounters and, consequently, to low frequency of mating during the observations.

The variation in the patterns of activity of the studied hermit crabs should be taken into account in the design of further experiments. The duration of experiments with hermit crabs, such as those focusing shell selection, may vary among studies from 12 h (Grant and Ulmer 1974) to 24 h or more (Abrams 1978, Turra and Leite 2002). Most hermit crab species have peaks of activity/inactivity during a 24 h period, i.e., they exhibit circadian rhythms of activity (Hazlett 1966, present study). In this way, it is recommended that experiments that require active crabs and, especially those, in which the data are obtained only at the end of the trials, such as shell selection, should be done in at least a 24 h period. Experiments with a minimum duration of 24h certainly will have active individuals of all the studied species. However, since *P. criniticornis* and *C. antillensis* have a relatively higher activity than the other two species (see Fig. 1), behavioral interspecific comparisons that do not take this variation into account may be misleading. Observations during shorter or more restricted periods of time should be made in the periods of high activity of all species. In this way, more precise and accurate interspecific behavioral comparisons among these species could be achieved in nocturnal experiments. Species like *P. criniticornis*, which have high activity during a 24 h period, are more adequate for behavioral studies or as educational tools once observations can be made over the entire day.

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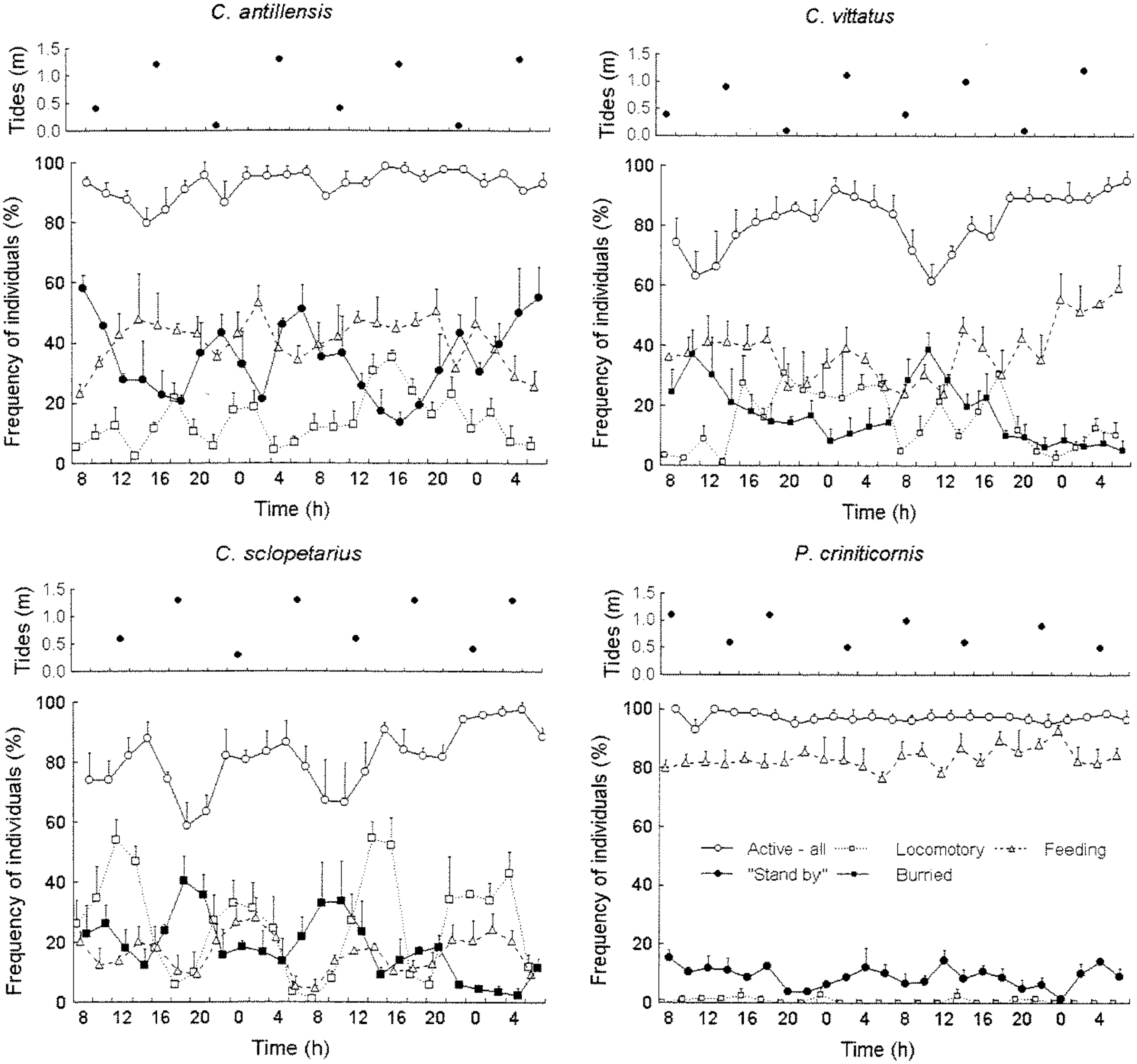

Fig. 1. Behavioral patterns displayed by the hermit crabs *Clibanarius antillensis*, *C. sclopetarius*, *C. vittatus* and *Pagurus criniticornis* during a 48-hour period. Points are the mean percentage of individuals (+SE) displaying each of the most conspicuous behaviors based on the 3 replicates (pools). Frequency of active individuals are the pooled frequencies of crabs displaying the following behaviors: locomotory, feeding, reproductive, agonistic and 'stand by' - stopped in the sediment surface. The height of high and low tides during the observation period is shown for each species. The periods of the days were characterized as follows: Morning – 06:00 to 12:00h; Afternoon – 12:00 to 19:00h; Evening – 19:00 to 20:00h; Night (nocturnal) – 20:00 to 05:00h; Dawn – 05:00 to 06:00h; Available daylight: ±14h.

Capítulo 2

Reproductive behavior of intertidal hermit crabs in South-eastern Brazil

Abstract

The reproductive behavior of four intertidal hermit crab species (*C. antillensis*, *C. sclopetarius*, *C. vittatus* and *Pagurus criniticornis*) was studied in São Sebastião Channel, South-eastern Brazil. The overall behavior followed previous descriptions for diogenid and pagurid hermit crabs but some particular differences were recorded among them. Agonistic encounters for females and guarding behavior were more common to *C. sclopetarius*, *C. vittatus* and *Pagurus criniticornis*. During guarding, males clearly grasped female shell aperture and female chelipeds and eyestalks. This oblique guarding/accessing behavior (OGAP) has never been described for hermit crabs and was more common to *C. sclopetarius* and *C. vittatus*. *Pagurus criniticornis* did not present the typical jerking behavior of pagurids. Multiple copulations within a single pair were recorded in all species while in only one occasion a female of *C. vittatus* was observed copulating with two males. In the post-copulatory behavior females were held by males for some time and, after that, they remained inactive and sometimes were left totally or partially buried in the substrate. In mating pairs of all species males were larger than females and two individuals (out of 23) of *C. antillensis*, one (out of 5) of *C. sclopetarius* and one (out of 3) of *C. vittatus*, which courted successfully as males, were demonstrated to be intersex, with gonopores in both third and fifth pair of pereopods.

Keywords: Hermit crabs, *Clibanarius*, *Pagurus*, reproductive behavior, courtship, guarding, intersex.

Introduction

Hermit crabs have a very complex behavioral repertoire related or not with their characteristic dependence on shells. They present elaborated shell investigation behaviors in order to access shell quality (see Elwood and Neil 1992 for a review) and employ agonistic displays that are characteristic to both shell and reproductive fights (Hazlett 1966). Finally, hermit crabs present mating behaviors with which males locate females and court and copulate with them.

Hazlett (1966) did pioneer observations on the behavior of hermit crabs and revealed that agonistic and reproductive displays were a family-related characteristic although reproductive behaviors presented higher variability among species of the same family. In general, the most common behavior of diogenid hermit crabs is rotating/shaking the female, while male jerking the female toward him is frequent in pagurids (Hazlett 1966). More information on the reproductive behavior became available along the years (Hazlett 1968, 1972, 1975, 1986, 1996a, 1996b, De Wilde 1973, Helfman 1977, Asakura 1987, Harvey 1990, Goshima *et al.* 1998), elucidating many questions related to the complex life-style of hermit crabs. Questions like, how do the crabs copulate even using a shell, how long fecundation lasts, how many times a single pair copulate and how many males can copulate with a single female could be answered with the findings of these studies.

Behavioral studies on Brazilian hermit crabs are recent (Turra and Leite 2000a, Turra and Denadai 2002, Chapter 1). The few information on the reproductive behavior of species found in Brazilian coast is presented in the studies of Hazlett (1966, 1972, 1996a, b) on Caribbean and Southern USA hermit crab populations. In these studies, this author observed only one successful mating of *C. antillensis* and partially described the sexual behavior of *C. sclopetarius* (synonym of *C. cubensis* – Forest and Saint Laurent 1967). The reproductive behavior of *C. vittatus* was described in detail only recently (Hazlett 1996b) while that of *Pagurus criniticornis* has never been observed. The present study describes in detail the pre and post-copulatory behavioral patterns of populations of these four species in South-eastern Brazil and compares them with previous descriptions.

Material and methods

The studied species are Atlantic intertidal and shallow subtidal hermit crabs (Forest and Saint Laurent 1967) that coexist in the intertidal region of Pernambuco Islet and adjacent mud flat (23°49'S, 45°24'W) in the mainland part of the São Sebastião Channel, northern coast of São Paulo State, South-eastern Brazil (Turra *et al.* 2000). A large amount of individuals (>200) of each species was collected in this area and maintained in the laboratory in plastic pools with running seawater. The bottom of the pools was filled with sediment from the collection site and the crabs were fed daily with fresh fish or mussel meat.

A total of 50h of observations on the reproductive behavior were carried out from March to April 2001 and were homogeneously distributed among species and periods of the day (morning, afternoon and night). The pools were observed systematically in these periods until a courting pair was identified. Attention was then paid only to the pair and all movements and displays, as well as the time they lasted, were recorded. The behavioral patterns of the crabs were observed and described according to Hazlett (1966, 1972, 1996a, b). For schemes of the most representative behaviors see Hazlett (1966). Observations ceased when the pair separated without copulating. Nocturnal observations were carried out using red lights to prevent alterations in crab behavior (Hazlett 1966). After copulation, males and females were removed from their shells and measured (shield length, mm) to establish a proportionality ratio between their sizes. Observation on both successful and unsuccessful courtships were used to describe the reproductive behavior of the studied species. A total of 23 complete successful courtships were recorded for *C. antillensis*, 5 for *C. sclopetarius*, 3 for *C. vittatus* and 7 for *P. criniticornis*.

Results and Discussion

Clibanarius antillensis

There were two possible outcomes when a male of *C. antillensis* accessed a female: (1) the female could withdraw into her shell and remain inactive or (2) the female escaped. When the female tried to escape, the male could hold her shell and, sometimes, exhibited a guarding/accessing behavior. This behavior was more commonly shown by *C. sclopetarius* and *C. vittatus* than by *C. antillensis* and is described in detail below. One pair of *C. antillensis* was reported in this guarding position until the female molted. Non-interested females could try to escape when males released them briefly during male-male agonistic encounters. However, fights among males for females were less common to *C. antillensis* than to *C. sclopetarius* and *C. vittatus*. When a male was well succeeded in courtship (or after a female have molted), he manipulated/rotated her shell until their shell openings were positioned in front of each other, i.e., in the opposed position according to Hazlett (1966). Then the male held the female shell with his ambulatory legs (second and third pairs of pereopods), while chelipeds remained inside or in contact with the opening of her shell. Some females had molted recently as evidenced by the exuvia or by their soft exoskeleton, while other courted females presented hardened exoskeleton and no exuvia. This suggests that copulation may be or not associated to female molting in this species. Males were generally larger than females in the observed mates (mean size ratio: 37%, range: 1 to 100%). In two successful courtships the "males" presented gonopores in both the third and fifth pair of pereopods, thus evidencing an intersex condition (Turra and Leite 2000b). Males that were extremely larger than the female could lift her up entirely from the substrate and/or hold her shell almost completely inside the opening of his relatively larger shell. The pairs could remain in this position for hours as well as more than one day.

After that, males presented some behavioral displays that could alternate with each other. They did frequent up and down cheliped tapping movements to grasp/pick female chelipeds or the internal lip of her shell. One or both chelipeds could be used in such display, which was characterized by the chelae staying in a right angle with carpus. The first ambulatory legs of males could also be used to grasp female chelipeds or shells in this pre-copulatory behavior. Cheliped tapping was alternated with closure of female shell aperture by male chelipeds (one or both). Also alternating with cheliped grasping and tapping, male *C. antillensis* presented gentle and violent rocking movements as previously described for this species and for *C. tricolor* by Hazlett (1966). Gentle rocking was alternated with cheliped tapping while violent rocking was recorded as a response of males to a quick retraction of the female into the shell. During violent rocking the male could also tap both internal and external lip of female shell aperture. The frequency of violent rocking was enhanced as copulation approached. In some cases, these behaviors were also alternated with female shell rotation by males.

Cheliped and antennular movements of female near the eyestalk/antennular portion of male signaled him that she was ready for copulation. Then, male and female eased out from their shells and copulated for a few seconds (2 to 3 seconds). During copulation, ambulatory legs intermingled each other and male (on the basis of the fifth pair of pereopods) and female gonopores (on the basis of the third pair of pereopods) touched each other to complete spermatophore transfer. As a consequence, males became more exposed than female. Two or more subsequent copulations were reported in one pair, alternated with up to 17 attempts. In each trial the female eased out from and retracted abruptly into her shell. Male violent rocking followed each female retraction.

Few post-copulatory behaviors were observed in *C. antillensis*. After copulation, males could grasp the aperture of female shells up to 2 to 3 hours. Then, males pushed females away and could exhibit some aggressive behavior (in general cheliped extensions) toward them. After that, males frequently walked away while females remained inactive.

Females which were removed from their shells right after they had been released by the males had eggs in pleopods and hardened exoskeleton. This demonstrates that males guard females until the latter have externalized the eggs as suggested by Hazlett (1966). One female recently released by a male was removed from her shell and immediately began to actively remove the eggs (not rounded yet) from the pleopods with her chela.

Hazlett (1966) observed only one complete courtship of *C. antillensis* and suggested it was very similar to that described for *C. tricolor*. The detailed observation of the reproductive behavior of *C. antillensis* in the present study confirms that it occurred exactly as described for *C. tricolor* (Hazlett 1966). More than one copulation with a single male could occur in *C. antillensis* while copulation with more than one male, which had been recorded for *C. tricolor* (Hazlett 1966), was not reported in this species.

Clibanarius sclopetarius

Males of *C. sclopetarius* generally located ripe females during occasional encounters while others were able to track them at distance. Males were also capable of locating buried or partially buried females while others were attracted to courting pairs. When males located females they could court females in two ways, which could alternate with each other. First, by turning the female shell into the opposed position and doing up and down cheliped tapping movements similar to those of *C. antillensis*. Males could also grasp/pick female shell aperture. Second, males could grasp/pick females during guarding displays. In general, the male turned the female shell down with the aperture facing the bottom and partially covered her shell with his two left ambulatories (Fig. 1). Their shells stayed in an oblique position with almost a right angle between shell aperture planes. The left cheliped of male was generally laid on the external lip of female shell aperture. The right cheliped (and less frequently first and second right ambulatory legs) was used to grasp/pick the external lip of the female shell and female chelipeds and eyestalks. Grasping/picking was more conspicuous when females tried to escape from males. Females could also extend their chelipeds and grasp male chelipeds. The relative position of the mating pair was very similar to the guarding behavior described for *C. vittatus* by Hazlett (1996b), although males grasping females were not reported in this previous study. This behavior was named oblique guarding/accessing position (OGAP, see Fig. 1 for scheme). When competing males approached the courting pair the defending male immediately started to guard female in OGAP and also displayed agonistic behaviors toward attacking males. Alternatingly, defending males constantly grasped/picked female as exposed above.

The way grasping occurred during OGAP seemed to be related to the shell species used by males. When using elongated medium spire shells, such as those of *Stramonita*, *Chicoreus* and *Cymatium*, males remained in an erect position in relation to the substrate, i.e., with ventral part parallel to the bottom surface. In contrast, when living in globose low spire shells, such as those of *Astraea*, males remained in an oblique position in relation to substrate, with their left side laying laterally on the sediment surface. These crabs could not hold up themselves with their ambulatories when in OGAP. The shell external lip held up the animal so that males could use their free left ambulatory legs to grasp/pick females. It was not evaluated in the present study if individuals of *C. sclopetarius* in elongated shells were in disadvantage in relation to those in globose ones. However, the effect of poorly adequate shell types or sizes on the reproductive success of males was clearly demonstrated for *Diogenes avarus* and *Clibanarius virescens* (Hazlett 1996a) and for *C. vittatus* (Hazlett 1996b).

Male agonistic behaviors to defend females were very similar to those described for diogenid hermit crabs by Hazlett (1966). Competing/attacking males generally approached the courting pair abruptly. The defending males tried to push them away by ambulatory raises and cheliped extensions. Attacking males were generally caused to roll. In more extreme fights the defending male displayed ambulatory pokes toward the eyestalks of the attacking males. Cheliped presentation was not recorded in such fights.

The accessing behaviors of males (opposed position and OGAP) could last from few hours to more than one day prior to copulation. They were alternated with each other and with female shell rotation as described for *C. vittatus* by Hazlett (1996b). After that, the pair could separate, the defending male could be displaced by attacking males or the defending male could win the contest and turn definitively the female into opposed position. Unsuccessful mates were recorded for *C. sclopetarius* and were associated to the inability of males in courting and defending females.

Before copulation, males could rock females violently. Then, females started to touch male on chelipeds, eyestalks and antennules with their own antennules, indicating they were ready for copulation. Then, the pair eased out from their shells and intermingled their ambulatories. The male put his gonopores in contact with the female gonopores during 5 to 10 seconds and then they returned abruptly to their shells. After copulation, females did in and out movements in the shell without touching shell aperture (10 to 15 times during 1 to 2 minutes). This behavior was also reported in other species as *Pagurus miamensis* (Hazlett 1966, synonym of *P. brevidactylus*) and both *P. criniticornis* and *C. vittatus* in the present study (see below). Then, male generally grasped female shell aperture violently and returned to OGAP. More than one copulation within a pair were common in this species and could be alternated with males moving females in opposed position or OGAP. In general, males were larger than females (mean size ratio: 42%, range: 28 to 59%). After copulation, males then alternated guarding (*sensu* Hazlett 1996b) and OGAP while females generally tried to be released. Males could grasp female chelipeds and shell lip and siphonal canal when in OGAP. Then, males could help females to burrow into the sediment with their ambulatory legs, thus leaving females inactive and partially or completely buried. Females also used their ambulatories to cover their shells with sediment. This post-copulatory behavior had never been described for hermit crabs (but see *C. vittatus* below) and could last up to one hour. Females inspected after pair separation presented egg masses in early developmental stages (not cleaved) attached to their pleopods.

There were no evidences of female molting prior to copulation and none of the successful mated females had soft exoskeleton. One individual that successfully copulated with a female was classified as intersex as it presented gonopores in both third and fifth pair of pereopods.

Clibanarius vittatus

The pre-copulatory behavior of *C. vittatus* was very similar to that described for *C. sclopetarius* (above) and for another population of *C. vittatus* (Hazlett 1996b). Males of *C. vittatus* courted females either in the opposed position or in the OGAP (Fig. 1). Cheliped tapping was evident when in opposed position with males grasping/picking female shell rim or female chelipeds, ambulatories and/or eyestalks. While alternating between these two behaviors (positions), males could rotate female shells. Sometimes females stopped in the opposed position but with their ventral part facing upwards. Courtship could stop due to female rejection by the male or during fights. Agonistic interactions among males were very common and frequently dominated by the largest individuals. When attacked by other males, the defending male sometimes interrupted the guarding of the female, which was able to go away.

Violent rocking movements (as recorded for *C. antillensis*) could occur when the pair was in the opposed position. This seemed to be a response of males to females that were trying to be released. Such rocking movements were alternated with cheliped tapping. In some situations, males were observed holding females in the opposed position while pushing them away with cheliped extensions. During cheliped tapping, males were also seen picking/grasping female chelipeds, ambulatories and eyestalks.

Females signaled to males they were ready for copulation by touching male in the eyestalk region with their antennules. Multiple copulations within a single pair were common in this species and each attempt lasted up to nineteen seconds. The pair could remain in opposed position or OGAP between subsequent copulations. In one case, copulation occurred four times (4 to 10 sec.) within a seventeen-minute interval. In another case, a male copulated with the female and, right after loosing it to another male, the female also copulated with the attacking male. After copulation both male and female returned rapidly to their shells. Females could exhibit constant and fast in and out movements (4 to 5 times) in the shell aperture similarly to *C. sclopetarius*. Post-copulatory behavior was also very similar to that shown by *C. sclopetarius*. The pair could alternate between guarding (*sensu* Hazlett 1996b) and OGAP and the male could help the female to bury. When the pair separated, females were generally left inactive and partially buried in the sediment. Females inspected after pair separation presented egg masses (not cleaved) attached to their pleopods.

Courtship was not reported to be associated to female molting as no exuvia were found and courted females had hardened exoskeleton. Males were always larger than females in pairs that copulated successfully (mean size ratio: 16%, range: 9 to 22%). One intersex individual was recorded successfully copulating with a female as in *C. antillensis* and *C. sclopetarius*.

Pagurus criniticornis

The reproductive behavior in *P. criniticornis* partially followed the pagurid pattern described by Hazlett (1966) and differed substantially from the *Clibanarius* species described above. Right after males had approached females they rotated female shells until they were in the opposed position (similarly to *Clibanarius* species). Then the male grasped/picked the female chelipeds and ambulatories with his chelipeds and, less frequently, ambulatory legs. Prior to copulation, the male could hold the female with his minor or major chelae in the shell aperture lip or in the left ambulatory legs. The pair remained frequently in an oblique position (similar to OGAP of *Clibanarius*, Fig. 1). Males frequently dragged female about over the bottom even when no competing males were present. The characteristic pagurid jerking behavior (for description and scheme see Hazlett 1966) was not recorded in this species. When stopped, females could grasp/pick male chelipeds with both their chelipeds and ambulatory legs. Males could also grasp female chelipeds, ambulatories and eyestalks with their major chelae. In addition, males could rotate female shells counter-clockwise before copulation. In one situation, a male was reported helping the female to molt by picking and pulling exuvia as previously described for *C. tricolor* by Hazlett (1966). However, molting was not seen in all pairs and most courted females had hardened exoskeleton. Males of successful courting pairs were always larger than females as in the other studied species (mean size ratio: 25%, range: 14 to 35%). Agonistic interactions among males were recorded and the defending males generally used their right large cheliped to defend the females.

Copulation could occur up to four times within a single pair and could last from 10 to 36 seconds. Copulation time showed to be longer than in the *Clibanarius* species. Prior to copulation, males rested their chela inside female shell aperture. Females signaled to males they were ready for copulation by touching male eyestalk region with their antennules. Then the pair eased out from their shells and put gonopores in contact. In two cases, the female was using a shell with a large hole in the body whorl, through which she eased out and copulated. After copulations females could retract abruptly into their shells and males grasped them violently in shell aperture and chelipeds. Females also did in and out movements in the shell aperture during few seconds. After that, the pair generally remained at the shell aperture. Sometimes males introduced their chelipeds inside the aperture of female shells and grasped their chelipeds. Counter-clockwise rotations were also recorded between subsequent copulations.

In the post-copulatory behavior, males could also rotate female shells and pull them by holding their shells with the minor or major chelae. Then, the pair alternated between opposed position and OGAP, always with simultaneous grasping movements. In one case, the male was observed using his large cheliped to bury the female. Males could also release females with the aperture of their shells turned upwards.

General comments

In this study, males were always larger than females in courting pairs although females could be slightly larger than males as demonstrated for *C. tricolor* by Hazlett (1966). The proportionality between the sizes of males and females may vary considerably but it reflects the dimorphism in the size structure in hermit crab populations (Turra and Leite 2000b). The size dimorphism in courting pairs and in populations was probably selected by the advantages large males have over the smallest ones in copulating with females (Harvey 1990).

Intersexuality in the three studied *Clibanarius* species was first reported by Turra and Leite (2000b) and nothing is known about the proximate and ultimate factors related to intersexuality in hermit crabs. However, intersex individuals certainly are able to successfully copulate females. The frequency of intersex males copulating females was higher than the frequency of intersex in the three studied *Clibanarius* species in nature (see Turra and Leite 2000b). This may indicate that intersex males are more aggressive or that they are more efficient in locating and defending ripe females than "typical" males.

Goshima *et al.* (1998) showed that males of *Pagurus filholi* are not able to access neither ripeness nor fecundity of females that are not ready for copulation and probably court and mate with the first ripe female they encounter. In addition these authors showed that males may recognize females which are within about 5 days from spawning. Such information is not available for the studied hermit crab species but the observations showed that a pair may be formed more than 24 hours before copulation. However, once a given female is being courted it may give off reproductive pheromones, as in *C. vittatus* (Hazlett 1996a), that stimulate male reproductive activity. In this way, males may benefit from pairing early with females but pheromone release by females may enhance the chances of agonistic encounters with competing males. This may explain the high frequency of agonistic encounters recorded for at least *C. sclopetarius* and *C. vittatus*. The oblique guarding/accessing position (OGAP) recorded in the studied species (except for *C. antillensis*) may represent an adaptation to deal with competitors once the defending males still courted the females while defending them from up to five attacking males. As Ridley (1983) pointed out, pre-copulatory-guarding behavior is highly correlated with a short receptive interval in females. Once females are thought to be receptive after molting or spawning (Hazlett 1966) guarding seems to be a successful strategy as a pre-copulatory behavior in hermit crabs.

Unsuccessful mating occurred frequently in both *C. sclopetarius* and *C. vittatus*. According to Hazlett (1966, 1996b), the reasons courtships finish are: (1) males go away; (2) females go away; (3) females dropped (male inability in holding female shell); (4) male-male fights. In this last situation, defending males may loose females to attacking males or females may go away because they were not guarded adequately. Despite male-male contests were frequent, the winner could go away without fecundating the female. It probably occurred because the former male paired with a female whose physiological status was not close to ripeness.

The comparison of the reproductive behavior between the present study and previous descriptions for other locations reveal that the general behavioral pattern is very similar among populations. However, rotation in *C. sclopetarius* and *C. vittatus* was not as conspicuous as described in the typical diogenid pattern (Hazlett 1966, 1996a). This behavior was frequently observed only in *C. antillensis*. On the other hand, previous descriptions for *C. sclopetarius* and *C. vittatus* did not record the evident pre and post-copulatory oblique guarding/accessing behavior (OGAP) shown in the present study. Female in and out movements in shell aperture after copulation were also not recorded in previous descriptions for the studied species (Hazlett 1966, 1996a). In addition, the post-copulatory behavior of *C. sclopetarius*, *C. vittatus* and even *P. criniticornis* showed that female could be left inactive and partially or totally buried after being released by the male.

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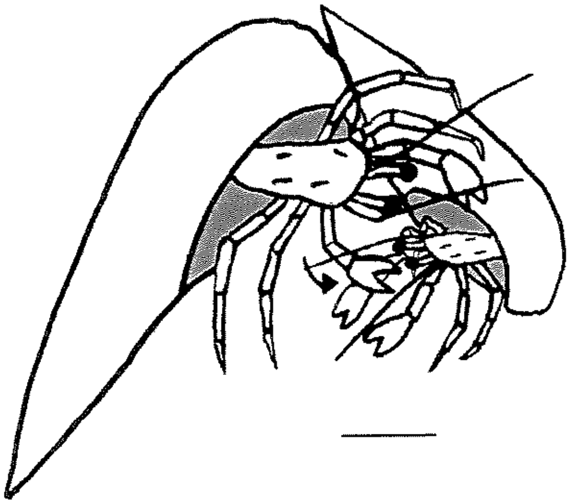

Fig. 1. Scheme of the opposite guarding accessing position (OGAP) presented during both pre and postcopulatory behavior by *C. sclopetarius*, *C. vittatus* and *P. criniticornis*. Note that male (largest individuals) grasp/pick female (smallest individual) with his chelipeds while holding female shell. (Scale line 5 mm)

Capítulo 3

Intersexuality in hermit crabs: Is it part of a protogynic hermaphroditism mechanism?

Abstract

This study addresses the function of intersex individuals in three intertidal hermit crab species (*Clibanarius antillensis*, *C. sclopetarius* and *C. vittatus*) and provides a description of the morphology of the gonopores of laboratory-reared intersex individuals before and after molting. Observations on the reproductive behavior revealed that intersex individuals of these hermit crab species copulated successfully as males. Intersex individuals showed morphological alterations indicating closure of the female gonopores after molting. These findings associated to information on behavior and population biology of hermit crabs suggest that hermit crab intersexuality may be part of a protogynic hermaphroditism. Because only protandric hermaphroditism has been recorded in decapods, this contribution highlights the importance of further studies in understanding the proximate and ultimate factors related to intersexuality in hermit crabs.

Keywords: Hermit crabs, *Clibanarius*, Diogenidae, intersex, reproduction, sequential hermaphroditism.

Introduction

Decapod crustaceans are typically gonochoristic organisms with a clear sexual dimorphism. Among the various external secondary sexual characters the gonopores are prominent on the basis of the third pair of pereopods in females and on the basis of the fifth pair in males. However, some individuals may have gonopores in both third and fifth pairs of pereopods and are called intersex. Intersexuality has been frequently described in decapod species such as the spider crab *Hyas coarctatus* (Hartnoll 1960), the fiddler crab *Uca pugilator*

(Zou and Fingerman 2000), the crayfishes *Parastacus pugnax* (Rudolph 1997), *Parastacus brasiliensis* (Almeida and Buckup 2000) and *Parastacus varicosus* (Rudolph *et al.* 2001), caridean shrimps (Buttler 1964, Yaldwyn 1966, Carpenter 1978, Bauer 1986), the mud-shrimp *Upogebia stellata* (Pinn *et al.* 2001), the mole crabs *Emerita analoga* (Barnes and Wenner 1968) and *Emerita asiatica* (Subramonian 1981), the oxystome crab *Ebalia tuberculata* (Manning and Holthuis 1981) and the hermit crabs *Clibanarius zebra* (personal observation of H. Snyder in Wenner 1972), *Eupagurus* sp. (Charniaux-Cotton 1975) and *C. antillensis*, *C. sclopetarius*, and *C. vittatus* (Turra and Leite 2000).

The presence of intersex individuals in natural populations may be evidence of sex reversal (Rudolph 1995, Almeida and Buckup 2000) although in the crayfish *Cherax quadricarinatus* (Sagi *et al.* 1996) and *Parastacus varicosus* and *P. pugnax* (Rudolph *et al.* 2001) intersexuality is associated with non-functional hermaphroditism. Functional sequential hermaphroditism is defined when both male and female reproductive systems are functional during different periods of the life of an individual (Sagi *et al.* 1996). While protandric hermaphroditism is well documented among decapod crustaceans any record of protogyny (see Brook *et al.* 1994) is available. Sequential protoginic hermaphroditism may be found only among peracarids (Lang 1958, Brook *et al.* 1994). The hypothesis of protogyny in hermit crabs was suggested by Wenner (1972) to explain the absence of males in the smallest size classes of the hermit crab *Clibanarius zebra* and complete absence of females in the largest ones. This hypothesis was never tested and the presence of ootestis in *Eupagurus* sp. (Charniaux-Cotton 1975) may suggest a non-functional sequential hermaphroditism in hermit crabs. The present study aims to discuss the relationship between intersexuality and sequential protogynic hermaphroditism in hermit crabs based on morphological, behavioral and populational information.

Material and methods

Hermit crab species

The three hermit crab species studied here coexist in the intertidal of the Araçá region, São Sebastião Channel, South-eastern Brazil (23°49'S, 45°24'W). All are common intertidal and shallow subtidal species in tropical and subtropical areas from the east coast of USA to Southern Brazil (Forest and Saint Laurent 1967) and partitionate microhabitats in the study site (Turra *et* *al.* 2000). *Clibanarius sclopetarius* and *C. vittatus* have similar sizes and are larger than *C. antillensis* (Turra and Leite 2000). All three species present the anomalous sex-ratio pattern (sensu Wenner 1972): males and females are equally abundant in the smallest size classes, females are more frequent in the intermediate classes, and males predominate in the largest ones (Turra and Leite 2000). The frequency of intersex individuals in the studied populations is low, not exceeding 7% of the total number of individuals sampled (Turra and Leite 2000).

Experiments

Observations on the mating behavior of the three *Clibanarius* species were conducted to identify successful mates (females laid eggs after copulation). A large number (>200) of individuals of each species from the Araçá region were maintained in plastic pools (1.8 m x 1.3 m for *C. sclopetarius* and *C. vittatus* and 0.6 m x 0.5 m for *C. antillensis*) with running seawater under a natural day-night photoperiod at the laboratories of the Centro de Biologia Marinha of Universidade de São Paulo (CEBIMar-USP). Observations were made mainly at morning and afternoon and were based on a previous description of reproductive behavior for diogenid hermit crabs (Hazlett 1966). Mating pairs were isolated, removed from their shells, sized (shield length, mm), and inspected for gonopores on the basis of the third (female) and fifth (male) pereopods. Intersex individuals were characterized by presenting gonopores of both males and females.

Intersex individuals were then maintained isolated in a closed water circulating system and fed industrialized fish food each three days in the laboratory. Observations were done daily, and exuvia removed after each molting cycle until crab death. Line drawings of the ventral portion of the exuvia were done to document any morphological modification in the gonopores.

Results

During behavioral observations on the three hermit crab intertidal species a total of twenty successful mating were recorded for *C. antillensis*, five for *C. sclopetarius* and three for *C. vittatus*. One intersex of *C. sclopetarius*, one of *C. vittatus* and two of *C. antillensis* successfully mated as males. The reproductive behavior of these individuals did not differ from that of "typical" males and all these functional male intersex hermit crabs were larger than the females they courted (Table 1).

The morphology of the gonopores of the intersex individuals of the three species is shown in Fig. 1. In *C. vittatus* the intersex individual had both male and females open gonopores. The female gonopore of this species presented a rounded papilla and was neither totally nor partially calcified. The female gonopores of the two individuals of *C. antillensis* were not totally open. One individual (Fig. 1, left pair) of this species had a partially calcified small opening on the basis of the right third pereopod and a partially calcified larger opening on the basis of the left third pereopod. The latter opening showed a round scar marking the position (diameter) of the gonopore before molting. Another individual of *C. antillensis* (Fig. 1, right pair) had only a partially calcified opening on the basis of the left third pereopod. The *C. sclopetarius* intersex individual had a small opening with a round scar on the basis of the right third pereopod and a larger totally open gonopore in the left pereopod. The male gonopores of the intersex individuals of these three species were completely open. In all individuals the female opening tended to close due to growth of the exoskeleton from the edge to the center of the gonopore (Fig. 1). The male gonopore remained unaltered in all specimens.

Discussion

Sex reversal in crustaceans is supposed to have evolved independently in different peracarid groups (Brook *et al.* 1994). As a consequence, one can argue that the ecological and evolutionary role of intersexuality may also differ among crustacean groups, as recorded for the frequency of intersexuality in different decapod species. Only one intersex individual was recorded in a population of the mud-shrimp *Upogebia stellata* (Pinn *et al.* 2001), while all individuals of the crayfish *Parastacus varicosus* and *P. pugnax* are intersex (Rudolph *et al.* 2001). Frequency of intersex may vary from 1.2 to 17.0 % in *Cherax quadricarinatus* (Medley and Rouse 1993, Sagi *et al.* 1996) and from 2.5 to 15.8 % in *Samastacus spinifrons* (Rudolph 1999). An one-year field survey revealed 10 (out 393 individuals, 2.5%) intersex in *C. antillensis*, 2 (out 391, 0.5%) in *C. sclopetarius* and 8 (out 125, 6.5%) in *C. vittatus* (Turra and Leite 2000). The function of intersex individuals is poorly reported in the literature but also show a marked variation among species. The intersex individuals of the isopod *Armadillidium vulgare* (Rigaud and Juchault 1998) are sterile while those of the crayfish *Cherax quadricarinatus* are functional males with arrested pre-vitelogenic ovaries (Sagi *et al.* 1996, Khalaila *et al.* 1999). In the crayfish *Parastacus varicosus* the intersex individuals may function as male or female. In the three *Clibanarius* species studied here, intersex individuals may function as fertile males, although a broad investigation of all possible states (external morphology and function) of intersexuality in these populations is not available yet. Intersex hermit crabs also seems to be stronger competitor for mates than "typical" males (see Chapter 2).

Intersexuality is common in crustaceans and is proximately associated with the plasticity in secondary sexual characters under endocrine control of the androgenic gland, which is responsible for sex differentiation and sexual characteristics in crustaceans (see Sagi *et al.* 1997, for a recent review). Thus, any factor influencing the activity of the androgenic gland will affect the hormonal balance. In fact, factors such as pollution (Depledge and Billinghurst 1999), combination of photoperiod and temperature in populations with environmental sex determination (Dunn *et al.* 1996), genetic abnormalities (Hough *et al.* 1992) and external (Ginsburger-Vogel 1991) and intracellular (Juchault *et al.*, 1991, Rigaud and Juchault 1998) parasites are reported to cause intersexuality in peracarids. In decapods, the external stimuli involved in intersexuality are still to be identified.

Despite only a few records were obtained, there was a high similarity in morphological alterations undertaken in the observed individuals of the three species of *Clibanarius* studied here. Moreover, the frequency of intersex individuals in natural population (Turra and Leite 2000) and in courting pairs (Chapter 2) also show that intersexuality is a common feature to the studied hermit crabs. Despite protogyny in crustaceans is known only among peracarids (Brook *et al.* 1994) and intersex individuals may be found in both functional (Rudolph 1995, Almeida and Buckup 2000) and non-functional hermaphrodites (Sagi *et al.* 1996, Rudolph *et al.* 2001), the above arguments enable further discussion on this subject. Thus, below it is presented a rationale examining intersexuality as part of a protogynic hermaphroditism in hermit crabs based on the closure of the female's gonopore, sexual dimorphism, size of intersex individuals in relation to males and females and population sex ratio.

Sagi *et al.* (1996) maintained intersex individuals of the crayfish *Cherax quadricarinatus* for several months and characterize them as non-functional hermaphrodites because they did not record any modification in gonads or in the external morphology, having always an active testis and an arrested ovary. However, gonopore modification is reported in hermaphrodite species (Almeida and Buckup 1997, Mascetti *et al.* 1997). In this way, the closure of the females' gonopores in intersex *Clibanarius* might suggest that these individuals could have been primarily females although there is no information on the early history of the male gonopores. The record of intersex females (the opposed situation than that recorded here) that have been copulated successfully would certainly strengthen this hypothesis.

Charnov (1982) pointed out that sex ratio shows a strong tendency to be biased toward the first sex, i.e., males in protandric species and females in protogynic species. In fact, the overall sex ratio in the three studied *Clibanarius* populations is markedly skewed toward females (Turra and Leite 2000). Despite the high overlap in the size distribution of males and females in these populations males are on average larger than females. The average sizes of the intersex individuals of the three *Clibanarius* populations show a tendency to be intermediate between males and females (Turra and Leite 2000), thus indicating that sex reversal would be experienced by the largest females in the population. In fact, intersex individuals in protandric pandalid species (Butler 1964) and in the protandric crayfish *Parastacus brasiliensis* (Almeida and Buckup 1997) are also of intermediate size in relation to males and females.

Because growth in crustaceans occurs through molts and is thought to be indeterminate, individuals that have changed their sex should have larger average sizes (or at least be of equal sizes) than the first sex, i.e., females would be larger than males in protandric species and males would be larger than females in protogynic species. In fact, females of protandric caridean shrimps (Butler 1964, Yaldwin 1966, Wenner 1972, Carpenter 1978) and anomuran Hippidae (Barnes and Wenner 1968, Subramonian 1981) are larger than males, while in protogynic isopods (Burbanck and Burbanck 1974, Brook *et al.* 1994) and tanaids (Modlin and Harris 1989, Highsmith 1983, Stoner 1986) females are larger than males. In hermit crabs, including the *Clibanarius* species studied here (Turra and Leite 2000), males are on average larger than females.

The occurrence of protogyny in the studied hermit crab populations may be also supported by the size advantage model (SAM, Ghiselin 1969). The SAM predicts that the fitness of "second" sex increases more rapidly with size. This means that sex reversal would be favored by natural selection when the reproductive success of a given individual as male or female is strongly associated with its age or size (Ghiselin 1969, Charnov 1982, Warner 1975). In hermit crabs fecundity is considered a measure of the reproductive success of females and is strongly associated with their size (Mantelatto and Garcia 1999, Turra and Leite 2001). However, Harvey (1990) showed that large males may have a size advantage over small ones when competing for mates. In addition, egg incubation by females enables a lower genetic contribution to next generations in relation to males, which may copulate with many females during the time a single female is prevented for mating due to egg brooding. Thus, males may copulate more times than females during their life span. A consequence of this postulated higher reproductive success of large-sized males is the proportionality in the sizes of males and females that copulate successfully (Hazlett 1989). Bertness (1981) suggested that male superiority in competition for shells in relation to females is the product of sexual selection for males to attain larger sizes than females. Thus, despite large-size females have a greater fecundity, large-size males may be stronger competitors for shells (Bertness 1981) and mates (Harvey 1990), copulating more times during a given period of time than females of the same size and than small-sized males. This suggests an evolutionary advantage of an eventual protogynic hermaphroditism in hermit crabs, although the relationship between size and reproductive success is also true for gonochoristic species.

Despite these arguments suggesting a relationship between intersexuality and protogynic hermaphroditism in hermit crabs, the report of an ootestis in *Eupagurus* (*Pagurus*) (Charniaux-Cotton 1975) may evidence a non-functional hermaphroditism in hermit crabs. Thus, it is important to investigate whether intersexuality in the studied hermit crabs is indeed part of a hermaphroditic process or represent only an aberrant sexuality. It is also important to understand the role of the androgenic gland (see Charniaux-Cotton 1965, 1975, Sagi *et al.* 1997, Sagi and Khalaila 2001) in gonad maturation and definition of secondary sexual characters. One should also identify the stimuli involved in such phenomenon. The fact is that the ultimate and proximate factors in intersexuality in hermit crabs are still to be identified.

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Table 1. Shield length (mm) of females and intersex males of *C. antillensis*, *C. sclopetarius* and *C. vittatus* that copulated successfully.

Hermit crab	Intersex male (mm)	Female (mm)
C. antillensis (Ind. 1)	4.52	4.45
C. antillensis (Ind. 2)	4.91	4.25
C. sclopetarius	10.96	8.35
C. vittatus	8.97	7.49

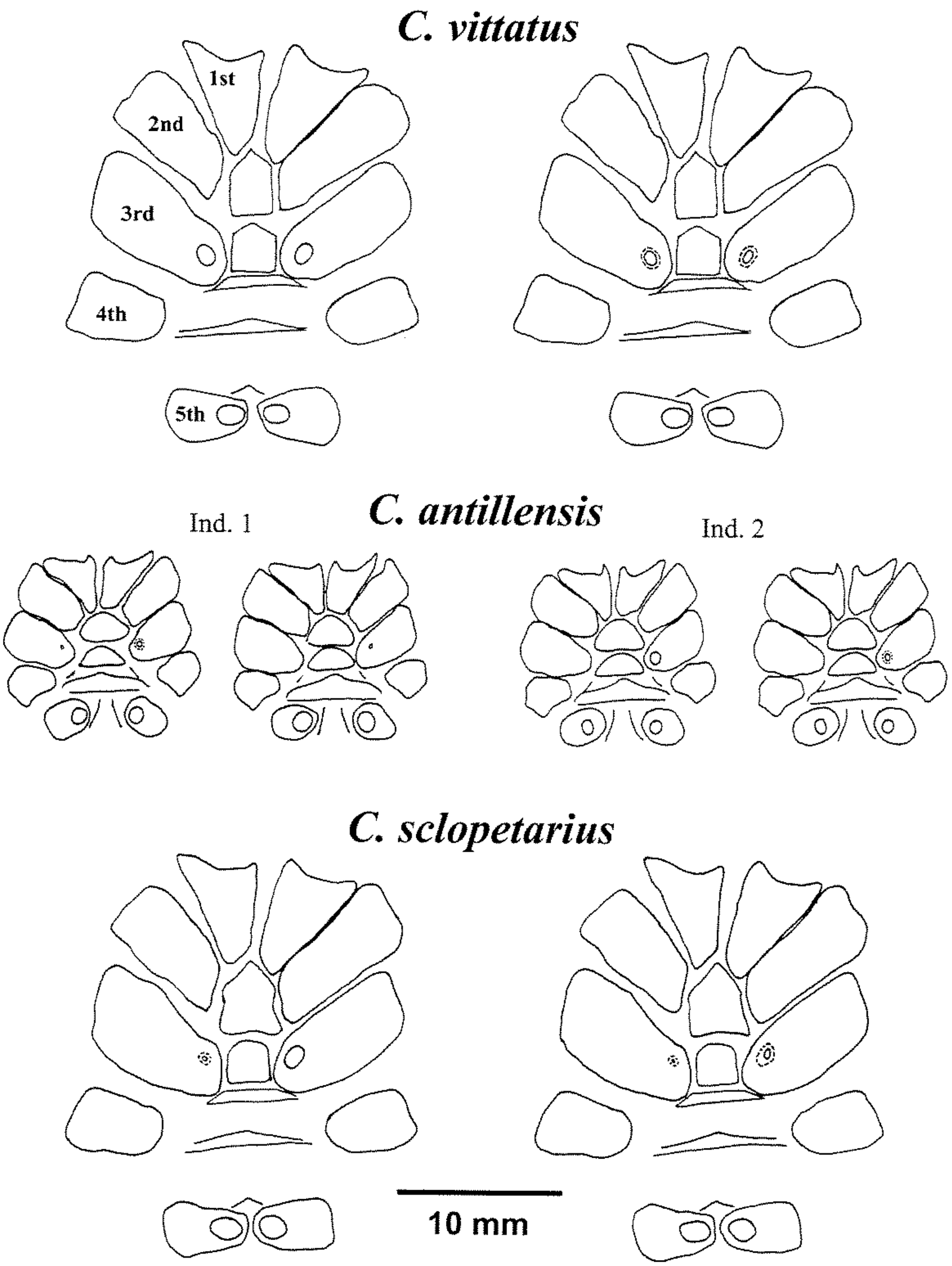

Fig. 1. Ventral view of the intersex individuals of the hermit crabs *C. vittatus*, *C. antillensis* (Ind. 1, left pair; Ind. 2, right pair), and *C. sclopetarius* illustrating the male (opening at the base of the 5th pair of pereopods) and female (opening at the base of the 3rd pair of pereopods) gonopores before (left drawings) and after (right drawings) molting. Numbers indicate the pereopods.

Capítulo 4

Embryonic development and duration of incubation period of tropical intertidal hermit crabs

Abstract

The complete macroscopic description of the embryonic development of the hermit crabs *Clibanarius antillensis*, *C. sclopetarius*, *C. vittatus* and *Pagurus criniticornis* and preliminary observations on the development of *Pagurus brevidactylus* and *Paguristes tortugae* were done. The overall characteristics of the embryonic development of the studied species was very similar and seven stages could be identified: Stage 1, Zygote and cleavage; Stage 2, Homogeneous mass (advanced cleavage and gastrulation); Stage 3, Initiation of the germinal disc (yolk-free area); Stage 4, Percentage of yolk-free area from 5% to 50-70%; Stage 5, Eye pigmentation (comma-shape) and heart beating; Stage 6, Percentage of yolk-free area from 70-80% to 95%) and eye development to a darkened irregularly-rounded area; Stage 7, Zoea visible and hatching. Three stages with equivalent developmental times could also be characterized: 1 – corresponding to stages 1, 2 and 3; 2 – corresponding to stage 4; 3 – corresponding to stages 5, 6 and 7. Differences were recorded in embryonic development of these species. Firstly, fresh eggs differed in size (*Paguristes tortugae* > *C. sclopetarius* = *C. vittatus* > *C. antillensis* = *P. criniticornis* = *P. brevidactylus*) and color (*C. antillensis*, *P. criniticornis* and *P. brevidactylus* – greenish/dark-yellow; *C. sclopetarius* and *C. vittatus* – dark-red/purple; *Paguristes tortugae* – light-red); after the first cleavage, which have originated an 8-cell embryo, the subsequent cleavages took on average 4 hours for the species of *Clibanarius* and 2-3 hours for *P. criniticornis*; the developmental time also depended on crab species (*C. sclopetarius* = *C. vittatus* > *C. antillensis* > *P. criniticornis* = *P. brevidactylus*); eye pigmentation started in the species of *Clibanarius* when the yolk-free area was about 50-60% in comparison to 70-80% in *P. criniticornis*. Finally, eye pigmentation in *P. criniticornis* took place relatively later in embryonic development, about 75% of the total developmental time, in comparison with 60-65% in the

Clibanarius species. Such differences suggest variations in reproductive strategy among species but the understanding of their proximate and ultimate factors need further experimentation.

Keywords: Reproduction, reproductive strategy, development, embryos, Crustacea, Anomura, *Clibanarius*, *Pagurus*, *Paguristes*.

Introduction

Hermit crabs are anomuran crustaceans that shelter their soft abdomen in gastropod shells. These shells also protect the eggs hermit crabs incubate in their pleopods. This is a critical period for both females and clutches, evidenced by the behavioral modifications undergone by females during egg-brooding (Neil and Elwood 1985). Egg loss during shell investigation and agonistic encounters may reduce female fitness. In addition, space limitation due to small shell internal volume may reduce the amount of eggs females can incubate (Fotheringham 1976). Thus, if shell volume is fixed, the larger the eggs the smaller the number of eggs that can be maintained inside the shells occupied by females. Besides these ecological consequences of different egg sizes, such variation may represent adaptations to various selective forces. In fact, relative sizes of eggs, larvae and juveniles and the duration of the embryonic and larval development are important components of the life history strategies of marine invertebrates (Hadfield and Strathmann 1996).

The duration of the embryonic development in decapods is highly variable and may be directly influenced by temperature (Wear 1974). The first descriptions of embryonic development of hermit crabs were made in very early studies (Rathke 1840, Mayer 1877, Faxon 1882, Jackson 1913, Krainska 1934, 1936, 1938, Scheidegger 1976; see review in Anderson 1982). Studies on the duration and macroscopic characterization of embryonic development are more recent and were conducted only in *Eupagurus* (*Pagurus*) *prideauxi* (Wear 1974), *Pagurus bernhardus* (Lancaster 1988), *Pagurus beringanus*, *P. caurinus*, *P. granosimanus* and *P. hirsutiusculus* (Nyblade 1974, 1987), *P. samuelis* (Coffin 1960, Nyblade 1974, 1987) and *Clibanarius olivaceous* (Kamalaveni 1949). Finally, field studies on the population biology and reproductive period of *Pagurus middendorffii* (Wada *et al.* 1995), *P. nigrofascia* (Goshima *et al.* 1996), and *P. lanuginosus* (Wada *et al.* 2000) enabled estimates on the duration of the embryonic development based on the presence of different embryonic stages along the year or reproductive season.

The study of Scheidegger (1976) with *Eupagurus* (*Pagurus*) *prideauxi* furnished the most complete description of the embryonic development of hermit crabs and identified thirteen stages from cleavage to larval hatching. Boolootian *et al.* (1959) identified ten stages in a macroscopic description of embryos of some brachyurans and anomurans species. Similarly, Lancaster (1988) distinguished seven developmental stages for the hermit crab *Pagurus bernhardus* based on external morphological characters. The macroscopic embryonic development of *Pagurus samuelis* was also studied but the eggs were not classified into stages (Coffin 1960). On the other hand, field studies on the reproductive biology of hermit crabs classify the clutches in a quite variable number of stages. Ameyaw-Akumfi (1975), Fotheringham (1980), and Turra and Leite (1999) separated the broods in 'with eyes' and 'without eyes'; Wada *et al.* (1995, 2000), Goshima *et al.* (1996) and Turra and Leite (2001) used a five-stage classification, while Negreiros-Fransozo *et al.* (1992) identified ten embryonic stages based on the study of Boolootian *et al.* (1959). Despite studies on the embryonic development of hermit crabs are limited, the post-embryonic (larval) development is well known for most Brazilian species (Lang and Young 1977, Hebling and Brossi-Garcia 1981, Brossi-Garcia and Hebling 1983, Hebling and Negreiros-Fransozo 1983, Brossi-Garcia 1987, Negreiros-Fransozo and Hebling 1987, Siddiqui *et al.* 1991).

The aim of this study was to record the morphology and size of embryos and the duration of the embryonic development of four intertidal hermit crab species (*Clibanarius antillensis*, *C. sclopetarius*, *C. vittatus* and *Pagurus criniticornis*; preliminary observations were taken for *Pagurus brevidactylus* and *Paguristes tortugae*). The sequence of the development was compared among species to propose a framework for macroscopic identification of the embryonic stages for further population or reproductive studies on hermit crabs.

Material and methods

The hermit crabs were collected along the São Sebastião Channel and maintained in plastic swimming pools (1.80 x 1.30m) with circulating seawater. The crabs were then observed and, after successful mating, the females were removed from their shells by breaking them gently. These females were then supplied with apex-less shells and maintained individually in small plastic aquaria (10 cm in diameter for the small-sized species – *C. antillensis*, *P. criniticornis*, *P. brevidactylus* and *P. tortugae* – and 20 cm in diameter for the large-sized ones – *C. vittatus* and *C. sclopetarius*) at 25°C, 34‰ and 12/12 h photoperiod in a incubation chamber. The water was changed daily and the crabs were fed with commercial fish food after each observation. Observations were taken daily (*Clibanarius* and *Paguristes*) or at 12h intervals (*Pagurus*) until hatching. These differences in observation time were based on previous information on total developmental time of the studied species and, as a consequence, enabled roughly equivalent observation periodicity. At each observation, the females were removed from their shells using a flexible rubber-coated wire to force them to leave shells through the aperture.

Ten embryos were removed in each time interval and macroscopic developmental features observed. These eggs were then fixed in aqueous Bouin solution for 24h, washed in water three times and preserved in alcohol 70%. Observations on preserved embryos enabled more detailed morphological descriptions. Fresh and preserved zygotes were measured to evaluate, through the Student t test (Zar 1999), the reduction in egg maximum diameter after preservation procedure. The mean size of the embryos in newly laid eggs (fresh embryos) was compared among species through ANOVA and Scheffé's post-hoc test to test the null hypothesis that the mean zygote size did not vary among hermit crab species (Zar 1999). In this analysis, the sizes of the ten eggs sampled in each clutch was averaged to produce a mean value of egg size that was used as replicate in interespecific comparisons.

Results

Egg size and developmental time

Egg laying took place up to one hour after copulation in all species. The size of the eggs (before cleavage) varied markedly among species (ANOVA, F=74.651, df=4, P<0.001; *P. brevidactylus* was not included in the analyses because egg size measurements were taken for only one individual – 0.357 mm). *Paguristes tortugae* presented the largest embryos (0.683 ± 0.021 mm, Scheffé's test: p<0.05 for all pairwise comparisons). *Clibanarius sclopetarius* (0.479 ± 0.039 mm) and *C. vittatus* (0.444 ± 0.019 mm) presented eggs of similar sizes (Scheffé's test: p>0.05) as well as the embryos of *C. antillensis* (0.354 ± 0.011 mm) and *P. criniticornis* (0.371 ± 0.003 mm: p>0.05). All other comparisons revealed differences in egg size. The maximum diameter of preserved eggs was reduced by a ratio of 3 to 12% in comparison to fresh embryos (Student t test, p<0.05 in all comparisons).

The duration of embryonic development varied markedly among species (ANOVA, F=922.275, df=3, p<0.001; Fig. 1). *Pagurus criniticornis* took on average 9.2 days to hatch, a shorter period than those of *Clibanarius* species (Scheffé's test: p<0.05 for all comparisons). The embryonic development of *Clibanarius antillensis* (18.2 days) was also shorter than that of *C.*

vittatus and *C. sclopetarius* (24.0 and 25.3 days, respectively; $p<0.05$ for both comparisons), which, in turn, presented similar duration ($p>0.05$). Preliminary observations were taken for other two common rocky shore hermit crabs, *Paguristes tortugae* and *Pagurus brevidactylus*, and revealed that the duration of the embryonic development of *P. brevidactylus* (9 days) is very similar to that of *P criniticornis*. The description of the embryonic development of *P. tortugae* could not be completed because females lost their few eggs during the experiment. The embryonic development of this species was demonstrated to be slower than that of *C. antillensis* but faster than those of *C. sclopetarius* and *C. vittatus* (see Table 1), suggesting that the total developmental time for this species would be between 20 and 26 days.

Morphological characteristics

The overall characteristics of the embryonic development of the studied species was very similar and enabled the identification of seven developmental stages (Table 1, Figs. 2-5).

Stage 1 – This stage was composed by the zygote and its subsequent cleavages (Fig. 2A-C, Fig. 3A-C, Fig. 4A-C, Fig. 5A-C). The fresh eggs of the studied species differed in color: *C. antillensis*, *P. criniticornis* and *P. brevidactylus* – greenish/dark-yellow; *C. sclopetarius* and *C. vittatus* – dark-red/purple; *Paguristes tortugae* – light-red. In all species, the first, second and third cleavages occurred almost simultaneously forming a 8-cell embryo. After that, subsequent cleavages took 4 hours for species of the genus *Clibanarius* and 2-3 hours for *P. criniticornis*, on average. The observation of cleavage was possible for up to 2 days (1 day for *P. criniticornis*) after copulation.

Stage 2 – The embryo was characterized as a homogeneous mass because cell number was very high and cell individualization became difficult (Fig. 2D-E, Fig. 3D-E, Fig. 4D-E, Fig. 5D-E).

Stage 3 – After some time (see table 1), a concentration of cells was observed in the eggs, evidencing the germinal disc, a grayish/pale yolk-free area (Fig. 2F-G, Fig. 3F-G, Fig. 4F-G, Fig. 5F-G). The yolk was broken up into cell-like granules.

Stage 4 – The percentage of the yolk-free area increased from 5% to up to 50-70% depending on the species (Fig. 2H-M, Fig. 3H-Q, Fig. 4H-P, Fig. 5H-O). In this period, some characteristics were recognizable in dorsal and lateral views such as the differentiation into cephalic and abdominal regions and the development of the cephalic lobe, which originates the eyes, first and second (bireme) antenna and mandible. The development of the buds of maxilla and maxilipeds could be observed through transparency in only some embryos.

Stage 5 – The dark eye pigmentation became evidenced (Fig. 2N, Fig. 3R, Fig. 4Q, Fig. 5P) and heart beats could be observed in all species.

Stage 6 – The pigmentation of the eyes increased during development from a comma-shape to an irregularly-rounded darkened area comprising almost 10% of the embryo lateral view. Such alterations in eye pigmentation were followed by variations in the percentage of yolk-free area from 70-80% to 95% (Fig. 2O-R, Fig. 3S-X, Fig. 4R-V, Fig. 5Q-R) but any evident alteration in embryo color (pigmentation) was recorded.

Stage 7 – The zoea became visible when more than 95% of the embryo lateral view was free from yolk. Hatching occurred and a small portion of yolk was still visible in the dorsal region of the zoea (Fig. 2S, Fig. 3W-Z, Fig. 4X-W, Fig. 5S).

Besides the morphological modifications during embryonic development a marked increase in egg size (maximum diameter) was recorded (see Figs. 2-5). Alterations in egg coloration were recorded in all species, with early stages being characterized by the color of yolk and the latter ones by the pale/whitish unpigmented embryo. Such alterations were a direct consequence of yolk consumption during development. The eggs of a single female developed synchronously, i.e., all incubated eggs were in the same developmental stage.

Discussion

Despite the general developmental sequence was very similar in the studied species some specific differences could be recorded. First, fresh eggs differed in size and color, suggesting that yolk amount and composition varied among species. Second, marked differences occurred in the cleavage. The almost immediate cleavage from zygote to the 8-cell stage is not an abnormality and may be explained by nuclear divisions not followed by superficial cleavages (Scheidegger 1976). Third, the developmental time also depended on crab species. Fourth, eye pigmentation occurred in the *Clibanarius* species when the yolk-free area was about 60% of the embryo lateral view in comparison to 70-80% in *P. criniticornis*. Finally, eye pigmentation in *P. criniticornis* took place relatively later in the embryonic development, at about 75% of the total developmental time, in comparison with 60-65% in the *Clibanarius* species (Fig. 6). Coffin (1960) and Lancaster (1988) showed that the beginning of eye pigmentation occurred almost in the middle of the development of *Pagurus samuelis* and *P. bernhardus*, respectively. On the other hand, the stages preceding eye pigmentation in *Pagurus middendorffii* (Wada *et al.* 1995), *P. nigrofasciata* (Goshima *et al.* 1996) and *P. lanuginosus* (Wada et al 2000) took about 70-80% of the total developmental time, being very similar to *P. criniticornis*. Such differences in duration of embryonic stages among species may have a direct relationship with relative egg size (amount of yolk), yolk composition and species-specific reproductive strategies, but further studies are necessary to evaluate these hypothesis.

In general, stages of embryonic development are characterized according to embryo morphology, size and coloration. Based on such characteristics, seven clearly identifiable stages were proposed for the studied species (see Figs. 2-6). Other field and laboratory studies classified hermit crab (and other decapods) embryos in a variable number of stages, which can be compared with the present classification (Table 2). Once classification was highly variable in these studies, there are stages that correspond to one or more stages proposed here. For example, Coffin (1960) did not report the presence of embryos with homogeneous masses prior to the formation of the germinal disc in *Pagurus samuelis*. The duration of the stages have been rarely taken into account in the classification of hermit crab embryonic development (Coffin 1960, Lancaster 1988). The present study showed that the most conspicuous egg stages have different developmental times. Thus, despite being clearly identifiable, they represent quite different periods in relation to total developmental time. This was also reported by Coffin (1960) and Lancaster (1988) in experimental studies and by Wada *et al.* (1995, 2000) and Goshima *et al.* (1996) in field surveys. Thus, if one would like to take developmental time into account in the classification of embryonic stages for the studied species, three stages with equivalent developmental times could be characterized (see Fig. 6): 1. corresponding to stages 1, 2 and 3; 2. corresponding to stage 4; 3. corresponding to stages 5, 6 and 7.

Turra and Leite (2001) studied the fecundity of *C. antillensis*, *C. sclopetarius* and *C. vittatus* and revealed that all the five developmental stages identified (see table 2) were well represented only in the clutches of *C. antillensis*, while those of the two other species were concentrated in the first one (yolk not consumed). It was hypothesized that such differences could be a consequence of species specific patterns of embryonic development, i.e., the first stage would be shorter in *C. antillensis* than in both *C. sclopetarius* and *C. vittatus*. The results of the present study do not support such hypothesis once the duration of stage 1 (*sensu* Turra and Leite 2001) is proportionally equivalent in the three species. In this way, the differences reported by

Turra and Leite (2001) may be associated to differences in reproductive activity among species, another important characteristic related to differences in the reproductive strategies of marine invertebrates (Hadfield and Strathmann 1996). *Clibanarius sclopetarius* and *C. vittatus* presented a seasonal reproductive pattern with a peak of ovigerous females in April while *C. antillensis* presented high frequency of ovigerous females throughout the year (Turra and Leite 2000). These results suggest that *C. sclopetarius* and *C. vittatus* may have few but synchronical broods per year (at least one brood per month) in comparison to the continuous breeding of *C. antillensis*. This synchronism in reproduction in these two species may have caused the skewed estimates of eggs in early developmental stages reported by Turra and Leite (2001).

According to Hadfield and Strathmann (1996), the sizes of eggs and larvae, as well as their developmental times, can vary greatly among species and even populations as a response to varying environmental conditions. Besides this reproductive plasticity, different reproductive traits evolved in closely related species. In fact, the abbreviated larval development of *Paguristes tortugae* (Hebling and Negreiros-Fransozo 1983) is probably associated with the large size of their eggs and their relatively longer embryonic development time. This strategy would force females to a longer incubation period, which can expose them to higher risks of predation and even desiccation once ovigerous females exchange shells less frequently and, as a consequence, may occupy worse shells than non-ovigerous females (Neil and Elwood 1985). In addition, the abbreviated larval development may prevent colonization of distant areas due to the time the larvae spend in the plankton is very small. On the other hand, the long duration of the larval development of *C. sclopetarius* (Brossi-Garcia 1987) and *C. vittatus* (Lang and Young 1977) is associated with their relatively (in relation to larval size) small-sized eggs and short embryonic development. Such species may be exposed to higher risks of predation in the plankton but are able to colonize farther areas than species with abbreviated larval development.

As observed above and by Nyblade (1974, 1987), egg size was inversely related to the overall duration of the embryonic development. However, there are few adequate data available to enable a general understanding of the variation in and, as a consequence, of the ultimate factors governing reproductive strategies in hermit crabs. The fast development of *P. criniticornis*, *P. brevidactylus* and even *C. antillensis* in comparison to *C. sclopetarius* and *C. vittatus* may be also caused by differences in yolk composition, evidenced by differences in egg coloration. Such information is also not available up to date and may elucidate some of the proximate factors related to different reproductive strategies employed by hermit crabs.

Finally, a reduction in egg diameter was evidenced after preservation procedure. Once egg diameter is an important feature related to the life strategy in crustaceans (Nyblade 1974, Hines 1982) and in invertebrates as a whole (Hadfield and Strathmann 1996), future studies should inform in which conditions egg measurements were taken. If egg measurements would be taken from fixed eggs, a parallel evaluation of the reduction in egg diameter due to fixation should be employed. Alternatively, one could use the dry weight of egg masses (see Nyblade 1974), which is probably less affected by the fixation procedure than egg diameter, as another method to evaluate female energetic investment per egg.

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Table 1. Comparison of the embryonic development of the four studied hermit crab species (*Clibanarius antillensis*, *C. sclopetarius*, *C. vittatus* and *Pagurus criniticornis*) including preliminary observations on two other species (*Pagurus brevidactylus* and *Paguristes tortugae*). C, cleavage; HM, homogeneous mass; GD, initiation of germinal disc (yolk-free area); %, percentage of yolk-free area; EP, eye pigmentation; HB, Heart beating; H, hatching; ?, the observation of the embryonic development stopped due to females lost all embryos.

	Time (days)																										
Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
C. antillensis	C	C	HM	HM	GD	<5%	5-10%	15-20%	20-30%	30%	40-50%	60-70% EP	70-80% EP HB	80% HB	80-85%	85-90%	95%	>95%	H	H							
C. sclopetarius	C	C	HM	HM	GD	GD	<5%	5%	5-10%	10%	10-15%	20%	20-30%	30-40%	40-50%	50-60%	50-60% EP HB	60-70% EP HB	70%	70-80%	80%	80-90%	90%	90-95%	95%	95% H	H
C. vittatus	C	C	HM	HM	GD	GD	5%	5-10%	10%	10-15%	15-20%	20-30%	30-40%	40-50%	50-60% EP	50-60% EP HB	60-70% HB	70-75%	75-80%	80%	80-85%	90%	95%	95% H	H		
P. tortugae	C	C	HM	GD	GD	5%	5-10%	10%	10-20%	20-30%	30%	40% EP HB	40-50% HB	?	?	?	?	?									
	½	1	1½	2	2½	3	3½	4	4½	5	5½	6	6½	7	7½	8	8½	9	9½								
P. criniticornis	C	C	HM	GD	<5%	5%	10-15%	20-30%	30%	40-50%	50%	60-70%	70% EP HB	70-80% HB	80-90%	90-95%	95% H	95% H	H								
P. brevidactylus							20%		40-50%		60-70%		70-80% EP		90% HB		95%		H								

Table 2. Comparison of stages of embryonic development of some species reported in laboratory and field studies to those described in the present study.

Stages (Present study)	Boolootian *et al.* (1959)[1]	Scheidegger (1976)[2]	Lancaster (1988)[3]	Wada *et al.* (1995)[4]	Goshima *et al.* (1996)[5]	Wada *et al.* (2000)[6]	Turra and Leite (2001)[7]
1	1 – 2	1		1	1	1	1
2		1 – 3	1	1	1	1	1
3	3	4 – 5	2	2	2	2	1
4	4	6 – 10	3	2 – 3	2 – 3	2 – 3	2
5	5	11	3	4	4	4	3
6	6 – 8	12	4 – 5	5	5	5	4
7	9 – 10	12-13	6 - 7	5	5	5	5

[1] Brachyurans – *Pachygrapsus crassipes*, *Hemigrapsus nudus* and *Pugetia producta*, and anomurans – *Emerita analoga* and *Petrolistes cinctipes*; [2] Hermit crab *Pagurus prideauxi*; [3] Hermit crab *Pagurus bernhardus*; [4] Hermit crab *P. middendorffii*; [5] Hermit crab *P. nigrofasciata*; [6] Hermit crab *P. lanuginosus*; [7] Hermit crabs *Clibanarius antillensis*, *C. sclopetarius* and *C. vittatus*.

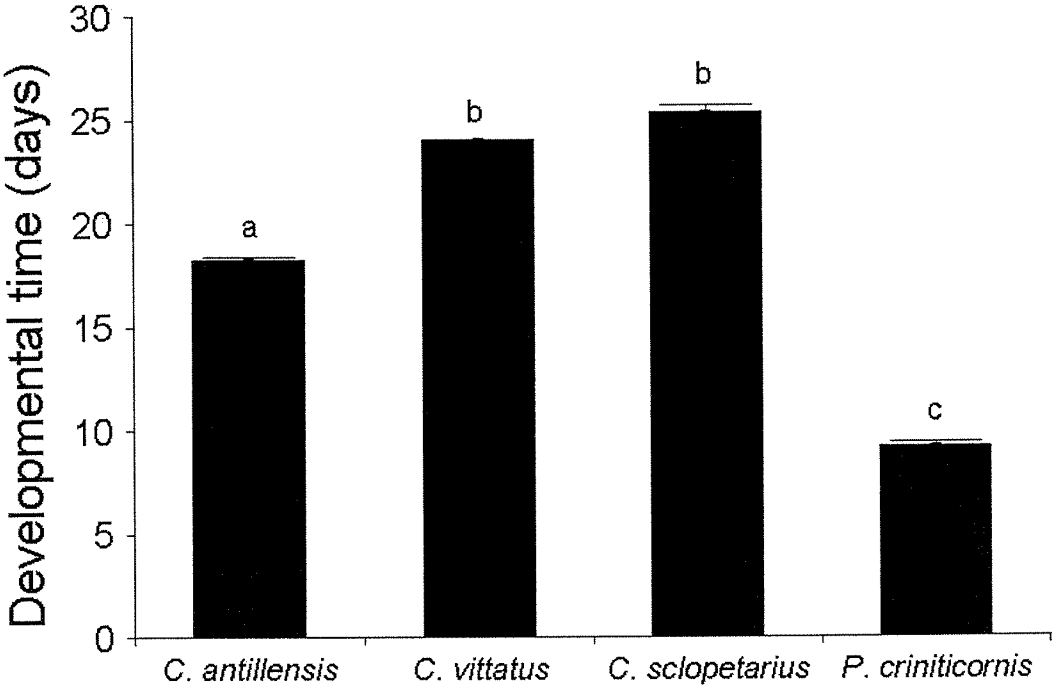

Fig. 1. Mean (+ 1 SE) duration time for the embryonic development of the hermit crabs *Clibanarius antillensis*, *C. sclopetarius*, *C. vittatus* and *Pagurus criniticornis* reared in the laboratory at 25°C. Superscript labels show the results of the post-hoc Scheffé's test.

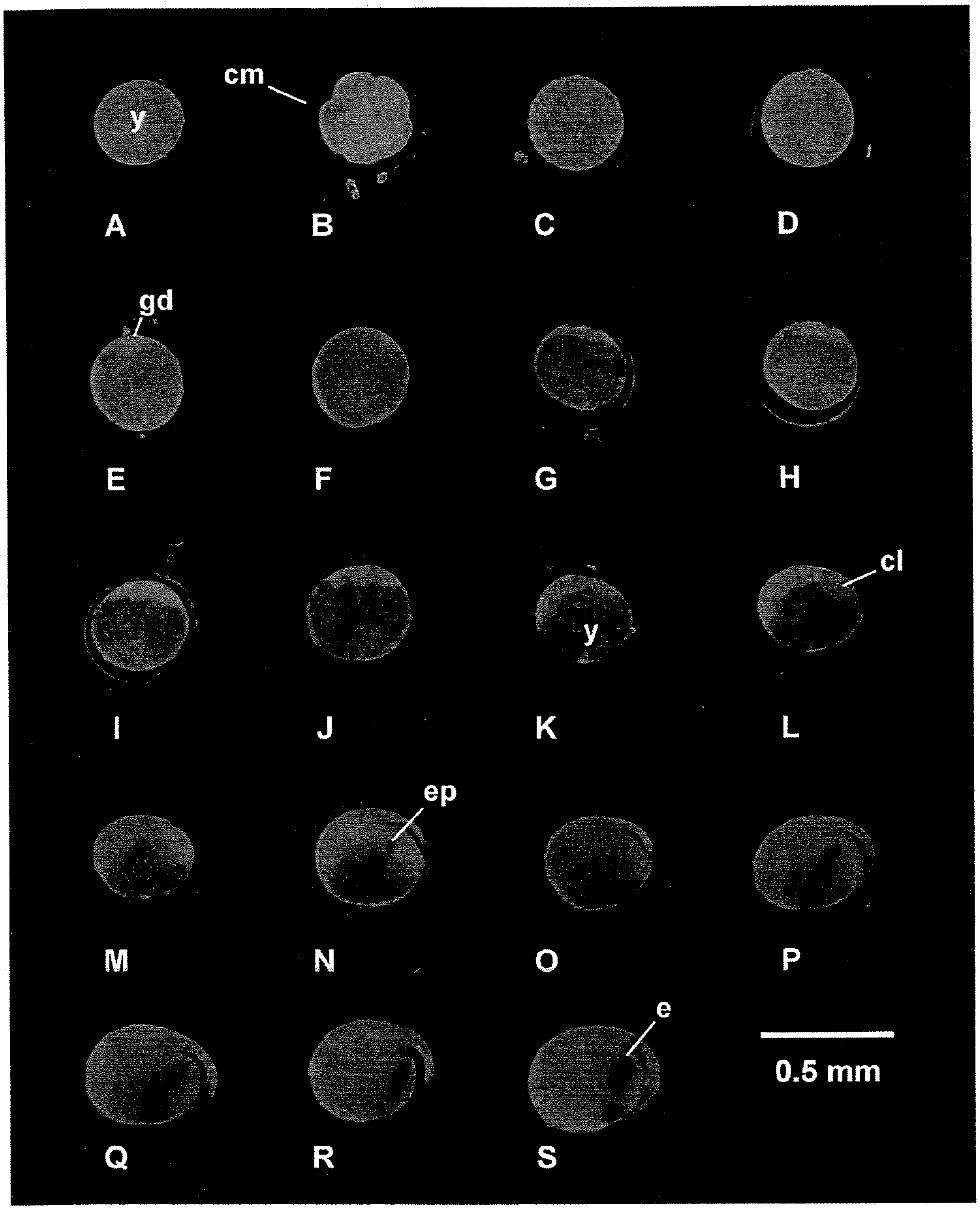

Fig. 2. Embryonic development of *Clibanarius antillensis*. A – zygote, B to S – lateral views of embryos in each subsequent day of observation. Stage 1, A – C; Stage 2, D – E; Stage 3, F – G; Stage 4, H – M; Stage 5, N; Stage 6, O – R; Stage 7, S. Labels: cl – cephalic lobe; cm – corionic membrane; e – eye; ep – eye pigment; gd – germinal disc (yolk-free area); y – yolk.

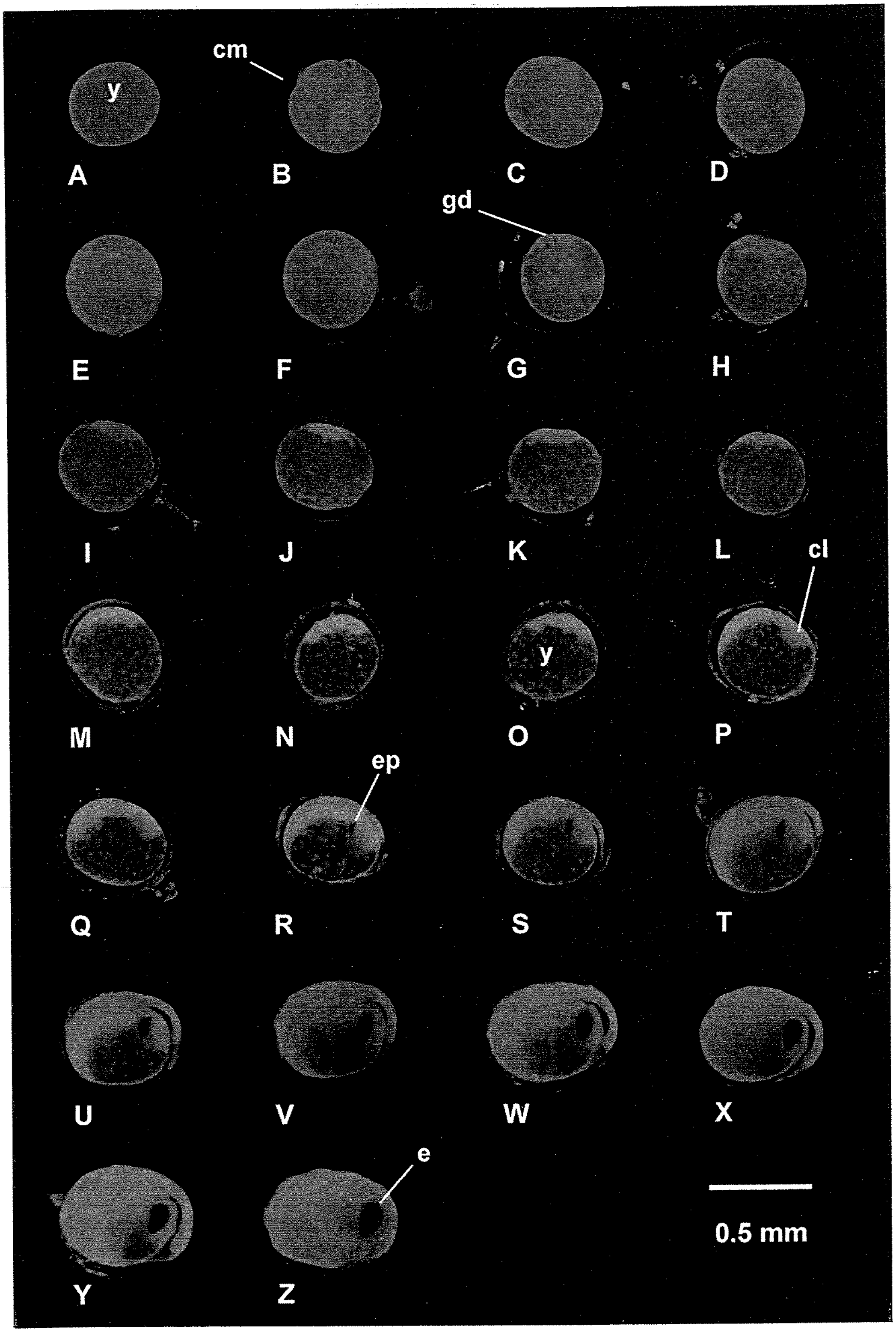

Fig. 3. Embryonic development of *Clibanarius sclopetarius*. A – zygote, B to Z – lateral views of embryos in each subsequent day of observation. Stage 1, A – C; Stage 2, D – E; Stage 3, F – G; Stage 4, H – Q; Stage 5, R; Stage 6, S – X; Stage 7, W – Z. Labels: cl – cephalic lobe; cm – corionic membrane; e – eye; ep – eye pigment; gd – germinal disc (yolk-free area); y – yolk.

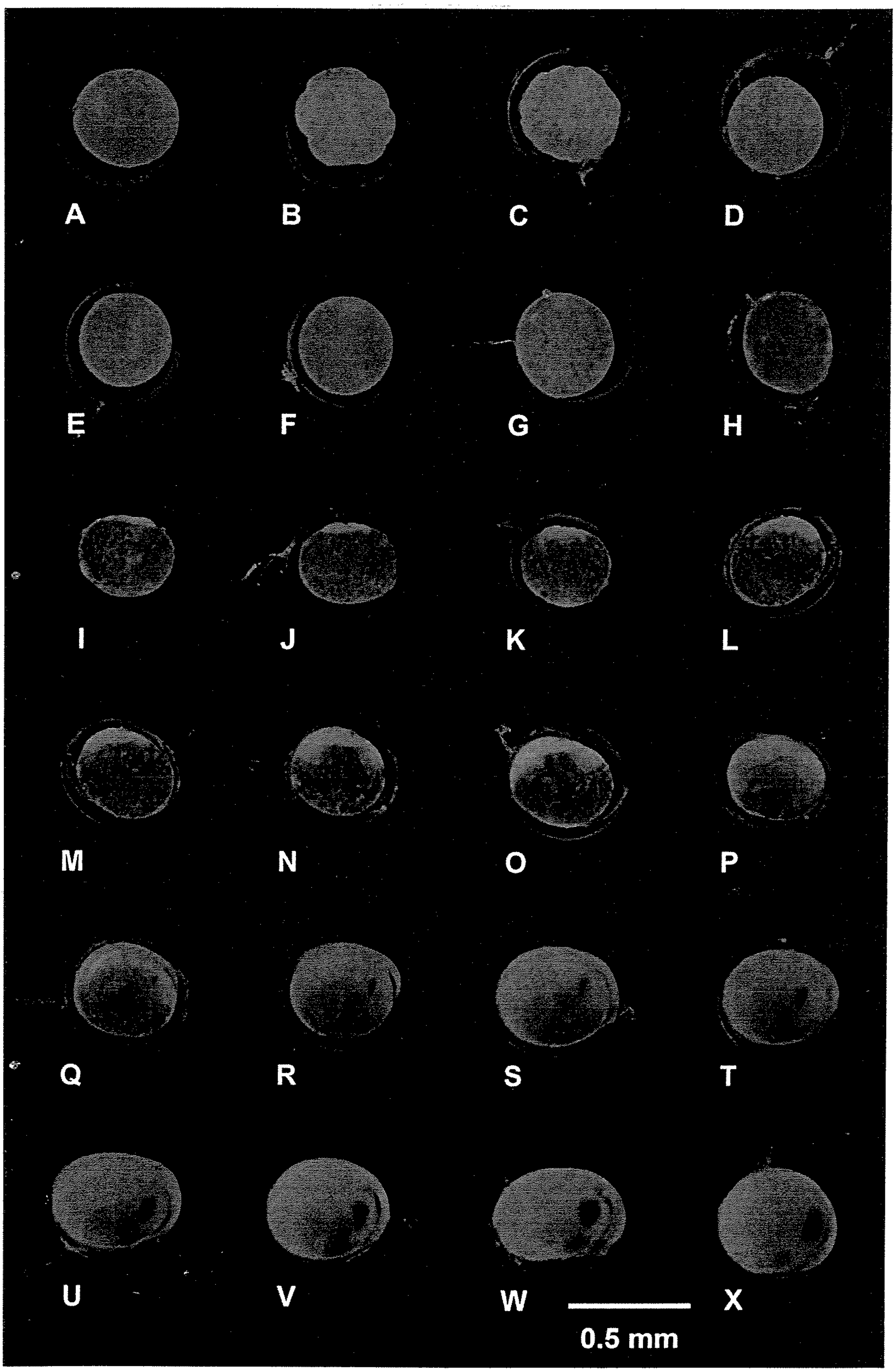

Fig. 4. Embryonic development of *Clibanarius vittatus*. A – zygote, B to W – lateral views of embryos in each subsequent day of observation. Stage 1, A – C; Stage 2, D – E; Stage 3, F – G; Stage 4, H – P; Stage 5, Q; Stage 6, R – V; Stage 7, X – W. Labels: cl – cephalic lobe; cm – corionic membrane; e – eye; ep – eye pigment; gd – germinal disc (yolk-free area); y – yolk.

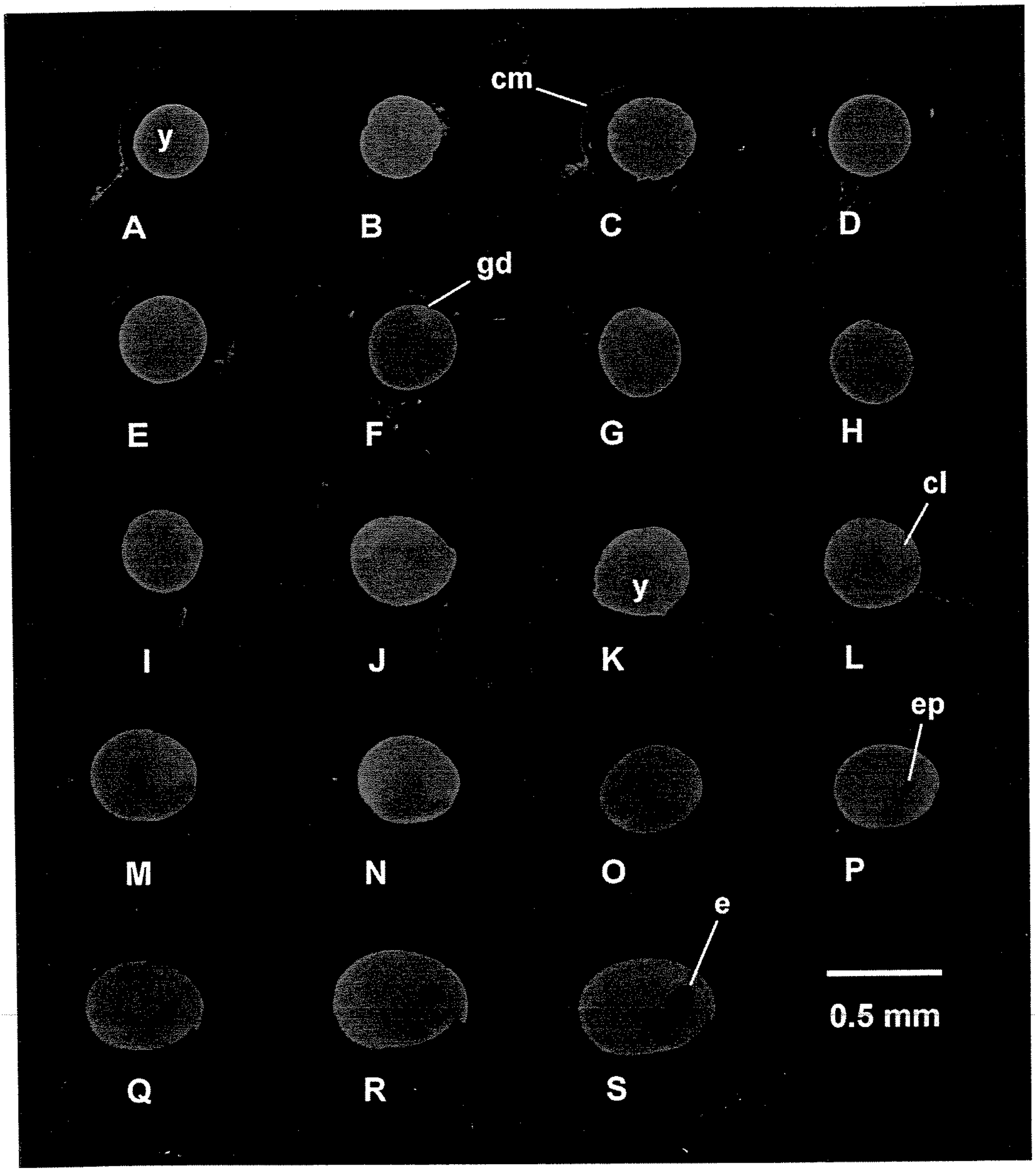

Fig. 5. Embryonic development of *Pagurus criniticornis*. A – zygote, B to S – lateral views of embryos in each 12 hour observation period. Stage 1, A – C; Stage 2, D – E; Stage 3, F – G; Stage 4, H – O; Stage 5, P; Stage 6, Q – R; Stage 7, S. Labels: cl – cephalic lobe; cm – corionic membrane; e – eye; ep – eye pigment; gd – germinal disc (yolk-free area); y – yolk.

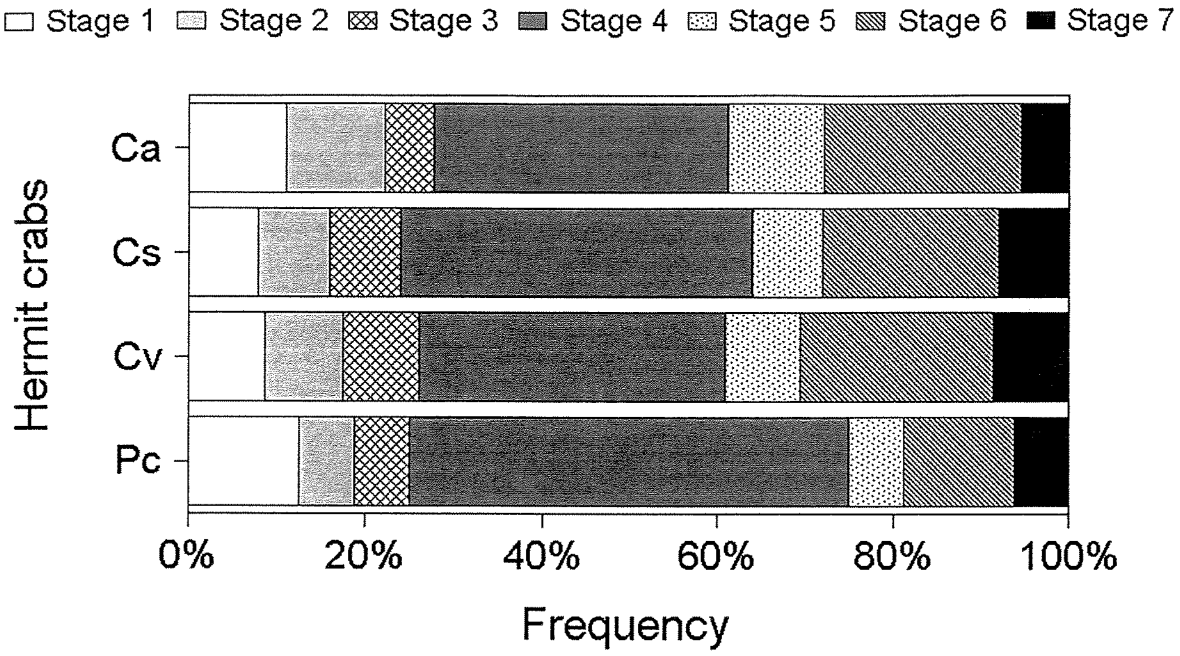

Fig. 6. Comparison of the duration of the most conspicuous embryonic stages identified for *Clibanarius antillensis* (Ca), *C. sclopetarius* (Cs), *C. vittatus* (Cv) and *Pagurus criniticornis* (Pc) reared in the laboratory at 25°C. Stage 1, Zygote and cleavage; Stage 2, Homogeneous mass; Stage 3, Initiation of the germinal disc (yolk-free area); Stage 4, Percentage of yolk-free area (5 to 50-70%); Stage 5, Eye pigmentation (comma-shape) and heart beating; Stage 6, Percentage of yolk-free area (70-80% to 95%) and eye development; Stage 7, Zoea visible and hatching.

Shell-size selection by intertidal sympatric hermit crab species

Abstract

This study evaluated the shell-size selection of three tropical intertidal hermit crabs, *Clibanarius antillensis*, *C. sclopetarius* and *C. vittatus* for their most used shells in nature (*Chicoreus senegalensis*, *Cymatium parthenopeum* and *Cerithium atratum*). Crab size and weight were highly and significantly related to all parameters of the selected shells. These relationships presented higher values of r^2 than those with the shells used, indicating that the crabs are occupying sub-optimal shells in nature. All shell variables were equally related with both crab size and weight. Negative allometric relationships were common and indicated that shells become proportionally smaller and lighter as crab size increases. The relationships between crab and shell dimensions did depend neither on crab nor on shell species. The only influence was recorded in the models fitted for aperture length and width, which showed to be more related to shell architecture than shell length or weight. There were different allometric relationships between dimensions of shells used in nature and selected by the hermit crabs in free access experiments, with the selected shells being proportionally heavier and presenting proportionally larger apertures than shells used in nature as shell length increases.

Keywords: Hermit crabs, shell-size selection, resource use, allometry, *Clibanarius*, diogenidae.

Introduction

The availability of resources may determine the realized niche of individuals and even populations. Hermit crabs depend on gastropod shells as shelters and their availability has been shown to influence crab abundance (Vance 1972, Spight 1977) and their patterns of shell utilization (Scully 1979, Leite *et al.* 1998). Shells may also modulate crab reproduction (Bertness

1981, Fotheringham 1976a, 1980), growth (Markham 1968, Fotheringham 1976b, Bertness 1981) and morphology (Blackstone 1985). Shell availability is thought to be low (Reese 1969, Vance 1972, Bach *et al.* 1976, Spight 1977, but see Scully 1979, Leite *et al.* 1998, Turra and Leite 2001), and more limiting for the largest individuals in natural populations (Spight 1977, 1985). In general, the lower the shell availability, the higher the influence they have on crabs.

Given the important role shells play in hermit crab biology, it is important to determine patterns of shell selection, i.e., identify the preferred shell sizes and species. Many studies described the size relationship between crabs and selected shells (Abrams 1978, Bertness 1980, Arantes 1994). This procedure enabled the calculation of the shell adequacy index by the comparison of optimal or preferred shells with the shells used in nature by the crabs (Vance 1972, Kellogg 1976, Bertness 1980, Gilchrist 1984, Turra in press). Other studies focused on preference of crabs for different shell types (Wilber 1990, Mesce 1993, Turra and Leite 2002).

Hermit crabs have adaptations to locate and obtain new shells (Mesce 1982, Rittschof *et al.* 1990) and exhibit elaborated behaviors associated to shell investigation (Reese 1962, 1963, Brown *et al.* 1993). It was demonstrated that various shell characteristics influence hermit crab preference. Conover (1978) verified the influence of angle of shell axis and position of the shell center of gravity on shell selection by the hermit crab *Pagurus pollicaris*. Floeter *et al.* (2000) suggested that crab tend to maximize shell volume in relation to shell weight when comparing preferred with used shells. Wilber (1990) concluded that shell size was more important in shell choice by the hermit crab *Pagurus longicarpus* than either shell species or presence of damages. Epibionts (Conover 1976, but see Hazlett 1984), background camouflage (Partridge 1980), presence of physical damages (McClintock 1985, Pechenik and Lewis 2000) and hydrodynamism (Hahn 1998) were also demonstrated to influence shell-species selection by hermit crabs. Finally, preference for shells in hermit crabs has been demonstrated to be also dependent on crab previous experience (Elwood *et al.* 1979, Hazlett 1992, 1996, but see Blackstone 1984) and on growth increment during molting (Wada *et al.* 1997).

This study aimed to describe the patterns of shell-size selection by three hermit crab species (*Clibanarius antillensis*, *C. sclopetarius*, and *C. vittatus*) that coexist in the intertidal of Araçá, South-eastern Brazil. Shell selection experiments were done for the most used gastropod shells in this area by these species (Turra and Leite 2002). The relationships of crab size and weight with shell parameters were examined to test the null hypotheses that crab and shell dimensions are positively and isometrically related with each other, that both crab size and weight are similarly correlated to all shell parameters, and that each shell parameters is similarly correlated to either crab size or weight for each combination of crab and shell species. Such models were also compared to field data (Turra and Leite 2002) to test the null hypothesis that crab and shell dimensions are equally related in both situations. The relationships of crab size and weight with shell parameters were compared between crab and shell species for *C. sclopetarius* and *C. vittatus* to test the null hypothesis that shell-size selection by hermit crabs did depend neither on shell type nor on crab species. The relationships between shell length and the other shell variables were calculated for the shells selected in the present study and for those used in the field by the three studied species (data from Turra and Leite 2002) to test the null hypothesis that shell parameters are equally related in both situations.

Material and methods

The hermit crab species were collected in the intertidal of the Araçá region, São Sebastião Channel, South-eastern Brazil (23°49'S, 45°24'W). This is an intertidal flat with two Islets (Pernambuco and Pedroso) composed by many substrate types, from mud to cobbles and rocky shore (Turra *et al.* 2000).

The shell selection experiments were conducted in the coastal laboratory of Centro de Biologia Marinha of Universidade de São Paulo (CEBIMar-USP). A system of plastic trays (30 x 45 x 7 cm) with running seawater and bottom filled with sand was built. The number of crabs (replicates, 19 to 35 individuals) varied among each combination of crab and shell species (see table 1). One crab was maintained in each tray (according to García-Berthou and Hulrbert, 1999) and the shell used after 24 h was considered the preferred one. Sizes of crabs were variable and corresponded to the size range recorded in nature (Turra and Leite 2002). The number and size of shells of each tested gastropod offered to each individual were also variable and depended on the relative size of the crabs. Ten empty shells of *Chicoreus senegalensis* or *Cymatium parthenopeum* were presented to the relatively large-sized *C. sclopetarius* and *C. vittatus*. For the small-sized *C. antillensis*, twenty empty shells of *Cerithium atratum* were used in each replicate. After the experiment, the crabs were removed from the selected shells and then measured (shield length, mm) and weighed (wet weight, g). Shells were also measured (mm) in relation to shell length, shell width, aperture length and aperture width (for scheme see Vance 1972). Shell weight (g) was recorded after drying for 24 h at 80 °C.

The relationships between crab size (or weight) and shell parameters were estimated using the power function ($y=ax^b$) for each combination of crab and shell species. The determination coefficients (r^2) obtained for these relationships were compared among shell parameters using a multiple comparison procedure of correlation coefficients (the square root of the r^2 values were taken for this analysis) for the Student t test (Zar 1999), to test the null hypothesis that all shell dimensions are related to crab size or weight with the same strength. The Student t test was also employed to compare the determination coefficients of the relationships with crab shield length and weight for each shell parameter to test the null hypothesis that crab size and weight can be equally used to estimate the parameters of shells selected by the crabs. The Student t test was used to test the null hypothesis of isometry between crab and shell dimensions: $b=1$ (linear vs. linear dimensions or cubic vs. cubic dimensions); $b=3$ (linear vs. cubic dimensions); $b=1/3$ (cubic vs. linear dimensions). This latter hypothesis is not common in the literature but is a natural consequence of relating a cubic (independent) variable to a linear (dependent) one. The equivalence of the regression lines was tested by comparing the values of b (slope) of the exponential models described above (Zar 1999). This test was employed for *C. sclopetarius* and *C. vittatus* by comparing the slope of the fitted models between shell species for each hermit crab. This procedure was also used to compare the slopes of the models obtained for each shell species (*C. senegalensis* and *C. parthenopeum*) between crab species. These comparisons of slopes (b) between crab and shell species were done to test the null hypothesis that shell selection by hermit crabs did depend neither on shell type nor on crab species.

The power function was also used to establish the relationships between crab size and weight and between length (as an independent variable) and the other parameters of the shells selected in the present study and used by the crabs in the field (data from Turra and Leite 2002; only intact shells were considered in this procedure). The Student t test was employed to contrast the values of r^2 and slope (b) between the relationships for selected and used shells. This regression model was also used to estimate the relationships between crab size (shield length) and the dimensions of the shells used in the field and those selected in a previous study conducted by Turra and Leite (2002).

Results

All three species of hermit crabs showed positive and significant relationships with the dimensions of the preferred shells, with larger/heavier individuals selecting larger/heavier shells

(Tables 1 and 2). The determination coefficients of the models fitted for all combinations of crabs and shells were generally high ($r^2>0.7$) and did not vary among the different shell dimensions/parameters investigated. The smallest values of r^2 were recorded for the relationships of size and weight of *C. antillensis* with the parameters of *Cerithium atratum* and of size and weight of *C. vittatus* with weight and aperture length and width of shells of *C. parthenopeum*. These values of r^2 did not vary between the models relating crab length or crab weight to each shell parameter for each combination of crab and shell species (Table 1 vs. Table 2; Student t test, $p>0.05$ for all comparisons of regression coefficients).

The t test for allometry revealed that isometry occurred only in relationships between shield length of *C. vittatus* and *C. sclopetarius* and some shell dimensions of *C. parthenopeum* and between weight of *C. vittatus* and some shells dimensions of *C. parthenopeum* (Table 1 and 2). In general, negative allometry was recorded in the fitted models (Table 1).

The slope of the relationships of shield length and crab wet weight of *C. vittatus* and *C. sclopetarius* with shell dimensions were compared between shell species (within each crab species) and between crab species (within each shell species) (Table 3). The results showed that slope did vary neither between crab species nor between shell species for most of the comparisons. Differences in slope were recorded only in relationships using shell aperture length and/or width (Table 3). During growth, *Clibanarius vittatus* selected shells of *C. parthenopeum* with proportionally larger apertures than shells of *C. senegalensis*. Similarly, *C. sclopetarius* selected shells with proportionally larger and wider apertures in relation to shells of *C. senegalensis* as crab size increases. In addition, *Clibanarius sclopetarius* preferred proportionally smaller but wider apertures of *C. parthenopeum* than *C. vittatus* as crab size increases. Significant and isometric fits were recorded between crab shield length and weight of the three studied species (Fig. 1; Student t test for allometry: $p>0.05$ for all comparisons).

The parameters of shells of *C. senegalensis*, *C. parthenopeum* and *C. atratum* selected in free choice experiments (present study) and used in the field by the studied hermit crab species (data from Turra and Leite 2002) were well correlated to shell length (Table 4). In general, the relationships recorded for the shells used in the field were as strong as (similar values of r^2, $p>0.05$ for all comparisons) those recorded in shell selection experiments. The better adjusts for the selected shells of *C. senegalensis* in relation to those used in nature were the only exception (Student t test, $p<0.05$ for all comparisons). The length of selected shells of these three gastropod species showed negative allometry with shell width and isometry with shell weight (except by the negative allometry in shells of *C. parthenopeum*) and aperture length and width (Table 4). The length of shells used in nature showed negative allometry with shell weight. Aperture length of *C. senegalensis* and *C. atratum* used in nature showed negative allometry with shell length while the aperture length of *C. parthenopeum* was isometrically related to shell length. In general, the slopes (b) of the models fitted for the parameters of shells used in the field were generally lower than those of the models fitted for the parameters of shells selected in free choice experiments ($p<0.05$ for all comparisons; exception for aperture length of *C. parthenopeum*) and presented equal values of r^2 ($p<0.05$ for all comparisons; except for the lower values of r^2 for *C. senegalensis* in the field). This evidences that selected shells were proportionally heavier and presented proportionally larger apertures than shells used in nature as shell length increases.

The relationships of crab size and shell parameters were also calculated for the data presented in Turra and Leite (2002). The fitted curves for these field data (Table 5) showed lower values of r^2 (all < 0.7) than those calculated for the selected shells in the present study (see Table 1, Student t test, $p<0.05$ for all comparisons).

Discussion

Size relationships with the preferred shells

The relationships between crab size/weight and the parameters of the shells were significant and positive as in other studies of shell-size selection (Abrams 1978, Bertness 1980, Turra and Leite 2002). Similar values of r^2 were recorded between the relationships fitted with the two crab dimensions (size and weight) as dependent variables. Shell dimensions generally showed negative allometry with crab variables while isometry was recorded only between size of *C. sclopetarius* and *C. vittatus* and some dimensions of *C. parthenopeum*. Positive allometry was not observed in the fitted models for selected shells by the studied species. Data on the relationships between crab shield length and dimensions of the shells used in nature (Table 5; data from Turra and Leite 2002) revealed similar patterns. In general, hermit crabs use and select proportionally smaller, narrower-aperture and lighter shells of *C. senegalensis*, *C. parthenopeum* and *C. atratum* as crab size increases. However, this variation in allometry in relationships between different shell variables reinforces the arguments used by Gilchrist (1984) on the caution with non-linearity in the relationships between crab size and the parameters of used and selected shells in the calculation of shell adequacy indexes (Vance 1972, Kellogg 1976, Bertness 1980). In contrast, all shell variables were equally correlated with crab size/weight indicating that different shell dimensions can produce similar estimates of crab size. This was a direct consequence of the high correlation between shell parameters (see Table 4). Moreover, these results may also evidence that the crabs are not accessing particular shell characteristics as argued by Abrams (1978), Conover (1978) and Floeter *et al.* (2000). If so, one would expect better correlations with a given variable in relation to others.

Shell selection vs. shell use in nature

Many studies have been carried out to contrast shell use (size or species) in nature with shell selection patterns. In general, hermit crabs use sub-optimal shells in the field, which is evidenced by low r^2 values for the relationships between crab and shell dimensions (Scully 1983, Turra and Leite 2001, 2002, Turra in press), by the extent that crabs retract into their shells (VAI, visual adequacy index, Abrams 1978, Turra in press), and by the comparison between the sizes of used and expected optimal shells (SAI, Shell Adequacy Index, see Vance 1972, Bertness 1980, Turra in press).

The power functions fitted for the data on shell use in nature (Turra and Leite 2002) showed lower values of r^2 (see Table 5) than those calculated for the selected shells in the present study, reinforcing shell limitation in the field for the studied crabs. These low values may be also a consequence of the frequent utilization of shells with encrustation and physical damages in nature (Turra in press). In general, crab size showed negative allometry with all variables of shells used in nature (except for the isometry recorded in shells of *C. parthenopeum*). These results also showed that the three hermit crab species used relatively lighter (smaller) shells in nature as crab size increases in comparison to those selected in free access experiments. This may evidence a stronger shell limitation for *C. sclopetarius* and *C. vittatus* than for *C. antillensis*. In fact, this agrees with the assumption that shell limitation is stronger for the largest individuals in a given population (Spight 1977, 1985).

When crabs are exposed to an unlimited shell supply, the selected shells present a clear pattern for the allometric relationships between their dimensions (all studied gastropod species showed similar patterns, see Table 4). The values of r^2 were also generally higher for selected than used shells. When the relationships of shells used in the field by the studied hermit crabs (see Turra and Leite 2002) are calculated, the model with shell aperture tended to be negatively allometric rather than isometric (Table 4). In this way, selected shells were, in general, relatively heavier and presented larger apertures than shells used in nature as shell length increases. The values of r^2 were also similar in these two situations, except by the significantly lower values of r^2 of the shells of *C. senegalensis* used by the crabs in the field. As relative shell weight can be considered a measure of shell architectural defense against shell predators (Bertness and Cunningham 1981), the preference for relatively heavier shells than those used in nature may reflect an adaptation against predator pressure, which is considered high in the study site due to the high abundance of xanthid crabs and blue crabs (A. Turra unpublished data). Shell aperture was a parameter strongly related to shell architecture (Turra and Leite 2002, present study, see below) and probably may constraint crab morphology more than shell length or weight (see Chapter 6). Thus, selection of shells with relatively larger apertures may prevent (or delay) shell influence on crab form.

Influence of shell architecture on size relationship between hermit crabs and selected shells

Shells of similar architectures as *C. senegalensis* and *C. parthenopeum*, which are both elongated and medium spire, may present similarity in the choice by hermit crabs in relation to shell length and weight. Shell aperture had a more evident effect on crab-shell size relationships and seemed to be more strongly correlated to shell architecture than shell length (see Table 3). These results may lead one to argue that only shell length and weight can be compared pooling different shell types. Field data reinforce this assumption. Orians and King (1964) found good fits between crab size (carapace width) and shell greatest length. Turra and Leite (2002) showed that the relationships between crab size and the weight of shells of *C. senegalensis* and *C. parthenopeum* used in nature by *C. sclopetarius* and *C. vittatus* did depend neither on shell nor on crab species. Wilber (1990) showed that relative shell size was a more important shell characteristic than shell species in shell selection by hermit crabs. However, what happens with shell size selection if shells with quite different architectures, i.e., globose, elongated, and high spire, are offered to the crabs is still to be tested (but see Chapter 6). Abrams (1978) also questioned the comparison of architecture-related shell dimensions among different shell types. He argued that taking several shell measurements and employing multivariate techniques (see Kuris and Brody 1976, Mitchell 1976) would lessen the problem.

Differences between methods

Despite shell selection experiments are a fundamental tool in understanding the ecology and the behavior of hermit crabs, studies on their experimental design are recent (Liszka and Underwood 1990, Wilber 1993, García-Berthou and Hulbert 1999). Wilber (1993) argued that testing shell preferences without individual crabs as replicates (pseudoreplication) does not lead to different results from a design where shells are offered to single crabs independently. More recently, García-Berthou and Hulbert (1999) questioned Wilber's arguments and evidenced the problems that pseudoreplication brings to such studies. Turra and Leite (2002) used the methodology proposed by Wilber (1993), offering a large amount of shells of the most used species in nature to groups of 30-40 hermit crabs and calculating size relationships between crabs and preferred shells through linear regressions. These data were reanalyzed using the exponential model (see Table 5) to enable comparisons with the present study, where each individual of each hermit crab species was treated as a replicate and to which a pool of shells of a single species was offered.

The relationship of the selected shells for this previous study (Turra and Leite 2002) revealed variable values of r^2 in relation to the present study (Table 5). Such comparisons showed that the methodology employed in the present study lead to better fits for the relationships of crab shield length and shell weight between *C. vittatus* and *C. senegalensis* and between *C. sclopetarius* and both *C. senegalensis* and *C. parthenopeum*. In contrast, the relationships between *C. vittatus* and *C. parthenopeum* and between *C. antillensis* and *C. atratum* calculated in the present study showed poorer fits than those in the previous study. The differences in the results between the two studies may be due to: 1. The strength of the relationship (r^2) depend on the shell type (lower values for *C. parthenopeum* in relation to *C. senegalensis* for both *C. vittatus* and *C. sclopetarius*, see Table 1); 2. Different shell types may have different allometric relationships among their dimensions and thus cause noise in the crab-shell relationships (the values for *C. sclopetarius* increased when shells were treated separately in the present study) thus reducing the r^2 values; 3. The wider variation in size of *C. antillensis* used by Turra and Leite (2002) probably caused the higher values of r^2 in that study. The largest crabs of this species, which do not use shells of *C. atratum*, were not included in the present study.

The values of b for the selected shells by *C. antillensis* in the former study was higher than that in the present study, indicating that this species selected proportionally heavier shells in that study as crab size increases (Student t test, $p<0.05$). This was probably caused by the inclusion of larger individuals and larger shell types in this former study. The opposite situation occurred for *C. vittatus* and *C. sclopetarius* (Student t test, $p<0.05$ for both comparisons), which selected proportionally lighter shells in the former than in the present study as crab size increases. This again may be caused by the use of many shell types in the former study, i.e., utilization of shells of *Stramonita haemastoma* and *Leucozonia nassa*, which were frequently selected by the crabs (Turra and Leite 2002), may brought noise to the analysis.

Despite these arguments, shell-size selection was demonstrated to depend on crab sex (Arantes 1994). The patterns of shell preference may also vary between sites with different shell supplies (Scully 1979). Moreover, shell-size preference is also influenced by the past experience of the crabs (Elwood *et al.* 1979, Hazlett 1992, 1996). Once shell availability (Leite *et al.* 1998) and the composition of the hermit crab ensemble (Bach *et al.* 1976, Leite *et al.* 1998) may vary among areas, the same hermit crab species may be exposed to quite different shell supplies and have quite different past experiences. All these arguments suggest that further studies on shell-size selection, and consequently, the calculation of shell adequacy indexes (see Vance 1972, Bertness 1980, Gilchrist 1984), should be based on data from each sex and for each study location (if studying more than one site). Moreover, despite the size relationships involving crab size or weight and weight of the selected shell were demonstrated to be independent on shell architecture, different shell species may be used by crabs of different sizes (see Turra and Leite 2002). However, this may introduce noise into the analysis (as discussed above for *C. antillensis*) and may lead to biased estimates of shell-size preferences of hermit crabs. In this way, these calculations should be done for each shell type independently, at least for the commonest types, and compared to the correspondent field data on shell use by the hermit crabs to calculate shell adequacy index. The present study reinforces that studies on shell-size selection should be conducted considering each crab as a replicate as proposed by García-Berthou and Hurlbert (1999).

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Table 1. Regression analysis ($y=ax^b$) between the shield length (mm) of the hermit crabs *Clibanarius vittatus*, *C. sclopetarius*, and *C. antillensis* and shell length (mm), shell width (mm), aperture length (mm), aperture width (mm) and shell weight (g) of the shells of *Chicoreus senegalensis*, *Cymatium parthenopeum* and *Cerithium atratum* selected by these crabs in free access experiments. Number of observations is shown in brackets. The two-tailed Student t test was employed to test the null hypothesis for isometry (b=1 or b=3)

Shell parameters	r^2	p	Equation	b
C. vittatus in *C. senegalensis*† (n=33)				
Shell length	0.757	<0.001	$y=15.251x^{0.677}$	$<1^{***}$
Aperture length	0.735	<0.001	$y=4.234x^{0.712}$	$<1^{*}$
Aperture width	0.754	<0.001	$y=3.016x^{0.743}$	$<1^{*}$
Shell weight	0.733	<0.001	$y=0.359x^{1.900}$	$<3^{***}$
C. vittatus in *C. parthenopeum* (n=25)				
Shell length	0.744	<0.001	$y=9.083x^{0.941}$	$=1^{ns}$
Shell width	0.702	<0.001	$y=5.996x^{0.859}$	$=1^{ns}$
Aperture length	0.691	<0.001	$y=2.603x^{1.042}$	$=1^{ns}$
Aperture width	0.601	<0.001	$y=3.073x^{0.751}$	$=1^{ns}$
Shell weight	0.569	<0.001	$y=0.201x^{2.133}$	$=3^{ns}$
C. sclopetarius in *C. senegalensis*† (n=19)				
Shell length	0.886	<0.001	$y=15.558x^{0.675}$	$<1^{***}$
Aperture length	0.873	<0.001	$y=4.637x^{0.674}$	$<1^{***}$
Aperture width	0.903	<0.001	$y=3.075x^{0.737}$	$<1^{***}$
Shell weight	0.895	<0.001	$y=0.260x^{2.064}$	$<3^{***}$
C. sclopetarius in *C. parthenopeum* (n=25)				
Shell length	0.826	<0.001	$y=9.836x^{0.880}$	$=1^{ns}$
Shell width	0.824	<0.001	$y=7.177x^{0.749}$	$=1^{ns}$
Aperture length	0.716	<0.001	$y=3.957x^{0.806}$	$<1^{*}$
Aperture width	0.760	<0.001	$y=2.216x^{0.900}$	$=1^{ns}$
Shell weight	0.825	<0.001	$y=0.158x^{2.248}$	$<3^{*}$
C. antillensis in *C. atratum* (n=34)				
Shell length	0.604	<0.001	$y=16.307x^{0.486}$	$<1^{***}$
Shell width	0.647	<0.001	$y=6.369x^{0.491}$	$<1^{***}$
Aperture length	0.612	<0.001	$y=6.351x^{0.502}$	$<1^{***}$
Aperture width	0.503	<0.001	$y=3.718x^{0.511}$	$<1^{***}$
Shell weight	0.635	<0.001	$y=0.197x^{1.633}$	$<3^{***}$

† Shell width was not evaluated in this shell species because of shell spines. *, $p<0.05$; ***, $p<0.001$; ^{ns}, not significant.

Table 2. Regression analysis ($y=ax^b$) between the weight (g) of the hermit crabs *Clibanarius vittatus*, *C. sclopetarius*, and *C. antillensis* and shell length (mm), shell width (mm), aperture length (mm), aperture width (mm) and shell weight (g) of the shells of *Chicoreus senegalensis*, *Cymatium parthenopeum* and *Cerithium atratum* selected by these crabs in free access experiments. Number of observations is shown in brackets. The two-tailed Student t test was employed to test the null hypothesis for isometry (b=   or b=1)

Shell parameters	r^2	p	Equation	b
C. vittatus in *C. senegalensis*[†] (n=33)				
Shell length	0.802	<0.001	$y=48.492x^{0.225}$	$<^{***}$
Aperture length	0.809	<0.001	$y=14.237x^{0.241}$	$<^{***}$
Aperture width	0.813	<0.001	$y=10.775x^{0.244}$	$<^{***}$
Shell weight	0.797	<0.001	$Y=9.304x^{0.619}$	$<1^{***}$
C. vittatus in *C. parthenopeum* (n=25)				
Shell length	0.808	<0.001	$y=47.201x^{0.283}$	$=^{ns}$
Shell width	0.766	<0.001	$y=27.003x^{0.258}$	$<^{*}$
Aperture length	0.742	<0.001	$y=16.164x^{0.309}$	$=^{ns}$
Aperture width	0.629	<0.001	$y=11.448x^{0.222}$	$<^{*}$
Shell weight	0.621	<0.001	$y=8.423x^{0.636}$	$<1^{*}$
C. sclopetarius in *C. senegalensis*[†] (n=19)				
Shell length	0.858	<0.001	$y=49.827x^{0.203}$	$<^{***}$
Aperture length	0.858	<0.001	$y=14.817x^{0.204}$	$<^{***}$
Aperture width	0.897	<0.001	$y=10.938x^{0.224}$	$<^{***}$
Shell weight	0.898	<0.001	$y=9.277x^{0.610}$	$<1^{***}$
C. sclopetarius in *C. parthenopeum* (n=25)				
Shell length	0.843	<0.001	$y=46.014x^{0.264}$	$<^{*}$
Shell width	0.849	<0.001	$y=26.610x^{0.226}$	$<^{***}$
Aperture length	0.759	<0.001	$y=16.190x^{0.245}$	$<^{***}$
Aperture width	0.798	<0.001	$y=10.684x^{0.273}$	$<^{*}$
Shell weight	0.842	<0.001	$y=8.111x^{0.671}$	$<1^{***}$
C. antillensis in *C. atratum* (n=34)				
Shell length	0.622	<0.001	$y=39.583x^{0.181}$	$<^{***}$
Shell width	0.620	<0.001	$y=15.125x^{0.165}$	$<^{***}$
Aperture length	0.551	<0.001	$y=15.173x^{0.161}$	$<^{***}$
Aperture width	0.485	<0.001	$y=9.122x^{0.169}$	$<^{***}$
Shell weight	0.631	<0.001	$y=3.486x^{0.542}$	$<1^{***}$

[†] Shell width was not evaluated in this shell species because of shell spines. *, $p<0.05$; ***, $p<0.001$; ^{ns}, not significant.

Table 3. Comparison of slopes (b) of the relationships of shield length (mm) and weight (g) of the hermit crabs with the parameters of the selected shells [length (mm), width (mm), aperture length (mm), aperture width (mm) and weight (g)] between shells (*Chicoreus* vs. *Cymatium*) and crab species (*C. sclopetarius* vs. *C. vittatus*), through the Student t test. Degrees of freedom are shown in brackets.

Comparison	t	p
Between shell species (*Chicoreus* vs. *Cymatium*)		
C. vittatus (df=50)		
Shield length vs.		
Shell length	-1.42	ns
Shell aperture length	-4.44	<0.001
Shell aperture width	-0.17	ns
Shell weight	-1.58	ns
Crab weight vs.		
Shell length	-0.12	ns
Shell aperture length	-0.37	ns
Shell aperture width	0.18	ns
Shell weight	-0.04	ns
C. sclopetarius (df=36)		
Shield length vs.		
Shell length	-1.34	ns
Shell aperture length	-2.15	<0.05
Shell aperture width	-3.81	<0.001
Shell weight	-1.62	ns
Crab weight vs.		
Shell length	-0.40	ns
Shell aperture length	-0.71	ns
Shell aperture width	-1.22	ns
Shell weight	-0.56	ns
Between crab species (*C. sclopetarius* vs. *C. vittatus*)		
Chicoreus senegalensis (df=44)		
Shield length vs.		
Shell length	0.01	ns
Shell aperture length	0.80	ns
Shell aperture width	0.18	ns
Shell weight	-1.53	ns
Crab weight vs.		
Shell length	0.13	ns
Shell aperture length	0.32	ns
Shell aperture width	0.24	ns
Shell weight	0.03	ns
Cymatium parthenopeum (df=42)		
Shield length vs.		
Shell length	0.30	ns
Shell width	1.02	ns
Shell aperture length	2.69	<0.05
Shell aperture width	-2.65	<0.05
Shell weight	0.73	ns
Crab weight vs.		
Shell length	0.04	ns
Shell width	0.13	ns
Shell aperture length	0.31	ns
Shell aperture width	-0.37	ns
Shell weight	-0.09	ns

Table 4. Regression analysis ($y=ax^b$) between the parameters of the shells [Independent variable: shell length (mm); dependent variables: shell width (mm), aperture length (mm), aperture width (mm) and shell weight (g)] of *Chicoreus senegalensis*, *Cymatium parthenopeum* and *Cerithium atratum* selected in free access experiments (present study) and used by the crabs in the field (data from Turra and Leite 2002). Data on shell use and selection by different crab species were pooled: *C. senegalensis* - *C. sclopetarius* and *C. vittatus*; *C. parthenopeum* - *C. sclopetarius* and *C. vittatus*; *C. atratum* - only *C. antillensis*. Number of observations is shown in brackets. The two-tailed Student t test was employed to test the null hypothesis for isometry (b=1 or b=3)

Shell parameters	r^2	p	Equation	b
Selected shells				
C. senegalensis† (n=51)				
Aperture length	0.922	<0.001	$y=0.305x^{0.994}$	$=1^{ns}$
Aperture width	0.904	<0.001	$y=0.209x^{1.018}$	$=1^{ns}$
Shell weight	0.888	<0.001	$y=0.001x^{2.817}$	$=3^{ns}$
C. parthenopeum (n=49)				
Shell width	0.916	<0.001	$y=1.011x^{0.855}$	$<1^{***}$
Aperture length	0.866	<0.001	$y=0.374x^{0.982}$	$=1^{ns}$
Aperture width	0.866	<0.001	$y=0.311x^{0.929}$	$=1^{ns}$
Shell weight	0.837	<0.001	$y=0.001x^{2.366}$	$<3^{***}$
C. atratum (n=31)				
Shell width	0.801	<0.001	$y=0.819x^{0.780}$	$<1^{*}$
Aperture length	0.740	<0.001	$y=0.613x^{0.869}$	$=1^{ns}$
Aperture width	0.586	<0.001	$y=0.371x^{0.864}$	$=1^{ns}$
Shell weight	0.808	<0.001	$y=0.001x^{2.624}$	$=3^{ns}$
Shells used in the field ‡				
C. senegalensis				
Aperture length (n=69)	0.756	<0.001	$y=0.499x^{0.871}$	$<1^{*}$
Shell weight (n=55)	0.733	<0.001	$y=0.001x^{2.405}$	$<3^{*}$
C. parthenopeum				
Aperture length (n=23)	0.832	<0.001	$y=0.208x^{1.173}$	$=1^{ns}$
Shell weight (n=20)	0.784	<0.001	$y=0.001x^{2.317}$	$<3^{*}$
C. atratum				
Aperture length (n=190)	0.701	<0.001	$y=0.656x^{0.851}$	$<1^{*}$
Shell weight (n=162)	0.665	<0.001	$y=0.001x^{2.218}$	$<3^{***}$

† Shell width was not evaluated in this shell species because of shell spines; ‡ Shell and aperture width were not evaluated in the field samples taken by Turra and Leite (2002). *, $p<0.05$; ***, $p<0.001$; ^{ns}, not significant.

Table 5. Regression analysis ($y=ax^b$) between the parameters of the shells [shell length (mm), aperture length (mm) and shell weight (g)] of *Chicoreus senegalensis*, *Cymatium parthenopeum* and *Cerithium atratum* used in the field and shield length (mm) of *Clibanarius vittatus*, *C. sclopetarius*, and *C. antillensis* (data from Turra and Leite 2002). This model was also fitted for the relationship between crab shield length and the weight of the shells selected in the free access experiments conducted by Turra and Leite (2002). Number of observations are shown in brackets. The two-tailed Student t test was employed to test the null hypothesis for isometry (b=1 or b=3)

Shell parameters	r^2	p	Equation	b
Shells used in the field †				
C. vittatus in *C. senegalensis*				
Shell length (207)	0.370	<0.001	$y=20.747x^{0.486}$	$<1^{***}$
Aperture length (206)	0.337	<0.001	$y=5.543x^{0.544}$	$<1^{***}$
Shell weight (205)	0.375	<0.001	$y=0.604x^{1.582}$	$<3^{***}$
C. vittatus in *C. parthenopeum*				
Shell length (26)	0.510	<0.001	$y=12.637x^{0.701}$	$<1^{*}$
Aperture length (23)	0.634	<0.001	$y=1.921x^{1.161}$	$=1^{ns}$
Shell weight (26)	0.091	<0.001	$y=3.801x^{0.586}$	$<3^{***}$
C. sclopetarius in *C. senegalensis*				
Shell length (163)	0.499	<0.001	$y=18.349x^{0.534}$	$<1^{***}$
Aperture length (161)	0.609	<0.001	$y=4.561x^{0.632}$	$<1^{***}$
Shell weight (169)	0.552	<0.001	$y=0.437x^{1.717}$	$<3^{***}$
C. sclopetarius in *C. parthenopeum*				
Shell length (26)	0.630	<0.001	$y=7.308x^{0.974}$	$=1^{ns}$
Aperture length (24)	0.652	<0.001	$y=2.141x^{1.147}$	$=1^{ns}$
Shell weight (28)	0.452	<0.001	$y=0.254x^{1.866}$	$<3^{*}$
C. antillensis in *C. atratum*				
Shell length (439)	0.285	<0.001	$y=17.259x^{0.404}$	$<1^{***}$
Aperture length (438)	0.257	<0.001	$y=6.547x^{0.444}$	$<1^{***}$
Shell weight (420)	0.180	<0.001	$y=0.359x^{1.202}$	$<3^{***}$
Shells selected in free access experiments				
C. vittatus				
Shell weight (37)	0.702	<0.001	$y=0.655x^{1.489}$	$<3^{***}$
C. sclopetarius				
Shell weight (35)	0.271	<0.001	$y=1.212x^{1.296}$	$<3^{***}$
C. antillensis				
Shell weight (37)	0.752	<0.001	$y=0.066x^{2.605}$	$=3^{ns}$

† Shell and aperture width were not evaluated in the field samples taken by Turra and Leite (2002). *, $p<0.05$; ***, $p<0.001$; ^{ns}, not significant.

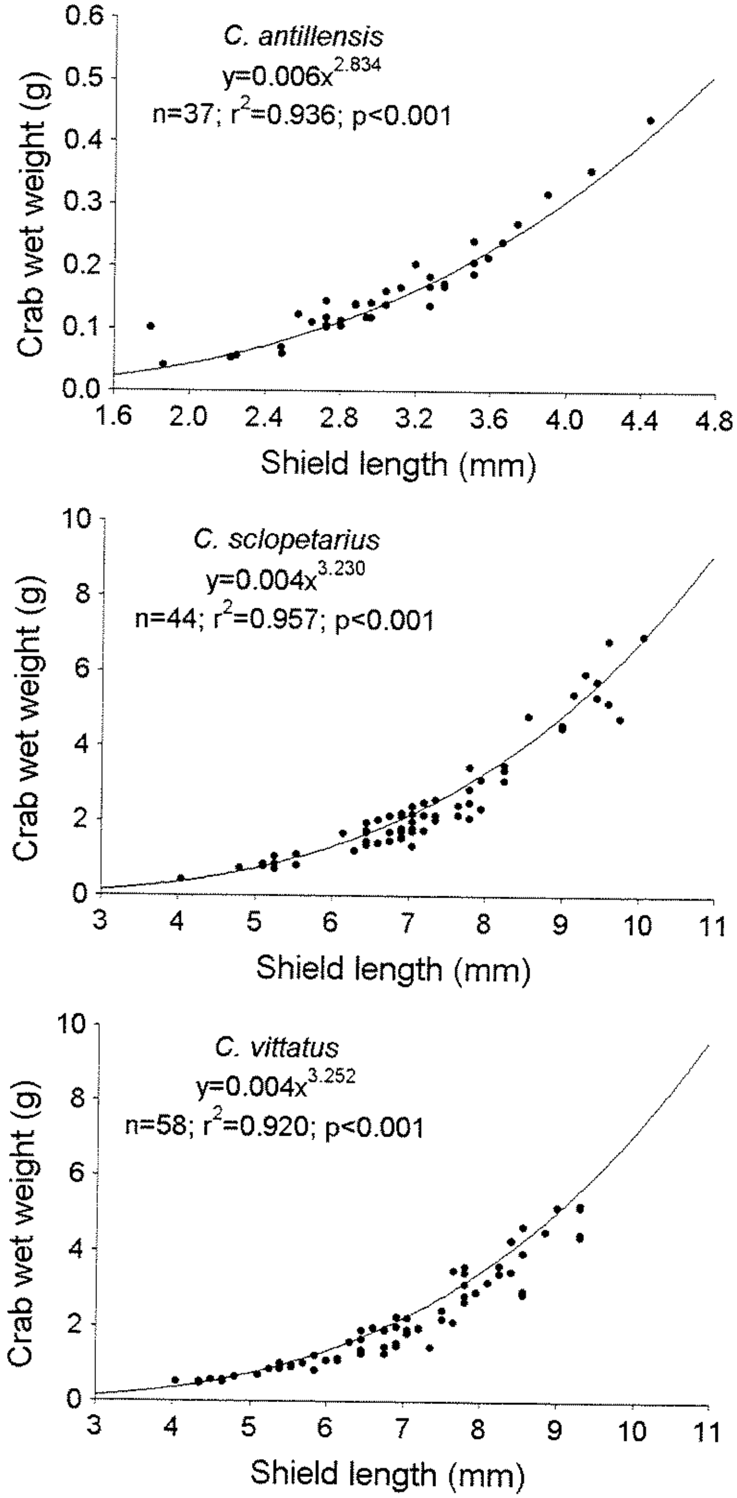

Fig. 1. Power relationships ($y=ax^b$) between shield length (mm) and wet weight (g) of *Clibanarius vittatus*, *C. sclopetarius*, and *C. antillensis* used in the shell choice experiments. Student t test for allometry: $p>0.05$ for all comparisons.

The molding hypothesis: linking shell use with hermit crab growth, morphology and shell-species selection

Abstract

The "molding hypothesis" (Elwood *et al.* 1979) suggests that shell-species selection by hermit crabs may be influenced by past experience in shell use through shell-imposed alterations in crab morphology. The present study was designed to understand how plastic is the shell-species selection pattern in hermit crabs. Shell use was demonstrated to influence crab growth and morphology. Individuals reared in shells of *Tegula* attained larger sizes than individuals in *Cerithium* or *Morula*. Crab growth was also dependent on crab sex once males attained larger sizes and presented longer intermolt periods than females. The most conspicuous influence on crab morphology was in dorso-ventral flattening: *Olivella>Morula>Cerithium>Tegula*. Shell treatment and crab sex also influenced length and width of the right chelae (*Tegula>Morula*; males>females). Crabs from all treatments and those collected in nature in shells of *Olivella* selected mainly shells of *Cerithium*. However, the previous experience of the crabs strongly influenced their shell-species selection pattern. Individuals maintained in *Morula* selected shells of this species more frequently than individuals reared in *Tegula* or *Cerithium*. The shell-species selection pattern of individuals reared in *Morula* was similar to that of the individuals collected in *Olivella* in nature. This influence of past experience was demonstrated to depend on the shell type once the patterns of shell-species selection of individuals reared in *Tegula* or *Cerithium* were identical. These differences in shell-species selection pattern reflected directly the differences in crab dorso-ventral flattening. Dorso-ventral compressed individuals (those in shells of *Morula* or *Olivella*) showed higher probability of selecting narrow-aperture shells than did rounded individuals (those in shells of *Tegula* or *Cerithium*). The weight of the selected shells at the end of the experiment (final weight) was not dependent on shell treatment, indicating that shell

weight depend more on crab size than on their previous experience with shells with different architectures.

Keywords: Phenotypic plasticity, shell-species selection, resource selection, growth, molding hypothesis, *Pagurus*

Introduction

Discussion on the relationship of environmental patchiness and predictability with natural selection has lead to the understanding of the evolution of habitat selection (MacArthur and Levins 1967). Natural selection favors habitat choice in a patchy environment with predictable cues. An elegant parallel was done with the shell use behavior of hermit crabs by Lively (1988). He hypothesized that each shell species was a different environment with particular architecture-related characteristics and that preference for shells was selected in hermit crabs in the evolutionary time. It is assumed that hermit crabs have intrinsic shell-species preferences. In contrast, shell availability in nature is highly unpredictable and variable within the geographic distribution of each species (Blackstone 1985, Leite *et al.* 1998). So, natural selection may have favored plasticity in habitat (shell) use in hermit crabs (Hazlett 1995). In addition, a given hermit crab species may be exposed to quite different shell supplies in habitats with quite different selective pressures, i.e., predation, hydrodynamism, structural complexity and aerial exposure. In this way, one would ask if preferences for shells might be a plastic character, i.e., are shell preferences habitat induced? This would be expected once behavior is a flexible phenotypic character highly influenced by the environment (West-Eberhard 1989). It was demonstrated that shell-species preferences might vary ontogenetically (Elwood *et al.* 1979, Blackstone and Joslyn 1984) but the extent to which it was due to availability or use of different shell types in different size classes is still to be investigated. Shell-size (Scully 1979) and shell-species (Blackstone 1985) preferences were demonstrated to vary between populations and past-experience was demonstrated to influence crab behavior (Jackson and Elwood 1989, Hazlett 1995) and shell-size selection (Hazlett 1992, but see Hazlett 1996). The "molding hypothesis" (Elwood *et al.* 1979, Elwood and Kennedy 1988) was proposed to explain such results. The modifications in crab preference after using a particular shell type would be due to the effect of shells in altering, i.e., molding, crab morphology. There are some records of dorso-ventral flattening in hermit crabs occupying narrow-aperture shells in nature (McLaughlin and Bailey-Brock 1975, Vermeij 1978) and experiments have showed the effect shell species has in shaping crab morphology

(Blackstone 1984, Blackstone and Joslyn 1984). The question on the effects of endogenous vs. exogenous factors on shell-species selection by hermit crabs is still controversial. There are indirect evidences that previous use of a certain shell species will predispose crabs to select them in future choices (Elwood *et al.* 1979, Hahn 1998). However, Blackstone (1984) and Elwood and Kennedy (1988) refuted this hypothesis for *Pagurus longicarpus* and *P. bernhardus*, respectively. The present study experimentally tested the molding hypothesis and showed that it may explain patterns of shell-species preferences in hermit crabs.

Material and methods

Species and study site

The São Sebastião Channel (South-eastern Brazil) is a sheltered area composed of different environments, from rocky shores to mangroves. Hermit crabs are very conspicuous in the channel, especially in the intertidal Araçá region (23°49'S, 45°24'W). *Pagurus criniticornis* is an intertidal and shallow subtidal heterochelic species (Forest and Saint Laurent 1967) that occupy mainly muddy substrates in this region (Turra *et al.* 2000). This species has high levels of activity during the entire day (Chapter 1) and occupies mainly shells of *Cerithium atratum* in the Araçá region (Leite *et al.* 1998, Chapter 7). The hermit crabs also occupy other shell species in the channel (Leite *et al.* 1998) such as *Morula nodulosa*, *Tegula viridula*, *Olivella minuta* and *Stramonita haemastoma*. These shells have different architectures, from high-spire (*Cerithium*) to medium- (*Morula*, *Olivella* and *Stramonita*) and low-spire (*Tegula*). Shell aperture is also highly variable among species, being rounded (*Tegula*), imperfectly rounded (*Stramonita* and *Cerithium*) or narrowed (*Olivella* and *Morula*).

Laboratory conditions and experimental design

A closed water circulation system was built to keep the hermit crabs. Six water tables (60 x 50 cm) were connected to a water reservoir in which the water was filtered and pumped. Thirty percent of the water was exchanged monthly and the salinity was fixed at 33 ‰. Water temperature was maintained at 23°C. The bottom of the water tables was filled with biological filter plates and an auxiliary underwater pump was installed in the center of the aquarium. The orientation of the pump was changed daily in a clockwise manner to homogenate water circulation. In each water table 110 hexagonal numbered porcelain cups (3 cm maximum diameter) were arranged in a beehive pattern. A total of 660 individuals (one in each cup) were used in the experiment. This high number of individuals was used to balance crab mortality during the experiment. These cups were covered with a 2 mm mesh using a silicon band and with a larger central hole (5 mm) to enable manipulation.

The individuals of *Pagurus* were collected manually during low tides in the Araçá region in May 1999, transported to the laboratory and separated into three groups. Small-sized individuals in shells of *Olivella minuta* were used in the beginning experiment to control for crab size (shield length, 0.76 to 1.67 mm) and past experience in shell use. These individuals were immature once none of the females presented ripe ovaries at the end of the experiment. A large amount (>1000) of each tested shell species (shell treatments: *Cerithium*, *Tegula* and *Morula*) with sizes matching the size range of the hermit crabs was offered to each group in separate aquaria. After three days, the crabs, now in the preferred shell of each shell treatment, were introduced in the water tables. This procedure enabled a complete standardization of the relative crab/shell size in the beginning of the experiment. The position of the crabs was systematically determined in the water tables following the linear sequence *Cerithium/Tegula/Morula* from the first to the last cup available.

The crabs were reared in isolation and fed each three days with commercial phosphate-free fish food. Faeces and/or food remains were removed daily. The presence of exuvia was observed daily. The exuvia were removed with a pipette and preserved in alcohol 70% for posterior measurements (shield length, mm). As crab molted new and larger shells were not supplied, differently from Blackstone (1985). This procedure simulated a shell limitation situation. The crabs were reared under these conditions until they have molted six times.

Shell selection experiment

After six molts the crabs were submitted to a new shell selection experiment. The preference for shells of the three treatments (*Cerithium*, *Tegula* and *Morula*) was tested for each crab individually. The previous shell was marked to enable its identification after the experiment, twenty shells of each shell treatment and of variable sizes were offered to each crab for 24 h and then the preferred shell type was recorded. These experiments were conducted in circular glass cups (70 mm diameter x 100 mm height). A "control" treatment for the shell-species selection was also employed using individuals collected in shells of *Olivella* in nature. A true control was not possible because the tiny *Olivella* shells do not attain large enough sizes to enable shell selection after crabs have molted six times. The results for this species revealed the shell-species selection pattern of the individuals prior to experimentation. The crabs were then removed from the selected shell and the following measurements taken: shield length, shield width (widest portion), shield height (from sternum at the first pereopod to the highest part of the shield), length and width (widest portion) of the right and left chela (propodus). The sex of the crab was also recorded. The used (experimental) and selected shells were dried for 24 h at 100°C and then weighed.

Data analysis

The shield length of the exuvia sampled during the experiment was plotted against cumulative molting time using an extension of the Classical Freundlich or allometric model ($y=a+bx^c$) for each tested individual. The von Bertalanffy growth function (Fotheringham 1976a) could not be fitted due to crab size used in the experiment was of a very limited size range. The initial size was set as parameter (a). The growth parameter (c), the size of the crabs at the end of the experiment and the total cumulative molting time needed to undertake six molts were compared between shell treatments and crab sex through ANCOVA taking initial size as covariate. Post hoc pair-wise comparisons (Scheffé's test) were done among shells treatments. Pearson correlation analysis was employed to relate crab final size, cumulative time, growth constant (c), and crab initial size (a) with each other.

The influence of shell treatment and crab sex on crab morphology was done using ANCOVA with crab shield length (final) as covariate. Right-left chela asymmetry and right chelae shape (length/width) were also compared among treatments. This procedure enabled the comparison of crab dimensions taking the variation in size into account. ANCOVA was followed by the Scheffé's test for multiple pair-wise comparisons.

To evaluate the effect of past experience on shell-species selection pattern a 4x3 contingency table (shells used *vs.* shells selected; data of individuals collected in shells of *Olivella* in nature were included as a "control treatment") was built and a log likelihood G test was employed. Paired comparisons were done through partial G tests. A cluster analysis of the shell selection pattern was done comparing shell and "control" treatments using UPGMA, percent similarity measure and original data (Krebs 1989). The effect of shell treatment on shell-weight selection was also tested. Crabs that have selected shells of *Cerithium* and *Morula* were analyzed independently (only a few individuals selected *Tegula* and were not included in this analysis, see below). The weight of the selected shells and the increment in weight (final – initial shell weights) were compared among shell treatments and sexes through ANCOVA using crab shield length as covariate. The Scheffé's test for multiple pair-wise comparisons was also employed. Statistical analyses were based on Zar (1999) with the significant level fixed at 5%.

Results

Influence of shells on crab growth

A high variation in growth was recorded among the individuals of *Pagurus criniticornis* reared for six molts in the laboratory. The size increment varied from 0.216 to 0.810 mm and the cumulative time needed to undertake six molts varied from 73 to 182 days. Size tended to stabilize through time toward an asymptote (Fig. 1), which was evidenced by negative allometry indicated by the mean value of the growth constant being significantly smaller than unit ($c=0.58$; isometric relationship: $c=1$; Student t test, $p<0.05$). The initial size of the crabs was demonstrated to positively influence final size and cumulative time and to negatively influence the growth constant of the allometric model (Fig. 2, Pearson correlation: $p<0.05$ for all comparisons). In other words, larger crabs would present larger final sizes and longer intermolt periods but smaller values for the growth constant than smaller crabs. A significant variation in the initial size was recorded in the tested crabs (Table 1), with shells of *Cerithium* being selected by larger individuals than those of *Tegula* (Scheffé's, $p<0.05$). To eliminate the effect of variable initial sizes on growth analysis this parameter was considered as covariate in subsequent analysis. The final size was strongly dependent on shell treatment (*Tegula*=*Cerithium*, *Cerithium*=*Morula*, *Tegula*>*Morula*) and on crab sex (M>F). The cumulative time to undertake six molts depended on crab sex, with the intermolt period of males being longer than that of the females. A positive relationship was recorded between cumulative growth time and crab final size (Pearson correlation; $p<0.05$). The rate with which final size was attained (c) did depend neither on shell species nor on crab sex (Table 1). This growth parameter was negatively influenced by the cumulative growth time and crab final size (Pearson correlation; $p<0.05$ for both comparisons).

Influence of shells on crab morphology

The evaluation of the influence of shell type (treatment) on hermit crab morphology was done through covariance analysis. Shell morphology varied markedly among shell species: globose or low-spire (*Tegula*), elongated or medium-spire (*Morula*) and high-spire (*Cerithium*). They showed significant variations in their aperture width when aperture length was considered as covariate (ANCOVA; Shell species: F=853.732, df=2, $p<0.001$; aperture length: F=2220.006, df=1, $p<0.001$). *Morula* presented narrower apertures than *Cerithium*, which, in turn, presented narrower apertures than *Tegula* (Scheffé's: $p<0.05$ for all comparisons). A similar comparison revealed that shell weight also varied among species (*Morula*>*Tegula*>*Cerithium*, Scheffé's: $p<0.05$ for all comparisons) when the dry weight of the soft parts of the gastropod was taken as covariate (ANCOVA; Shell species: F=20.124, df=2, $p<0.001$; gastropod dry weight: F=9868.572, df=1, $p<0.001$).

The shield length of the individuals was considered as covariate so that variation in the other crab dimensions would necessarily take its variation into account. All tested variables were strongly dependent on shield length (Table 2). The sex of the crabs did not influence shield width and height, propod width of the left chelae and shape of the right chelae. Crab sex significantly influenced the lengths of the right and left chela and the width of the right chelae with male presenting larger/wider propods. Shell treatment influenced almost all crab dimensions (except length and width of the left chelae and right chelae shape). Despite no differences were found in the pair-wise comparisons for shield width among shell treatments, the individuals reared in *Morula* tended to be proportionally wider than those in *Cerithium*. Dorso-ventral flattening was strongly dependent on the shell treatment (*Morula*>*Cerithium*>*Tegula*; $p<0.05$ for all paired comparisons).

Shell treatment also had a significant influence on length and width of the right chelae (Table 2), with individuals in *Tegula* presenting the largest/widest chela and individuals in *Morula* presenting the smallest/narrowest ones. Individuals reared in *Cerithium* showed intermediate values between these two species. A significant influence of shell treatment on right-left chela asymmetry was recorded, with individuals in *Morula* presenting smaller chela asymmetry than individuals in *Tegula* and *Cerithium* (Scheffé's; $p<0.05$ for both comparisons). The results also indicate that shell treatment did not influence the differences in the studied variables between males and females (see Table 2; not significant interactions).

Influence of past experience on shell selection pattern

The preference for *Tegula*, *Morula* and *Cerithium* was tested for each individual subjected to the three shell treatments and for individuals collected in shells of *Olivella* in nature ("control"). In all situations the individuals of *Pagurus* selected mainly shells of *Cerithium* (Fig. 2). However, the results showed a significant influence of shell treatment in shell-species selection pattern (Fig. 2, G=27.66, df=4, p<0.001). Individuals maintained in *Morula* selected shells of this species more frequently than individuals reared in *Tegula* and *Cerithium* (*Morula vs. Tegula*: G=15.70, df=2, p<0.001; *Morula vs. Cerithium*: G=22.42, df=2, p<0.001). This influence of shell treatment was demonstrated to depend on the shell type once the patterns of shell-species selection of individuals reared in *Tegula* and *Cerithium* were identical (G=1.60, df=2, ns).

The pattern of shell-species selection of the individuals collected in nature using shells of *Olivella* was closer to that of the individuals reared in *Morula* (G=6.68, df=2, p<0.05) than to those of the individuals reared in *Tegula* (G=12.20, df=2, p<0.001) and *Cerithium* (G=11.70, df=2, p<0.005) (Fig. 3). This was reinforced by cluster analysis for their shell selection patterns (Fig. 4).

Influence of shells on shell-weight selection

The weight of the shells of *Cerithium* and *Morula* selected at the end of the experiment depended more on crab size than on shell treatment or crab sex (Table 3), A positive association between weight increment and crab size was recorded (Pearson correlation, p<0.05), i.e., the larger the crabs, the larger the increment in shell weight at the end of the experiment. The weight increment (final – initial shell weight), on the other hand, depended on shell treatment for individuals that selected both *Cerithium* and *Morula*. For the individuals that selected *Cerithium*, those reared in *Tegula* and *Cerithium* showed similar increments in weight but larger than the weight increment recorded for those individuals reared in *Morula*. This same tendency was recorded for the individuals that selected *Morula*, although no significant differences were found in the multiple pair-wise tests.

Discussion

Growth and morphology

It is largely accepted that inadequate/small (Markham 1968, Fotheringham 1976a, b) or relatively heavy (Bertness 1981a) shells reduce crab growth. More recently, Bertness (1981a) and Blackstone (1985) showed that shell architecture might modulate hermit crab growth, with individuals attaining larger sizes in low than in high-spire shells. The present study afforded similar results: shell type was demonstrated to influence crab growth and morphology. Individuals reared in low-spire shells (*Tegula*) were shown to grow more than individuals maintained in medium-spire ones (*Morula*), although any difference was recorded between them and the high-spire (*Cerithium*) shells. These results may suggest that not only general shell architecture is responsible for regulating crab growth. Factors like relative aperture width (*Tegula*>*Cerithium*>*Morula*) may explain the results better.

Shell treatment influenced crab final size but not the time needed to undergo six molts. Such differences in the final size among shell treatments were probably caused by the slight tendency of higher growth rate (higher size increment after molting) recorded for individuals reared in *Tegula*. The high "within-shell treatment" variation in growth constant was caused by the variable initial sizes of crabs used in the beginning of the experiment, which caused noise in "among-shell treatment" comparisons.

Population studies revealed sexual dimorphism in interidal hermit crabs with males being larger than females (Turra and Leite 2000). Such differences are generally attributed to the higher female investment in reproduction (egg production and incubation), which have a direct trade off with somatic growth (Fotheringham 1976b, Bertness 1981a, b). Laboratory studies (Fotheringham 1976b, Blackstone 1985, but see Fotheringham 1976a) have demonstrated the higher growth rates of males in relation to females and Harvey (1990) emphasized that such sexual differences are favored by selection once large-sized crabs are better competitors for mates and shells than small-sized ones. However, the larger sizes attained by males were a consequence of the longer growth time, i.e., longer intermolt period, experienced by such individuals instead of different growth rates. This means that size increment after molting did not depend on crab sex and was a direct function of the duration of intermolt period (see Fig. 1). Females molt more frequently than males but the rate at which size increases with time is the same for males and females. In fact, the positive relationship recorded between growth time and crab final size support this assumption. This apparent contradiction with the studies cited above is probably a consequence of the use of small-sized and immature individuals in the present study. None of the females inspected after the experiment presented olive-green ovaries as those observed in mature females of this species in nature. This suggests that differences in crab growth between sexes are not significant in immature individuals, which are still not investing in reproduction. Such differences in crab growth between sexes would be expected if mature individuals have been used in the experiment.

The influence of shell treatments on crab morphology was more evident than the effect on growth itself. The most conspicuous effect of shell treatment (but not of crab sex) was on dorso-ventral flattening. Dorso-ventrally compressed crabs were recorded in narrow-aperture shells (*Morula* and *Olivella*). Dorso-ventral flattening have been previously observed in *Dardanus guttatus* (Vermeij 1978) and in *Clibanarius sclopetarius* and *Paguristes erythrops* (A. Turra, unpublished data). In fact, shells are known to influence the shape of hermit crabs (Selbie 1921, Goldschmidt 1940, McLaughlin and Bailey-Brock 1975, Blackstone and Joslyn 1984, Blackstone 1985). Similarly, results showed a tendency of individuals reared in *Morula*, a narrow-aperture shell, to present wider shields than individuals reared in *Cerithium*. Widened shields were also recorded as a response of previous usage of medium-spire shells of *Urosalpinx cinerea* by *Pagurus longicarpus* in comparison to individuals in low-spire shells of *Littorina littorea* and *Polinices duplicatus* (Blackstone 1985). As Blackstone (1985) pointed out, the molding hypothesis supposes that crab shape would change to better conform the shape of the inhabited shell. In fact, general shell architecture (low- vs. high-spire) seems to be very important in determining crab growth and shape. However, aperture shape, which represents the space hermit crabs use when not retracted into their shells, seems to be more important than general shell architecture in molding crab morphology as indicated by the correlation between crab dorso-ventral flattening and shell aperture shape.

Chelae length was also influenced by shell treatment and by crab sex. Individuals reared in *Tegula* showed larger (in length and width) right chelae than individuals in *Morula*. Individuals in *Cerithium* presented intermediate values. Individuals reared in the low-spire *Tegula* and in the high-spire *Cerithium* presented higher chela asymmetry (right/left) than individuals reared in *Morula*. Blackstone (1985) also showed that individuals in medium-spire shells of *Urosalpinx cinerea* presented relatively larger chelae and larger chela asymmetry than individuals in low-spire shells of *Littorina littorea* and *Polinices duplicatus*. Blackstone (1985) also did not record any difference in right chelae shape (length/width of the right chelae) as a response of previous shell use. The differences between the findings of Blackstone (1985) and the present study reinforce the hypothesis that aperture shape is more important than shell shape in influencing crab morphology. Narrow apertures would limit chelae growth and, as a consequence, reduce right/left chela asymmetry. In addition, males presented larger (right and left) and wider (right) chela than females and showed a more pronounced chela asymmetry. Blackstone (1985) showed that males have a greater relative growth of claws than females. Such differences in chelae size and asymmetry between males and females is evident in heterochelic species, as *P. criniticornis*, and is important in the reproductive behavior (Hazlett 1966).

Shell-species selection

The behavioral repertoire presented by hermit crabs is enormous. Hermit crab behavior can be influenced by tides (Gherardi and Vannini 1993), temperature (Rebach 1974), period of the day (Hazlett 1966, Chapter 1), wind and beach slope (Barnes 2002) and presence of predators (Scarratt and Godin 1992, Hazlett and Rittschof 2000). The variability in shell use by hermit crabs is thought to be influenced by environmental factors such as shell availability (Bertness 1980, Leite *et al.* 1998) but crab preferences may also be important (Floeter et al 2000, Turra and Leite 2002). Previous studies have demonstrated that past experience of hermit crabs with shells may lead to shell related behavioral plasticity. In fact, learning is an important component in such modifications in crab-shell relationships. Crabs may learn how to deal with shells with occluded apertures (Jackson and Elwood 1989) and how to estimate the dimensions of optimal shells (Elwood *et al.* 1979, Hazlett 1992, 1996). In contrast, mechanisms to locate shells (Mesce 1982, Rittschof 1980) and to occupy the first shells in nature (Reese 1963) are thought to be innate in hermit crabs.

The small-sized individuals of *Pagurus* collected in nature in shells of *Olivella* ("control") and subjected to all shell treatments selected mainly the high-spire shells of *Cerithium*. Preference for high-spire shells is common to other hermit crab species (Reese 1963, Bertness 1980, Blackstone 1984, 1985, Blackstone and Joslyn 1984, Lively 1988, Turra and Leite 2002), mainly to small-sized individuals. This suggests that hermit crabs may have innate preferences for certain shell types as also argued by Reese (1963) and Elwood *et al.* (1979).

The question of whether or not future shell-species choice in hermit crabs can be influenced by their previous experience (Elwood *et al.* 1979) still is challenging once this hypothesis was not supported by the subsequent studies carried out by Blackstone (1984), Elwood and Kennedy (1988) and Hahn (1998). It was demonstrated in the present study that, despite crabs may present intrinsic preferences for certain shell types, previous experience with shells may influence their future choices. Individuals reared in medium-spire narrow-aperture shells of *Morula* were more dorso-ventrally flattened and changed to treatment shells more frequently than individuals reared in low-spire rounded-aperture *Tegula* and high-spire rounded-aperture *Cerithium*. The pattern of shell selection of the individuals reared in *Morula* was very similar to the individuals collected in nature in shells of the medium-spire narrow-aperture *Olivella*, which also presented a significant higher dorso-ventral flattening than individuals reared in *Tegula* and *Cerithium*. The results do not show that shell previous experience may alter completely crab preferences so that an abrupt switch in the preferred shell could occur. However, they explain differences in shell utilization and selection patterns between populations subjected to distinct shell supplies.

Shell-size selection

Elwood *et al.* (1979) and Hazlett (1992, 1996) have demonstrated that crabs reared in low-adequacy shells selected relatively larger shells than crabs in adequate ones. In the present study the crabs were reared in different shell types but with similar adequacies (all crabs were in optimal shells at the beginning of the experiment). The weight of the shells selected at the end of the experiment (final weight) was demonstrated to be more dependent on crab size than on shell treatment (shell architecture) or crab sex. Bertness (1980) suggested that size relationships between hermit crabs and gastropod shells should take gastropod architectural differences into account. However, recent studies (Turra and Leite 2002, Chapter 5), revealed that the relationships between hermit crab size and the weight of the shells used in nature and selected in free-access experiments did not depend neither on crab nor on shell species. Such differences might be significant if shells with quite different architectures would be taken into account as suggested by Bertness (1980) and in Chapter 5. The present study compared the shell-weight selection among quite different shell types (*Tegula*: globose; *Morula*: elongated; *Cerithium*: high-spire) and recorded the same results as in Chapter 5 thus emphasizing that the relationships between crab size and shell weight did not depend on shell architecture.

Consequences of preference switches due to shell utilization history

The results of this study enable the following question: Why the crabs do not select shells of *Tegula* as these shells allow the crabs to grow more than other shell types? Bertness (1981b) found similar results for intertidal hermit crabs in Panama, as they also exhibited low preference for low-spire shells which enabled higher growth (*Nerita*). In fact, shell characteristics may have conflicting advantages to hermit crabs (see Table 4). The low preference for the low-spire *Tegula* can be a consequence of higher protection offered by other shell types against predation (Borjesson and Szelistowski 1989, Kuhlmann 1992) and desiccation, which is a very important factor for intertidal hermit crabs (Taylor 1981, Turra and Denadai 2001).

Bertness (1981a) argued that individuals from subordinant hermit crab species may evolve preferences for shell types of inferior value on an evolutionary time frame while dominant species use and prefer optimal shells. The results in the present study show that such switch in shell preferences may occur in an ecological time frame, after only few molting cycles. *Clibanarius antillensis* generally coexists with *Pagurus criniticornis* in intertidal areas in South-eastern Brazil (Leite *et al.* 1998, Turra and Leite 2001, Turra *et al.* 2000) and dominates this latter species in shell fights (Chapter 7). Thus, *Pagurus* may be forced to use less preferred shells in nature. As growth proceeds, the individuals may be molded by the shells and thus switch their shell-type selection pattern. This would cause a reduction in competitive interactions between species and, as a consequence, would favor coexistence. Further, shell selection experiments would then reveal low overlap in shell selection pattern. However, it is important to note that such modifications would be a consequence of plasticity in phenotypic expression rather than evolution of shell preferences.

General comments

Shell-type selection patterns of hermit crabs are significantly influenced by the molding effect of previous used shells. This effect was dependent on which gastropod shell the crabs were reared and was quite evident in shells with narrow apertures (as *Morula*).

The studied crabs preferred the high-spire shells of *Cerithium* in all treatments and strongly rejected the low-spire shells of *Tegula*. This would not be expected once *Tegula* enabled crabs to attain larger sizes. This indicates that crabs are not taking only growth advantages into account when selecting shells as also proposed by Bertness (1981b). Factors such as protection against predators and/or desiccation may be more important in hermit crab choice.

As population consequences of changes in preferences for narrow-aperture and medium-spire shells (Bertness 1981a, c, Fotheringham 1976a), crabs may grow at lower rates, attain sexual maturity at smaller sizes and produce smaller clutch sizes but may also reduce the need of new and large shells. This is also fundamental in situations of low shell availability, which is a common situation to hermit crab ensembles (Reese 1969, Vance 1972, Bach *et al.* 1976, Spight 1977, but see Scully 1979, Leite *et al.* 1998, Turra and Leite 2001). In addition, crabs in inadequate shells are more susceptible to predation (Angel 2000) and desiccation (Reese 1969).

Finally, shell availability may influence shell use by hermit crabs in three ways: 1. hermit crabs generally use shells of the most abundant gastropod species in nature (Bertness 1980, Leite *et al.* 1998, Turra and Leite 2001); 2. utilization of low adequacy shells may cause hermit crabs to overestimate shell size in future choices (Elwood *et al.* 1979, Hazlett 1992, 1996), and 3. the continuous use of certain shell types, mainly those with narrow apertures, may alter crab morphology and, consequently, its shell selection pattern.

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Table 1. Covariance analysis for the initial size (shield length, mm), final size (shield length, mm), the cumulative growth time to undertake six molts and the growth constant (c) of the extended allometric model ($y=a+bx^c$) of the individuals of *Pagurus criniticornis* reared under different shell treatments (*Tegula*, *Morula* and *Cerithium*). Shell treatment and hermit crab sex are fixed factors and initial size is the covariate.

Model/Source of variation	N	df	F	p
Initial size (Shield length, a)	281			
Shell treatment		2	7.331	<0.001
Sex		1	0.500	0.480
Shell * Sex		2	0.946	0.390
Final size (Shield length)	281			
Initial size		1	818.737	<0.001
Shell treatment		2	5.428	0.005
Sex		1	21.730	<0.001
Shell * Sex		2	0.474	0.623
Cumulative time (for six molts)	281			
Initial size		1	70.963	<0.001
Shell treatment		2	0.002	0.998
Sex		1	6.632	0.011
Shell * Sex		2	1.765	0.173
Growth constant (c)	281			
Initial size		1	14.266	<0.001
Shell treatment		2	0.025	0.975
Sex		1	0.008	0.927
Shell * Sex		2	0.065	0.937

Table 2. Covariance analysis for the dimensions of the hermit crab *Pagurus criniticornis* reared under different shell treatments (*Tegula*, *Morula* and *Cerithium*). Shell treatment and hermit crab sex are fixed factors and shield length is the covariate.

Model/Source of variation	N	df	F	p
Shield width	387			
Shield length		1	2378.367	<0.001
Shell treatment		2	3.579	0.029
Sex		1	0.509	0.476
Shell * Sex		2	0.634	0.531
Shield height	369			
Shield length		1	961.731	<0.001
Shell treatment		2	27.120	<0.001
Sex		1	1.782	0.183
Shell * Sex		2	0.627	0.535
Right chelae length	363			
Shield length		1	522.870	<0.001
Shell treatment		2	7.357	<0.001
Sex		1	161.639	<0.001
Shell * Sex		2	0.702	0.496
Left chelae length	378			
Shield length		1	1355.543	<0.001
Shell treatment		2	1.991	0.138
Sex		1	49.580	<0.001
Shell * Sex		2	1.520	0.220
Right chelae width	366			
Shield length		1	205.339	<0.001
Shell treatment		2	5.245	0.006
Sex		1	56.660	<0.001
Shell * Sex		2	0.448	0.639
Left chelae width	380			
Shield length		1	802.885	<0.001
Shell treatment		2	3.079	0.048
Sex		1	0.357	0.551
Shell * Sex		2	1.136	0.322
Right-left chela asymmetry	295			
Shield length		1	30.300	<0.001
Shell treatment		2	13.419	<0.001
Sex		1	154.327	<0.001
Shell * Sex		2	0.457	0.634
Right chelae shape	363			
Shield length		1	12.082	<0.001
Shell treatment		2	1.065	0.346
Sex		1	1.300	0.256
Shell * Sex		2	1.549	0.241

Table 3. Covariance analysis for the weight (g) and weight difference (final – initial weights) of the shells selected by the hermit crab *Pagurus criniticornis* reared under different shell treatments (*Tegula*, *Morula* and *Cerithium*). Only crabs that selected shells of *Cerithium* and *Morula* were included in this analysis. Shell treatment and hermit crab sex are fixed factors and shield length is the covariate.

Model/Source of variation	N	df	F	p
Cerithium				
Shell weight	212			
Shield length		1	67.048	<0.001
Shell treatment		2	0.837	0.434
Sex		1	0.062	0.804
Shell * Sex		2	0.101	0.904
Weight difference (final – initial)	212			
Shield length		1	2.242	0.136
Shell treatment		2	18.641	<0.001
Sex		1	3.158	0.077
Shell * Sex		2	0.531	0.589
Morula				
Shell weight	54			
Shield length		1	30.201	<0.001
Shell treatment		2	0.363	0.698
Sex		1	0.207	0.651
Shell * Sex		2	0.226	0.799
Weight difference (final – initial)	54			
Shield length		1	6.470	0.014
Shell treatment		2	3.469	0.039
Sex		1	0.026	0.874
Shell * Sex		2	0.845	0.436

Table 4. Conflicting advantages of the three gastropod species studied here based on data in the present study and on literature information (after Bertness 1981b)

Parameter	*Tegula* (low-spire)	*Morula* (medium-spire)	*Cerithium* (high-spire)
Anti-predator defence[1]	+	+++	++
Influence on growth[2]	+++	++	+++
Influence on fecundity[3]	+++	+	++
Relative shell mass[4]	22.35	35.59	22.48
Depth to withdrawn[4]	2.47	4.34	4.88
Resistance to desiccation [5]	+	++	+++

+, small; ++, medium, and +++, high influence.

[1] Shell architectural defenses (form + investment in shell material) are based on data presented in Vermeij (1978), Bertness (1981b, c) and Chapter 8

[2] Results of the present study

[3] Based on results presented by Bertness (1981a)

[4] Calculation based on the model for shell-species selection proposed by Lively (1988): Relative shell mass – shell weight/dry weight of gastropod soft parts (adapted); Depth to withdrawn – shell length (apex to siphonal canal)/aperture width (at the narrowest point)

[5] Based on Bertness (1981b, c)

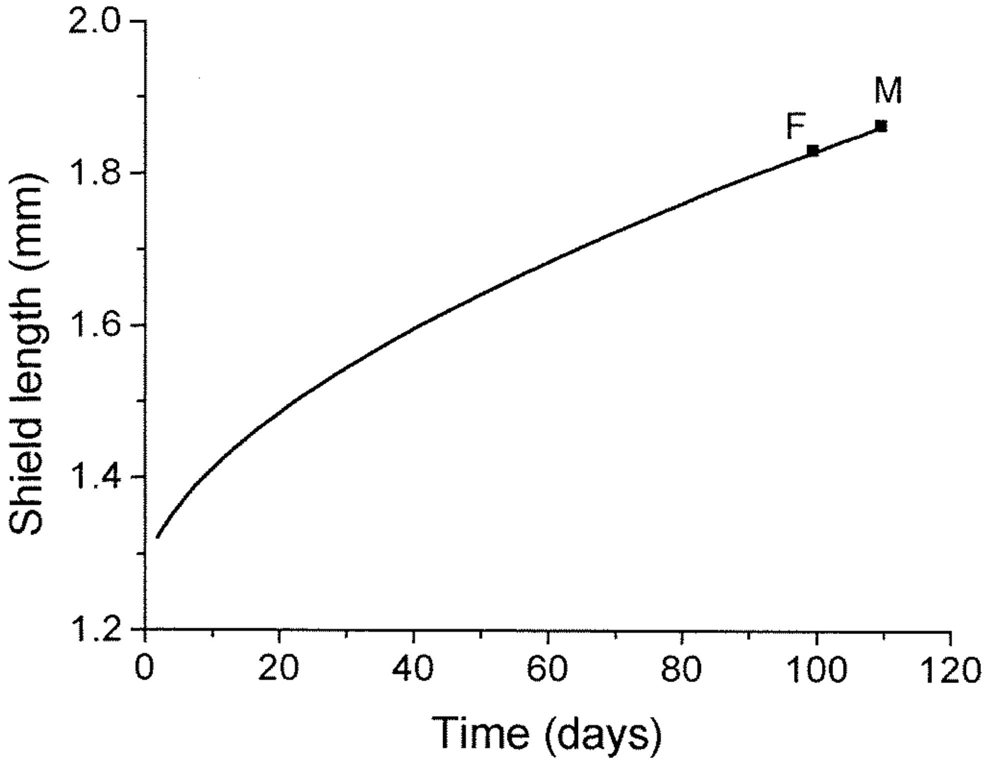

Fig. 1. Average growth curve ($y=a+bx^c$) for *Pagurus criniticornis* reared in the laboratory. The rate at which crabs grow depended more on crab initial size than on shell treatment or crab sex.. The simulation of the final size and growth time of males (M) and females (F) is also shown.

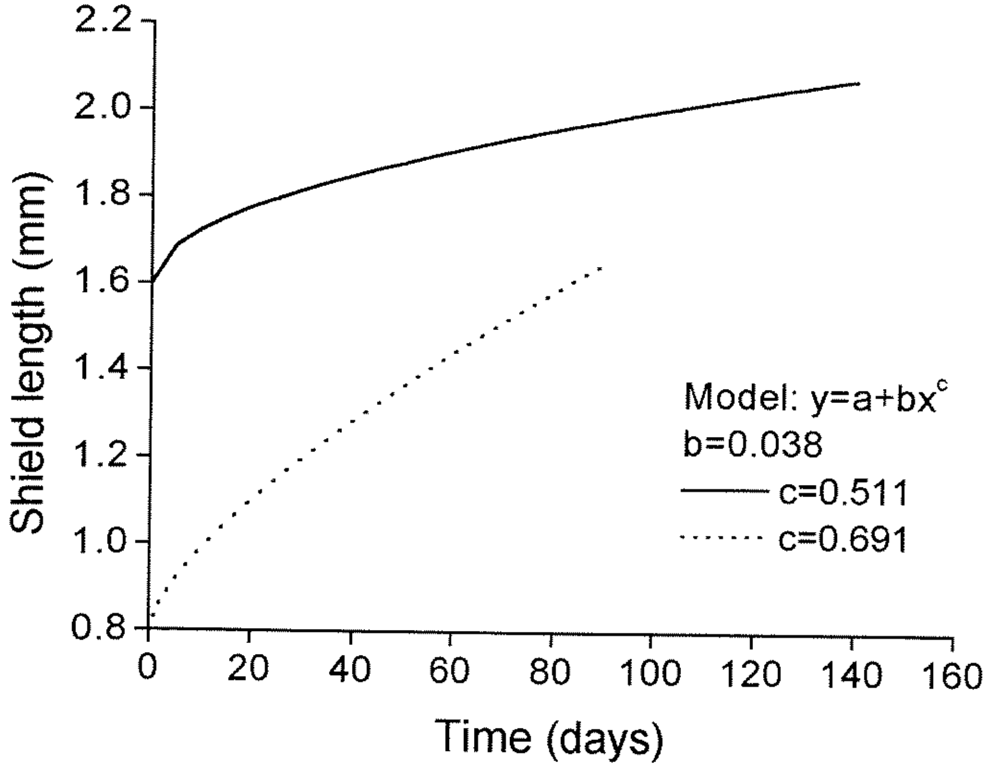

Fig. 2. Growth curves for *Pagurus criniticornis* showing the effect of initial size on crab growth. The values of growth constant (c) were obtained from values of a. The value of b=0.038 represent the average value for all individuals used in the experiment.

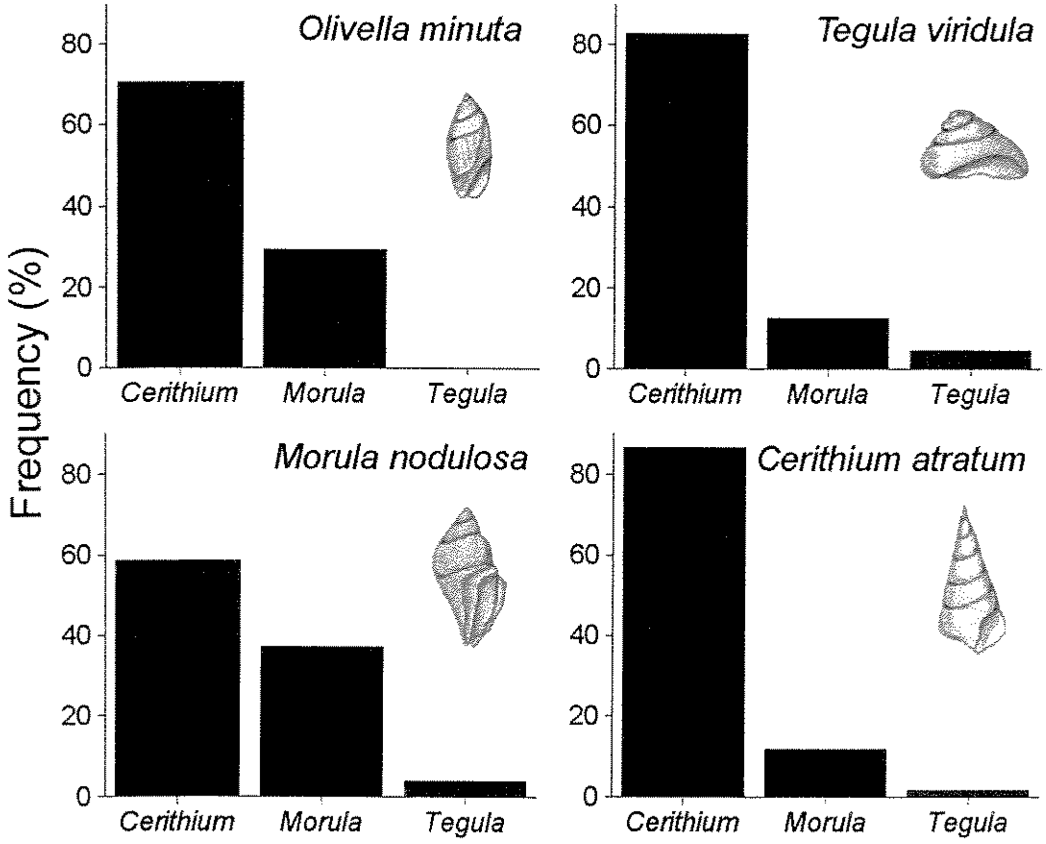

Fig. 3. Patterns of shell selection of *Pagurus criniticornis* collected in nature in shells of *Olivella* and reared under different shell treatments (*Tegula*, *Morula* and *Cerithium*). Drawings represent general shell morphology (architecture).

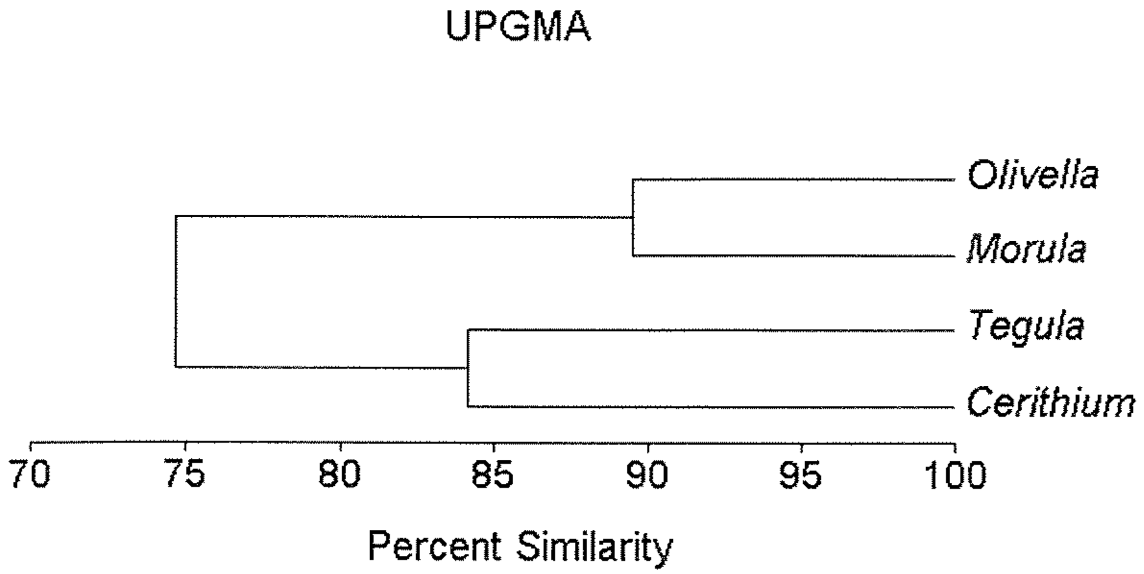

Fig. 4. Cluster analysis of the shell selection patterns of *Pagurus criniticornis* collected in nature in shells of *Olivella* and reared under different shell treatments (*Tegula*, *Morula* and *Cerithium*). UPGMA method, Percent similarity measure and original data were used in this analysis.

Interference and exploitation components in interespecific competition between sympatric intertidal hermit crabs

Abstract

Coexistence in hermit crabs is generally associated to variable degrees of niche overlap and resource partitioning among sympatric species. *Pagurus criniticornis* and *Clibanarius antillensis* are common hermit crabs in coastal Brazilian waters where they may occur in sympstry and present high overlap in shell use. Shells of the gastropod *Cerithium atratum* are the main resource used by them but they differ in the quality of shells used: *Pagurus* uses eroded shells and shells with physical damages in higher frequency than *Clibanarius*. Hypotheses based on differential competitive strategies were claimed to explain such pattern. *Pagurus* was demonstrated to be a more efficient exploitation competitor, reaching baits in simulated gastropod predation events more rapidly than *Clibanarius*. On the other hand, *Clibanarius* was a more efficient interference competitor, subordinating *Pagurus* during agonistic encounters. The poorer condition of shells used by *Pagurus*, the higher ability of this species to attend gastropod predation sites and the preference of the shell-breaking crab *Menippe nodifrons* for this hermit crab species may increase its risks of predation, revealing the disadvantages of such a competitive strategy. In contrast, species like *Pagurus* may have a key effect on hermit crab ensembles because it can enhance shell availability to species, such as *Clibanarius*, that have lower ability to find newly available by shell-breaking crabs and use them.

Keywords: Hermit crab ensembles, resource partitioning, competitive dynamics, *Clibanarius*, *Pagurus*

Introduction

Shells are an important resource for hermit crabs once they need to look for new and large shells as growth proceeds. Empty shells are generally in low supply in nature (Childress 1972, Vance 1972a, Kellogg 1976, Bertness 1980, but see Turra and Leite 2001) and may become a limited resource for hermit crabs. In fact, hermit crabs are generally reported occupying damaged shells and shells of non preferred shapes and sizes (Vance 1972a, Bach *et al.* 1976, Kellogg 1976, Turra and Leite 2002, Turra in press). The amount, size, type and quality of shells were demonstrated to influence population size (Vance 1972a, Spight 1977), growth (Markham 1968, Fotheringham 1976a, Chapter 5), morphology (Blackstone 1985, Chapter 5), fecundity (Childress 1972, Fotheringham 1976b, Bertness 1981a) and survivorship (Vance 1972b, Reese 1969, Bertness 1981b, Taylor 1981, Lively 1988, Angel 2000) of hermit crabs.

In this way, competition for shells is thought to be an important component in hermit crab fitness (Bach *et al.* 1976, Bertness 1981c, d) and was suggested to cause ecological separation of species in an evolutionary time frame (Bach *et al.* 1976). In general, many hermit crab species coexist in coastal areas and may present variable degrees of niche overlap (Grant and Ulmer 1974, Bach *et al.* 1976, Kellogg 1977, Abrams 1980, Turra *et al.* 2000, Turra and Leite 2001, 2002, Barnes 2002). Coexistence is generally associated with differences in sizes of coexisting species (Kellogg 1977) and partitioning in shell use (Kellogg 1977, Gherardi and Nardone 1997, Barnes 1999, Barnes and De Grave 2000, Turra and Leite 2001, 2002), shell preferences (Kellogg 1977, Gherardi 1990), microhabitat use (Vance 1972a, Kellogg 1977, Gherardi and Nardone 1997, Floeter et al. 2000, Turra *et al.* 2000, Barnes 2002, Turra and Denadai 2002), activity (Barnes 2002, Chapter 1) and tolerance against desiccation (Taylor 1981, Bertness 1981e, Turra and Denadai 2001). Once different hermit crab species may have similar preferences for certain resources that are in limited supply (Busato *et al.* 1998), they are expected to compete with each other.

Competition for shells may occur in two ways. First, crabs may use chemical cues to locate newly empty shells prior to shell loss due to burial, breakage or inaccessibility (Mesce 1982, Rittschof 1980). The ability of attending gastropod predation events was demonstrated to depend on hermit crab species (Hazlett 1996a, Hazlett *et al.* 1996). In fact, Busato *et al.* (1998) concluded that the shell utilization patterns in a Mediterranean hermit crab ensemble was due to better competitive abilities of *Clibanarius erythropus* in relation to *Calcinus tubularis* in finding and using new shells. This competitive strategy, where individuals or species are able to acquire resources more rapidly reducing resource availability to other individuals or species is called scramble (Nicholson 1954) or exploitation (Miller 1967). Second, hermit crabs may display ritualized agonistic shell fighting behaviors (Hazlett 1966) and subordinate other individuals of the same species (Hazlett 1966, Bertness 1981d) or of other species (Bach *et al.* 1976, Bertness 1981c, d). This strategy is named contest (Nicholson 1954) or interference (Miller 1967). Both strategies are claimed to explain resource partitioning and coexistence in hermit crab ensembles (Bach *et al.* 1976, Bertness 1981c, d, Busato *et al.* 1998), although dominance orders were not reported in some pairs of species (Mitchell 1975, Kellogg 1977, Abrams 1980, Gherardi 1990, Busato *et al.* 1998). In fact, Abrams (1980) argued that shell fighting is much less important than exploitative competition in hermit crabs.

Shell partitioning in hermit crabs is generally associated to utilization of different shell species (Kellogg 1977, Gherardi and Nardone 1997, Leite *et al.* 1998, Turra and Leite 2001, in press) or sizes (Vance 1972a, Bach *et al.* 1976). However, shell use by hermit crabs may differ in details, such as presence of physical damages (Gherardi and Nardone 1997, Turra in press) and epibionts (Gherardi 1990, Turra in press). The aim of this study was to test the hypothesis that coexistence in two sympatric intertidal hermit crab species in South-eastern Brazil is enabled by resource partitioning (shell damages) and that shell partitioning can be explained by different competitive strategies employed by each species in acquiring new shells.

Material and methods

Organisms

Clibanarius antillensis and *Pagurus criniticornis* are very common intertidal and shallow subtidal hermit crab species along the Brazilian coast and especially abundant in the Araçá region (23°49'S, 45°24'W), São Sebastião Channel, South-eastern Brazil (Leite *et al.* 1998). In this area, they are part of a hermit crab ensemble (Turra *et al.* 2000) and coexist with the gastropod *Cerithium atratum*. The shells of this gastropod are the most used by these two species (Leite *et al.* 1998) and by small-sized individuals of two other large-sized co-occurring hermit crab species (Turra and Leite 2002).

Shell partitioning in nature

A random sample through search effort (one hour of active search) was done in the intertidal zone of the Araçá region to evaluate the shell types used by *Pagurus* and *Clibanarius*. The percent similarity index (Krebs 1989) was calculated to address the overlap degree in shell use by the two species. The individuals in shell of *Cerithium* were observed with more detail to record the types of physical damages in their shells. All size classes recorded in nature were included in the analysis. The degree of physical damages was compared between crab species through the log-likelihood G test.

Attraction of P. criniticornis vs. C. antillensis to simulated predation events on gastropods

This experiment was conducted to test the null hypothesis that this two sympatric hermit crab species *Clibanarius* and *Pagurus* are equally attracted to simulated predation events on gastropods. The experiments were done in plastic trays (30 x 50 x 30 cm) with still water. Thirty four pairs (replicates) of individuals of the two species with similar sizes were selected. Each individual was located in one of the opposed sides of the tray. The side where each species was placed was randomly determined in each replicate. The hermit crabs were then caged using small two-inches PVC pipes (7 cm long) three hours before the experiment initiation. Prior to experiment initiation, one individual of *Cerithium atratum* was broken using pliers and placed in the central portion of each tray to simulate gastropod predation events. The hermit crabs were released from the cages 5 minutes after introduction of injured gastropods. This procedure was done at night, when both crab species are active (Chapter 1), and using a red light to minimize disturbance in crab behavior. The first species that reached the bait and the time needed to touch it after being released were recorded. Crab behavior was observed during the experiment. The null hypothesis of similarity in attending the baits was tested with the log-likelihood G test by comparing the times each species reached the bait first. The time each species needed to reach the bait was also compared between species through the Student t test.

Predation by Menippe - P. criniticornis vs. C. antillensis

The preference of a common intertidal predator, the xanthid crab *Menippe nodifrons*, for the two studied hermit crab species was tested. One crab of each species using a shell of

Cerithium was offered to a single shell-breaking predator. A total of nineteen replicates of this experiment was done in total. The hermit crabs were of similar sizes and were in optimal shells. They were maintained in an aquaria with an abundant supply of shells of variable sizes 24 h prior to experimentation. Predation experiments were conducted in small-sized plastic circular aquaria (250 mm in diameter) to prevent predator avoidance due to differences in locomotory ability between the hermit crabs. The first hermit crab species consumed was considered the preferred prey and the experiment was ended.

Shell transfer between P. criniticornis and C. antillensis

The possibility and the direction of shell transfer in shell-fights between *Pagurus* and *Clibanarius* was tested simulating three situations: 1. *Clibanarius* in sub-optimal shells and *Pagurus* in optimal shells; 2. both species in optimal shells; 3. *Pagurus* in sub-optimal shells and *Clibanarius* in optimal shells. The optimal shell treatment was prepared by exposing the crabs to a large shell supply with variable sizes 24h prior to experimentation. The low adequacy treatment corresponded to crabs in optimal shell that had their aperture peeled (sensu Bertness and Cunningham 1981) with pressure pliers until their chelipeds became exposed and closing shell aperture. This procedure tested the effect of shell inadequacy in the shell transfer between these two species. Fifteen individuals of each species were marked (distinct pattern for each species) and placed in a plastic tray (30 x 50 x 30 cm) with the bottom filled with sand and with circulating seawater. Three replicates were done for each treatment. Crab size and shell species (*Cerithium*) were controlled according to Bertness (1981d). The number of crabs of each species using shells marked with the pattern of the other species were recorded daily. Daily observations were done and the experiment was terminated after three days, when frequencies stabilized. This experiment was designed to test the null hypotheses that (1) shell transfer between these two species does not exist, and, if this hypothesis would be rejected, (2) shell transfer between these two species is independent of the shell adequacy and (3) there is no competitive dominant species. These two latter hypothesis were tested through ANOVA taking the frequencies of shell exchange at the end of the experiment (after 72h) into account. Some observations of shell fighting behavior were conducted during the experiment, based on Hazlett (1966).

Results

Shell partitioning in nature

A field sample revealed that individuals of *Pagurus* used 17 shell types in comparison to 8 used by *Clibanarius* (Fig. 1). Both species used mainly shells of *Cerithium atratum* and presented a high similarity in shell utilization pattern (percent similarity: 52.34%). *Pagurus* used more eroded (ribs and/or periostracum) and damaged shells than *Clibanarius* (Fig. 2) (G=17.47, df=1, p<0.001 and G=14.49, df=1, p<0.001). The most common damages were recorded in shell aperture followed by irregular perforations in shell wall. Perforations caused by drilling gastropod predators and perforations in the body whorl were also common.

Attraction of Pagurus vs. Clibanarius to simulated predation events on gastropods

The individuals of *Pagurus* were more frequently attracted to the baits than *Clibanarius* (28 vs. 6, G=15.45, df=1, p<0.001). In six replicates none of the species touched the baits (after two hours) despite the individuals were generally recorded walking in the cages. The time necessary to approach and touch the bait did not vary between species (Student t test; t=-0.638, df=32, p=0.528). In 17 replicates *Pagurus* reached the bait prior to *Clibanarius* have initiated its movement. The behavior of the crabs when approaching the baits was recorded. Both species generally avoided the neighborhood of the baits and hesitated in attacking them. Only 6 *Pagurus* did not hesitate in approaching baits. The variation in the time the crabs approached the baits was dependent on the time needed to start movement after being released and the degree of avoidance/hesitation.

Predation by Menippe - Pagurus vs. Clibanarius

The preference of a common intertidal predator, the xanthid crab *Menippe nodifrons*, for the two studied hermit crab species was tested. In nineteen replicates *Pagurus* was the first crab species consumed in fifteen of them while *Clibanarius* in only four (G=6.78, df=1, p<0.05).

Shell transfer between Pagurus and Clibanarius

A variable number of shell transfers between species was recorded among treatments (Table 1, ANOVA: F=60.100, df=2, p<0.001). *Pagurus* may obtain shells of *Clibanarius* but the degree to which shell transfer occurred when *Pagurus* was in sub-optimal shells was very similar to that recorded when both species were in optimal shells (Scheffé's test: p=0.929). In contrast, the rate of shell transfer between species was significantly higher when *Clibanarius* was in sub-optimal shells (p<0.001 for both comparisons). These results indicate that (1) shell transfer occurs between these two species, (2) shell adequacy influence crab aggressiveness and the rate of shell transfer and (3) *Clibanarius* is competitively dominant over *Pagurus*.

Despite no quantification of agonistic interactions between the two species have been made in the three treatments, the level of aggressiveness of the hermit crabs was enhanced when they were submitted to low adequacy shells. Some agonistic encounters were observed in the treatment of *Clibanarius* in low adequacy shells. Crabs approached individuals of *Pagurus* quickly and hold their shells. They generally rotated the shells of the defending crab and hold them in the opposed position, i.e., with shell apertures facing each other. *Clibanarius* rocked quickly *Pagurus* shell until the defending crab abandoned its shell and moved to the top of the shell of the attacking crab. The attacking crab changed to the new shell immediately and then the defending individual entered the poorly fitting shell.

Discussion

Despite the gastropod *Cerithium atratum* is highly abundant in the study region (Turra and Leite 2002), empty shells are rare and concentrated at the drift line of the tidal flat. Shell limitation to the studied hermit crab ensemble may also be inferred by the high utilization of severely damaged/encrusted and poorly fitting shells (Turra and Leite 2002, see also Bertness 1981d, Scully 1983). This resource limitation would cause stronger competition for shells between *Clibanarius* and *Pagurus* because both species prefer shells of *Cerithium* (Turra and Leite 2002, Chapter 6). In fact, both shell preference and availability are associated with their shell use patterns in nature (Leite *et al.* 1998) and are responsible for the relatively high overlap in shell use between them (52.34%). High overlap in shell use is common to hermit crab ensembles (Kellogg 1977, Abrams 1980, Gherardi and Nardone 1997, Turra and Leite 2001, 2002) and is considered inversely related to species coexistence (Hazlett 1981, Gherardi 1990).

As shell use by hermit crabs may differ in details (Gherardi 1990, Gherardi and Nardone 1997, Turra in press), coexistence in the study area may be enabled by differences in the quality of shells each species uses. In fact, *Pagurus* used proportionally more eroded and damaged shells than *Clibanarius*.

Competition for shells by these two species may be lowered in nature due to microhabitat partitioning (Turra *et al.* 2000). *Clibanarius* is generally associated to rocky and sandy substrates while *Pagurus* is proportionally more common in muddy substrates. Moreover, *Clibanarius* is able to occupy higher intertidal areas due to its higher tolerance against desiccation than *Pagurus* (Turra and Denadai 2001). Finally, despite these two species used manly shells of *Cerithium*, the largest individuals of *Clibanarius* also used other shell types (Turra and Leite 2002), thus reducing competition with *Pagurus*. Bertness (1981c) showed that habitat segregation between *Calcinus* and *Clibanarius* was, at least in part, consequence of the active escape response of *Clibanarius* from *Calcinus*. Once *Pagurus* was recorded avoiding the presence of *Clibanarius* in laboratory conditions (personal observation) this may also explain small-scale habitat partitioning in nature between them. In fact, low overlap in microhabitat use and crab size are associated to the higher influence in intra than interspecific competition (both interference and exploitation) on shell supply and, consequently, on shell utilization patterns by hermit crabs (Abrams 1980, 1981a). However, responses of hermit crabs to tidal cycles may force them to overlap strongly in microhabitat use as they look for refuges or form large clusters (Gherardi and Vannini 1993). This would increase both intra and interspecific competition. According to Abrams (1980), exploitation competition plays a major role in determining resource use by hermit crabs due to low frequency of shell exchanges reported between pairs of species. In addition , interference competition may have a less-than-expected deleterial effect on subordinant individuals or species once crabs may benefit from mutual exchanges (Spight 1977, Hazlett 1978, Chase *et al.* 1988, Gherardi and Vannini 1993).

Interspecific competition was demonstrated to play an important role in the shell utilization patterns of the studied species given the different competitive strategies employed by them. *Pagurus* was demonstrated to be a better exploitation competitor once it responded more rapidly than *Clibanarius* to cues from simulated predation events. The better quality of shells used by *Clibanarius* was demonstrated to be a consequence of its high ability in shell fights, i.e., in subordinating *Pagurus*. A similar situation was observed in a hermit crab ensemble in Panama (Bertness 1981c, d). The utilization of preferred shells in nature by *Calcinus obscurus* was attributed to the dominance of this species over *Clibanarius albidigitus* in interspecific shell fights. Despite *C. albidigitus* presented higher ability in exploiting new shells, they lost them to *Calcinus* in agonistic encounters. In contrast, shell use pattern of two sympatric Mediterranean hermit crabs (*Clibanarius erythropus* and *Calcinus tubularis*) was better explained by the greater ability of *Clibanarius* in finding and utilizing vacant shells once there was no dominance in interspecific interactions between them (Busato *et al.* 1998). Thus, despite shell utilization patterns by coexisting hermit crabs species may be directly influenced by their competitive strategies, hermit crab species composition would govern what strategy would play a major role in regulating their shell use pattern.

Bertness (1981d) argued that increased ability to find and utilize newly empty shells could represent the evolutionary consequences of being subordinant in interference competition. However, there are species with good exploitation ability coexisting with species with which shell transfers (Gherardi 1990, Hazlett *et al.* 1996) and specific dominances (Busato *et al.* 1998) were not reported. It becomes difficult to realize how such responses have evolved because hermit crab species composition is highly variable among sites, as well as shell availability and habitat complexity (Bach *et al.* 1976, Leite *et al.* 1998). In this way, one would expect highly variable population traits as a response to diffuse selective pressures. In addition, the average amount of interspecific exploitation (Abrams 1981b, c) and interference (Abrams 1981a) competition may vary among hermit crab ensembles once the degree of shell exchanges among pairs of species were demonstrated to depend on the degree of microhabitat overlap between species in different areas. Once dominance in shell fights between the same species may vary among areas (Abrams 1980), exploitation competition may prevail over interference. Moreover, the ability of exploitation competition is directly related to chemoreception and the degree to which hermit crabs attend gastropod predation sites, which was demonstrated to depend on starvation (Hazlett 1996b), shell fit (Rittschof *et al.* 1992, but see Rittschof and Hazlett 1997), and crab species (Hazlett 1996a, Hazlett *et al.* 1996) and size (Rittschof *et al.* 1992). One would suggest that such evolutive process have occurred in an earlier evolutionary time, and would be expected to generate family or genera related characters that are regulated locally. Studies on dominance order in hermit crabs indicate that, in general, species of the genus *Calcinus* subordinate those of *Clibanarius*, which, in turn, subordinate those of *Pagurus* (Bach *et al.* 1976, Bertness 1981c, Abrams 1982). Some species of *Clibanarius* may be better shell exploiters than species of *Calcinus* (Bertness 1981c, Gherardi 1990, Busato *et al.* 1998) but are less able to acquire new shells than those of the genus *Pagurus* (Bertness 1981c, Hazlett *et al.* 1996). Abrams (1982) showed that dominant species in general occupy lower areas in the intertidal region, attain larger sizes than subordinates and are less abundant. In fact, *Clibanarius antillensis* is less abundant (Turra *et al.* 2000) and attain larger sizes (personal observation) than *Pagurus criniticornis* but, in contrast, presents a tendency to occupy higher intertidal levels than *Pagurus* (Turra *et al.* 2000, Turra and Denadai 2001).

The exploitative competitive strategy exposes *Pagurus* to shell-breaking predators, thus enhancing its predation risks. This is aggravated due the fact that the preference of the common intertidal shell-breaking crab *Menippe* on *Pagurus* rather than *Clibanarius* and because crabs in poorly fitting (Vance 1972b, Angel 2000) and in damaged shells (Reese 1969), as those generally used by *Pagurus*, also exposing them to higher predation risks. The utilization of inadequate shells by *Pagurus* can also cause growth limitations (Markham 1968, Fotheringham 1976a, Bertness 1981a) and, as a consequence, reduce fecundity (Fotheringham 1976b).

McLean (1983) verified that prevention of shell loss in nature by hermit crabs might have important consequences for benthic communities. Hermit crab species that respond to chemical cues from gastropod predation events (Rittschof 1980) may be fundamental in making this resource available to other organisms. This situation might be applied to the studied population of *Clibanarius*, whose individuals are probable benefiting from the exploitation ability of *Pagurus*. One would expect that in the absence of *Pagurus* shell limitation to *Clibanarius* would be stronger. Introduction of new shells into hermit crab ensembles would cause a vacancy chain process (Chase *et al.* 1988, Osorno *et al.* 1998), which, in general, enhance shell adequacy to hermit crabs (Hazlett 1987). This would be especially important when direct shell transfer do not occur between species due to differences in their behavioral patterns (Gherardi 1990, Hazlett 1996a). Mutual attendance to gastropod predation sites would enable indirect interspecific shell exchanges between such species, with one species occupying the shell recently vacated by other species (Hazlett *et al.* 1996). In addition, *Pagurus* may enhance the amount of hard substrata (shells) available to sessile benthic organisms (Creed 2000) and organisms like octopuses, fishes and other hermit crabs that also use gastropod shells as shelters (McLean 1983) may also benefit from this behavior.

In general, hermit crabs are able to retain adequate shells in the presence of other species in poor quality shells, suggesting that dominance orders are not common between pairs of species (Abrams 1982). Higher frequencies of shell exchanges between species were associated to situations where marked asymmetric relationships (dominance) were reported, i.e., one species is clearly dominant (Abrams 1981a, 1982). This was the case of the relationships of *Clibanarius* and *Pagurus* studied here. *Clibanarius* was demonstrated to have a more evident shell holding potential than *Pagurus*, retaining its good quality shells even when *Pagurus* was in poor quality ones. This reinforces the theory of asymmetric contests of Maynard Smith and Palmer (1976) about resource distribution among competing species.

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Table 1. Number of shell transfers between the hermit crabs *Clibanarius antillensis* and *Pagurus criniticornis* in different shell treatments: 1. *Clibanarius* in sub-optimal shells and individuals of *Pagurus* in optimal shells; 2. both species in optimal shells; 3. *Pagurus* in sub-optimal shells and individuals of *Clibanarius* in optimal shells. Three replicates were done and observations were done in three subsequent days.

Treatment/Time	Replicates		
	1	2	3
Both species in optimal shells			
24h	0	0	0
48h	0	0	2
72h	2	3	1
Clibanarius in sub-optimal shells			
24h	7	8	8
48h	9	8	8
72h	11	9	10
Pagurus in sub-optimal shells			
24h	1	3	1
48h	1	3	1
72h	1	3	1

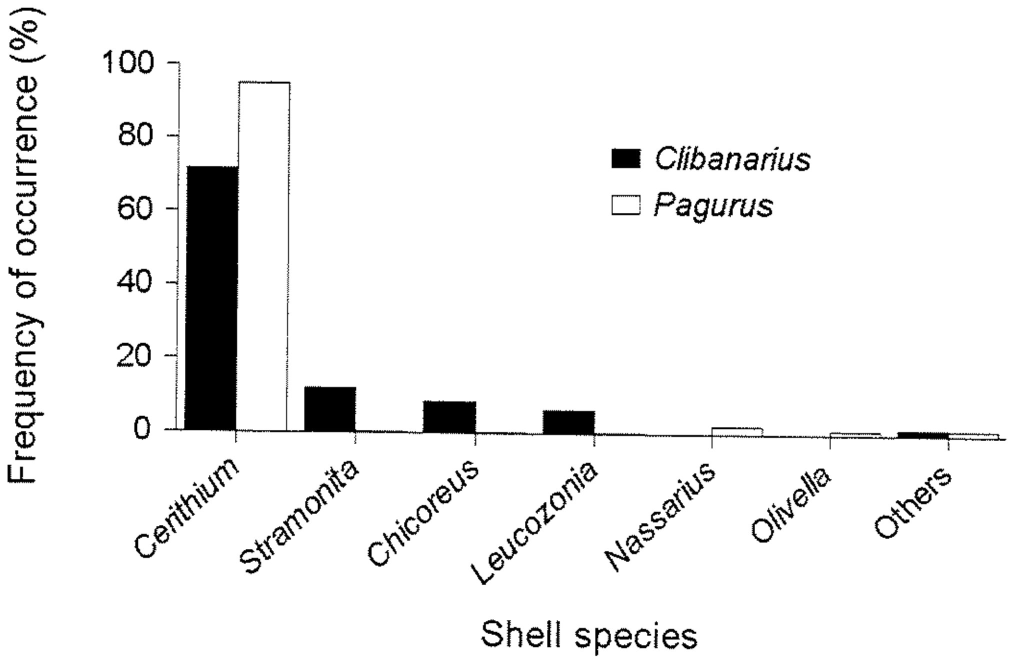

Fig. 1. Patterns of shell utilization of *Pagurus criniticornis* and *Clibanarius antillensis* in nature. "Others" represent 6 shell types for *Pagurus* and 2 for *Clibanarius*. Legend *Cerithium atratum*; *Stramonita haemastoma*; *Chicoreus senegalensis*; *Leucozonia nassa*; *Nassarius vibex*; *Olivella minuta*.

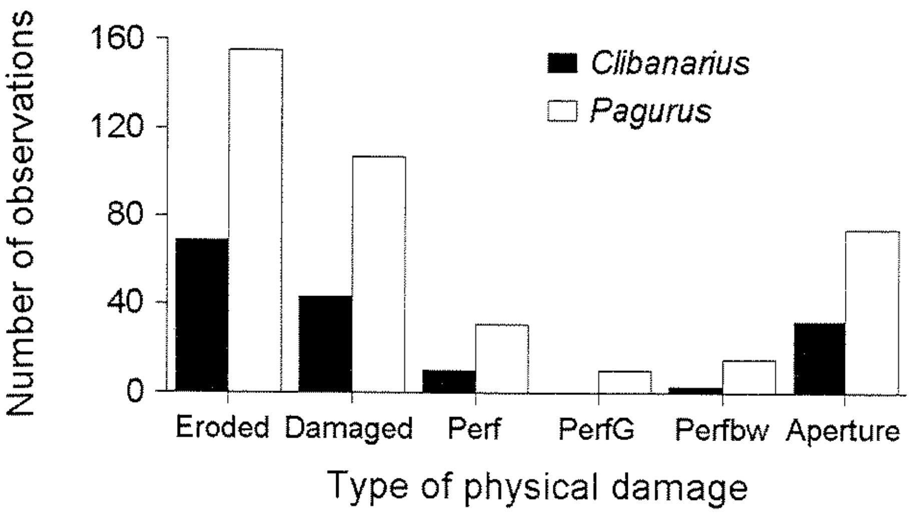

Fig. 2. Types of damage recorded in shells of *Cerithium* used by *Clibanarius* and *Pagurus* in nature. Eroded, shells with eroded periostracum or ribs; Damaged, shells with any kind of physical damage, Perf, irregular perforations in shell wall; PerfG, perforations caused by drilling gastropod predators; Perfbw, perforations in the gastropod body whorl; Aperture, aperture peeled.

Capítulo 8

Does predation by shell-breaking crabs on gastropods influence shell use by hermit crabs?

Abstract

Predation on gastropods is an important factor in organizing benthic communities and in influencing shell availability to hermit crabs. This study focuses on how reliable this effect would be. The predatory tactics and rates and the types of damage caused by four common intertidal and shallow subtidal predators (*Menippe nodifrons*, *Panopeus occidentalis*, *Eriphia gonagra* and *Callinectes danae*) in the gastropod *Cerithium atratum* were identified in the laboratory and compared to the damages recorded in the shells of this species used by the hermit crab *Pagurus criniticornis* in nature. Variable rates of predation within and among predator species were recorded with *Menippe* being the most important consumer. The number of *Cerithium* consumed was more dependent on crab size (weight) than on crab species. The mean number of unsuccessful attempts was dependent on crab species and was higher in *Callinectes* than in other species. Different crab species employed different predatory strategies on *Cerithium*. *Menippe* preyed almost exclusively through crushing while *Callinectes* and *Eriphia* through both peeling and crushing. Crushing was generally lethal and used on relatively smaller preys (except for *Menippe*) while peeling attempts yielded higher prey survival. The mean critical size of consumed shells of *Cerithium* did not vary among crab species although a significant effect of crab size was recorded. The shells used by *Pagurus criniticornis* in the field were generally damaged (68%), which were very similar to those recorded in preyed gastropods in the experiments. Peeling made shells available to medium and large-sized individuals while crushing produced shells to both medium (peeled shells without apex and high spires) and small-sized (only shell apex) hermit crabs. Prey selection experiments indicated that *Cerithium* is preferred to *Pagurus* by *Menippe* but this selection pattern could not be explained by differences in prey energetic value (biomass). Predation by *Menippe* and *Callinectes* on typical intertidal rocky shore gastropods (*Stramonita haemastoma*, *Morula nodulosa* and *Tegula viridula*) also showed a high variation within and between predator species. Both species preyed mainly upon *Stramonita* but *Menippe* consumed proportionally more *Tegula* than did *Callinectes*. Only *Menippe* consumed *Morula*. Such differences reflected the descending order in investment in shell architectural defense among these gastropod species (*Morula>Tegula>Stramonita*). *Menippe* also generally crushed these gastropods while *Callinectes* use both peeling and crushing. The predation strategy was also dependent on prey size: relatively smaller shells were crushed, medium sized were peeled and relatively larger shells were preyed through the aperture and sometimes left intact. Thus, intact shells used by hermit crabs may be also a result of successful predation events on relatively large-sized gastropods.

Keywords: Predation, shell availability, resources, shell utilization, intertidal, shell-breaking crabs, crushing, peeling, *Cerithium*, *Pagurus*, *Menippe*, *Callinectes*, *Eriphia*, *Panopeus*.

Introduction

Gastropods and hermit crabs are important components of intertidal and shallow subtidal communities (Leite *et al.* 1998). Hermit crabs generally shelter their soft abdomen in gastropod shells, which may directly influence their biology. Empty shells are generally in short supply (Childress 1972, Vance 1972a, Kellogg 1976, Bertness 1980, but see Turra and Leite 2001) and, despite hermit crabs have adaptations to find shells prior to their removal from the area (Rittschof 1980, Mesce 1982), shells are thought to be a limiting resource to hermit crabs. In fact, there are strong evidences supporting the positive effect of shell availability on hermit crab populations (Vance 1972a, Spight 1977, Wilber and Herrnkind 1984).

Inadequate shells may restrict crab growth (Markham 1968, Fotheringham 1976a, Bertness 1981a, Angel 2000), fecundity (Childress 1972, Fotheringham 1976b) and survivorship (Reese 1969, Taylor 1981, Angel 2000). Gastropod shells also provide protection against desiccation (see Turra and Denadai 2001) and predation (Bertness and Cunningham 1981), which was demonstrated to vary among gastropod species (Vermeij 1978, Bertness 1981b, Bertness and Cunningham 1981, Lively 1988). Once predation on shells is more dependent on mechanical limitations, i.e., dependent on relative strengths and sizes of crabs and shells (Zisper and Vermeij 1978, Juanes 1992, Yamada and Boulding 1998), hermit crabs may encounter refuge from predation when using shells larger than the critical size (sensu Vermeij 1978) attained by each gastropod species.

Bertness (1980) pointed out that the effective shell availability to hermit crabs will depend on the cause of gastropod dead. Desiccation and other non-destructive causes (e.g. parasitism) may produce intact shells to hermit crabs. Spight (1977) showed that predation on gastropods is followed by high rates of shell entry in hermit crabs ensembles. Gastropods and hermit crabs are subjected to various predatory species such as gastropods, fishes, octopus, lobsters and brachyuran crabs and predation on hermit crabs was demonstrated to be very important in reducing crab abundance in nature (Bertness and Cunningham 1981, Borjesson and Szelistowski 1989, Kuhlmann 1992). Batfishes may snap entirely small-sized shells and make intact shell available (Gibran and Castro 1999) while spiny puffer fishes completely crush their preys (Bertness *et al.* 1981). Drilling gastropods (Wilber and Herrnkind 1984) and octopus (McLean 1983, Cortez *et al.* 1998) produce holes in the preyed shells, which are avoided by intertidal hermit crabs in shell selection experiments (Pechenik and Lewis 2000). Predation by crabs or lobsters on both gastropods and hermit crabs may cause complete or partial destruction of shells (Shoup 1968, Rossi and Parisi 1973, Vermeij 1978, Zisper and Vermeij 1978, McLean 1983, Lau 1987, Bertness and Cunningham 1981, Angel 2000). Such predation may have a negative effect on hermit crab populations through reducing shell availability once damaged shells are strongly avoided by hermit crabs (Vance 1972a, Pechenik and Lewis 2000). However, despite Bertness and Cunningham (1981) estimated the number of damaged shells originated from shell-breaking predation used by hermit crabs, there are no data comparing the types of damage produced by shell crushing crabs and the physical condition of shells used by hermit crabs in nature to address the effect shell-breaking crabs have on shell use by hermit crabs.

The aim of this study was to understand the role shell predation by crabs play on the shell utilization patterns of hermit crabs in a tidal flat in South-eastern Brazil. The predatory tactics and types of damage caused by four common intertidal and shallow subtidal predators (*Menippe nodifrons*, *Panopeus occidentalis*, *Eriphia gonagra* and *Callinectes danae*) in the gastropod *Cerithium atratum* were identified in the laboratory and compared to the damages recorded in the shells of this species used by the hermit crab *Pagurus criniticornis* in nature. The consumption rate, number of successful attempts, rate of successful predation and the critical size of *Cerithium* were compared among these four shell-breaking crabs. The preference of *Menippe* between *Cerithium* and *Pagurus* was tested as well as the food reward offered by each prey species. The consumption rate and the critical size of the common intertidal rocky shore gastropods *Stramonita haemastoma*, *Morula nodulosa* and *Tegula viridula* preyed by *Menippe* and *Callinectes* were also evaluated. The investment in shell architectural defense was compared among the four studied gastropod species as an indirect measurement of their resistance to predation.

Material and methods

Consumption rate and critical size of Cerithium atratum

This experiment was designed to test the consumption rate of the gastropod *Cerithium atratum* by the crabs *Menippe nodifrons*, *Panopeus occidentalis* and *Eriphia gonagra* and by the blue crab *Callinectes danae*. These are very common intertidal and shallow subtidal species along the Brazilian coast, and especially abundant in the Araçá region (23°49'S 45°24'W), São Sebastião Channel, South-eastern Brazil. In this area they coexist with a hermit crab ensemble (Turra *et al.* 2000) and with the gastropod *Cerithium atratum* (Leite *et al.* 1998, Turra and Leite 2002). The shells of this gastropod species are the most used by the small-sized hermit crabs *Clibanarius antillensis* and *Pagurus criniticornis* in this area (Leite *et al.* 1998).

The consumption rate was defined as the mean number of preys consumed by each predator species by day. The predators (20 individuals of each species) were starved for 72h before experiment and then put in individual experimental tanks (40 x 70 x 40 cm) with running seawater. The bottom of the tanks was not filled with sand to avoid gastropod burying and thus confounding effects due to this behavior. The water level was maintained at 5 cm to avoid climbing and, consequently, predation avoidance by the gastropods. Individuals of *Cerithium* comprising the entire size range (15.50 - 34.10 mm) of the species in the field were collected, measured (shell length, mm), and individually marked. Twenty one-millimeter size classes were identified and one individual of each size class was offered to each predator (twenty individuals in total). Observations were made daily during 3 subsequent days. In each observation the consumed individuals of *Cerithium* were recorded and replaced in the tanks by another individual of the same size class. The critical size was the size of the largest prey consumed by a predator of a given species and size. The types of damage caused in the preyed shells were recorded. The mean consumption rate (preys/day) was compared among predator species using an one-way ANOVA (followed by a multiple comparison Tukey test, Zar 1999) to test the null hypothesis that the consumption rate is similar among predator species. ANCOVA (Zar 1999) was also employed using predator weight as covariate to evidence the effect predator size on the consumption rate. The one-way ANOVA was also employed to test the null hypothesis of equality in the mean number of unsuccessful attempts (living gastropods with damaged shells) and in the mean rate of successful predation (successful attempts/successful + unsuccessful attempts) among predator species. The null hypothesis that the critical prey size is the same for all tested individuals of each predator species was tested by ANCOVA using predator size as covariate.

Shell damages in Cerithium atratum used by Pagurus criniticornis in the field

The types of damage in shells of *Cerithium atratum* caused by the four predator species in the previous laboratory experiments were compared to the damages in the shells of this species used by *Pagurus criniticornis* in the field. The population was sampled by dredging (1 mm mesh size) the study area during high tides. The sediment was washed with seawater in 1 mm sieves. The shell species and the types of damage (based on laboratory observations) were recorded for all individuals.

Predation by Menippe - Pagurus criniticornis vs. Cerithium atratum

The preference of *Menippe nodifrons* between the gastropod *Cerithium atratum* and the hermit crab *Pagurus criniticornis* (in shells of *Cerithium*) was evaluated. One individual of each prey species with similar shell sizes (shell length) was offered to each predator in small tanks (0.25m in diameter) with running seawater. A total of twenty-four pairs were used in this experiment. Twenty-four hours prior to experimentation a large number of empty gastropod shells was offered to the hermit crabs to assure that they were in ideal/optimal shells. This procedure was undertaken to minimize the higher susceptibility to predation of crabs in sub-optimal shells (Angel 2000). The first species consumed by each crab was considered as the preferred prey. The number of preys of each species preferred by *Menippe* was compared through a log-likelihood G test to test the null hypothesis that this shell-breaking crab species does not show prey preferences.

Once the preference of the predator for a given prey may be a consequence of differences in biomass between preys, the null hypothesis that the weight of the soft parts of both gastropod and hermit crab are equally related to shell weight was tested. Thus, a field sample of gastropods was employed followed by measurements of shell weight and dry weight of soft parts. A shell-size selection experiment was done offering twenty shells of *Cerithium* of variable sizes to individuals of *Pagurus* of a wide size range. After 24 h, the crabs were removed from the selected shells and the dry weight of shells and crabs were recorded. The relationship ($y= a + bx$) between the shell weight and the dry weight of the gastropods (soft parts only) and hermit crabs (in optimal shells) was then compared through ANCOVA.

Critical size of shells of Stramonita haemastoma, Morula nodulosa and Tegula viridula

The critical size of other gastropod species (*Stramonita haemastoma*, *Morula nodulosa* and *Tegula viridula*, Fig. 1) was tested in another series of experiments using *Menippe nodifrons* and *Callinectes danae* as shell-breaking predators. These gastropod species are very abundant in the intertidal and shallow subtidal regions of rocky shores in the South-eastern Brazilian coast (Magalhães 2000) and are also frequently used by coexisting hermit crabs in the study site (Leite *et al.* 1998). The laboratorial structure used was the same as in previous procedures. Nine crabs (replicates) of each predator species were used. Eight preys of the entire size range sampled in the field were offered to each crab (shell length; *Stramonita*: 11.45 - 55.25 mm; *Morula*: 11.15 - 21.35 mm; *Tegula*: 3.20 - 17.55 mm). All gastropods were sized (shell length, mm) and individually marked prior to experiments. Observations were conducted daily during seven days and the number of preys consumed, their sizes and the types of damage were recorded. In each daily observation, if none of the shells have been preyed, smaller shells were added; if the largest one has been preyed, larger shells were added. This procedure was based on Bertness and Cunningham (1981). The number and size of all preyed gastropods was recorded for posterior comparisons.

Gastropod investment in architectural defense

A sample of individuals of all size classes of each gastropod species (*Cerithium atratum*, *Stramonita haemastoma*, *Morula nodulosa* and *Tegula viridula*) was used to relate shell weight (dependent variable) to the dry weight of animal soft parts. A linear equation ($y= a + bx$) was used to describe the relationship between these two variables. Covariance analysis (ANCOVA) was employed to address the null hypothesis that investment in shell material (shell weight) in relation to dry weight of soft parts did not differ among gastropod species. The slopes (b) of the fitted models were compared through a Tukey-type multiple comparison test (Zar 1999).

Results

Consumption rate and critical size of shells of Cerithium atratum

The four predator species showed different consumption rates on shells of *Cerithium* (ANOVA, F=4.009, df=3, p=0.011, Fig. 2). *Menippe* presented the highest consumption rate while *Panopeus* showed a tendency to consume fewer preys than the other species. The high variability within treatments (predator species) did not enabled clear comparisons among them (see Fig. 2). Crab weight was another source of variability in the analysis. ANCOVA revealed a non-significant influence of predator species and a significant effect of crab weight in consumption rate (ANCOVA; Treatment effect – crab predator species, F=0.514, df=3, p=0.674; Covariate effect – crab weight, F=6.437, df=1, p=0.013). These data indicate a stronger influence of crab weight than crab species in their consumption rate on *Cerithium*.

A variable number of unsuccessful predatory attempts was recorded for the four shell-breaking crabs (ANOVA; F=5.367, df=3, p=0.002). The lower values were recorded for the xanthid crabs (*Panopeus*, *Eriphia* and *Menippe*) while the blue crab *Callinectes* showed the highest mean number of unsuccessful predatory attempts. Differently from the mean number of gastropods consumed, the mean number of unsuccessful attempts did depend more on crab species than on crab weight (ANCOVA; Treatment effect – crab predator species, F=4.402, df=3, p=0.007; Covariate effect – crab weight, F=0.358, df=1, p=0.552). The successful predation rate (successful attempts/successful + unsuccessful attempts) also depended on crab species (ANOVA; F=4.444; df=3, p=0.008). However, when crab weight was taken into account, neither crab species nor crab weight explained the variation in the rate of successful predation among crab species (ANCOVA; Treatment effect – crab predator species, F=2.617, df=3, p=0.059; Covariate effect – crab weight, F=0.136, df=1, p=0.714).

Despite the differences in the consumption rate among species described above the predators used different strategies to break the shells of *Cerithium* (Table 1). *Menippe* almost exclusively "crushed" its preys while *Callinectes* and *Eriphia* showed equilibrium between crushing and peeling. *Panopeus* tended to use peeling more frequently than crushing as a predatory strategy. Perforation in the body whorl of the gastropod shell was a less frequent but successful predatory strategy employed by *Menippe* and *Callinectes*. Perforations in the body whorl were also frequently observed in one or two days before shell crushing. Crushing was always associated to gastropod death while peeling attempts showed significantly higher prey survivorship for all predators (76% to 91%). Apex breakage was recorded in lower frequencies and has never caused gastropod death in the experiment. In some cases, the gastropod was preyed and the shell left intact. This occurred only when shells were relatively large in relation to crabs. In such cases, as well as in successful peeling attempts, only the anterior part of the gastropod was consumed. In a descending order, crushing, peeling and perforations in the body whorl were the main predatory strategies employed by the studied predators. Figure 3 illustrates the damages caused by different predatory strategies in the shell of *Cerithium*. Crushing may have two outcomes: 1. the shells may be entirely broken with only a tiny portion of the shell apex remaining intact; 2. the apex and high spires of the shells may be removed and, sometimes, the aperture is also peeled.

The sizes of peeled and crushed shells for each individual predator are shown in Table 2. In most cases, peeled shells were larger or of the same size than crushed ones. A comparison between predatory strategies revealed that crushed shells were on average smaller than peeled ones for *Callinectes* and *Eriphia* (Student t test; t=-4.25, df=57, p<0.001 and t=-2.84, df=32, p=0.009, respectively). Non-significant tendencies of peeled shells being larger than crushed ones were recorded for *Panopeus* and *Menippe* (Student t test; t=-1.27, df=12, p=0.230 and t=-1.877, df=101, p=0.063, respectively) due to the low number of observations in *Panopeus* and the dissimilarity in use of crushing and peeling predatory strategies by both species.

Prey size was compared among crab species and revealed that all predator species were able to consume preys of almost all size classes used in the experiment (except *Panopeus*). There were non-significant differences in the mean prey size among predators (ANOVA; F=0.555, df=3, p=0.646; Table 2). The critical size of *C. atratum* was also determined for each predator by recording the size of the largest consumed individual (see Table 2). All predators were able to consume preys of the largest size class offered (except *Panopeus*). The average mean prey critical size did not vary among species (ANOVA; F=15.497, df=3, p=0.575), although a significant effect of crab weight did (ANCOVA; Treatment effect – crab predator species, F=1.239, df=3, p=0.308; Covariate effect – crab weight, F=6.658, df=1, p=0.014).

Shell damages in Cerithium atratum used by Pagurus criniticornis in the field

Analysis of a field sample revealed that 74% of the individuals of the hermit crab *Pagurus criniticornis* used shells of *Cerithium* in the Araçá region. Utilization of intact shells of this species was common (31.6% of individuals in shells of *Cerithium*) but they frequently presented damages (Fig. 4). Peeled shells were the most used by the hermit crabs, followed by peeled shells without both apex and high spires. A high proportion of this hermit crab population used only tiny shell apex. Perforations in the gastropod body whorl were more common than shells without apex. Damages caused by other predators, such as drilling gastropods, were less frequent than other types of damage but represent almost ten percent of the damaged shell used.

Predation by Menippe - Pagurus criniticornis vs. Cerithium atratum

This experiment tested the preference of *Menippe* between the gastropod *Cerithium atratum* and the hermit crab *Pagurus criniticornis* in optimal shells of *Cerithium*. *Menippe* showed a significant preference for the gastropod (18 out 24 replicates) in relation to the hermit crab (6 out 24 replicates; $G=6.28$, $df=1$, $p<0.05$).

The relationship of shell weight with dry weight of soft parts of the gastropods (soft parts only) and hermit crabs using optimal shells revealed that the data for *Pagurus* were more dispersed ($y=-0.0033 + 0.1023x$, $N=105$, $r^2=0.923$, $p<0.001$) than those for *Cerithium* ($y=-0.0003 + 0.0478x$, $N=82$, $r^2=0.938$, $p<0.001$) and showed a higher increase in the dry weight of soft parts (ANCOVA; y-intercept: $F=1.7849$, $df=1$, $p=0.183$; slope: $F=110.822$, $df=1$, $p<0.001$, Fig. 5). This means that *Pagurus* offer proportionally more biomass than *Cerithium* in relation to a similar predation effort (similar shell weights) and that this difference increases with shell size.

Critical size of shells of Stramonita haemastoma, Morula nodulosa and Tegula viridula

As in the experiments with *C. atratum*, high variability in the number of preys consumed was recorded within each predator species. High variation was also recorded among the gastropod species tested. *Morula nodulosa* was preyed only by *Menippe* and in a markedly lower rate (0.21 ± 0.31 $ind.day^{-1}$) than the two other gastropods. The consumption rate by *Callinectes* and *Menippe* on *Stramonita* (1.78 ± 1.59 $ind.day^{-1}$ and 0.91 ± 0.34 $ind.day^{-1}$, respectively) and *Tegula* (0.13 ± 0.16 $ind.day^{-1}$ and 0.39 ± 0.38 $ind.day^{-1}$, respectively) did not vary between predators (Student t test; *Callinectes*: t=1.61, df=16, p=0.128; *Menippe*: t=1.93, df=16, p=0.072), but both crabs showed preyed proportionally more upon *Stramonita* than *Tegula* (Paired t test; *Callinectes*: t=3.04, df=8, p=0.016; *Menippe*: t=3.36, df=8, p=0.001).

Different predatory strategies were used to prey upon these gastropod species (Fig. 6). *Menippe* generally crushed the shells while peeling was more common to *Callinectes*. Shells of *Morula* and *Tegula* were preyed by *Menippe* only through crushing, while both crushing and peeling were used by *Callinectes*. Most of the peeled shells have only small pieces of their apertures removed by the crab claw evidencing that predators break only the amount necessary to reach prey soft parts. Almost all peeled individuals have their opercula dilacerated. This was more evident in *Callinectes*, which introduced its slender and acute claws into shell aperture to destroy gastropod operculum and consume its anterior part. In some cases, crabs were able to consume the prey without breaking the shells (Fig. 6). This occurred frequently in very large shells in relation to crab size due to the easy introduction of the claws into shell aperture. In fact, these three predatory strategies (crushing, peeling and intact) were used by the crabs to prey upon individuals of *Stramonita* of different sizes (Table 3). Relatively smaller gastropods were crushed while peeling was used to prey upon relatively larger individuals. The largest consumed gastropods were preyed without damages in their shells.

Menippe was able to crush larger individuals of *Stramonita* than did *Callinectes* (Student t test; t=-2.75, df=9, p=0.026). *Menippe* also showed a tendency to prey shells of *Stramonita* of larger sizes through peeling and without causing damages (intact shells) of larger sizes than *Callinectes* (Peeled; t=-1.94, df=13, p=0.074, Intact, t=-1.37, df=7, p=0.214). This crab species was also able to prey upon individuals of *Tegula* of larger sizes than did *Callinectes* (Table 3). However, these two crabs were not able to prey upon the largest individuals of the three gastropod species offered to them. Non-significant correlations were recorded between the weight of both *Callinectes* and *Menippe* and the largest consumed (all strategies pooled), largest peeled, largest crushed and largest intact individual of *Stramonita* (Pearson correlation; p>0.05 for all comparisons).

Strong linear relationships were recorded between dry weight of soft parts and shell weight for all gastropod species (Fig. 7). ANCOVA rejected the null hypothesis of similarity among the slopes of the regression models and also showed a significant variation in the y-intercept values (ANCOVA; y-intercept: $F=5.888$, $df=3$, $p<0.001$; slope: $F=209.300$, $df=3$, $p<0.001$). The Tukey-type multiple comparison test revealed a descending order for regression coefficients ($p<0.05$ for all comparisons): *Morula>Cerithium>Tegula>Stramonita*. This indicates that individuals of species in the upper limit (*Morula*) invest more on shell material than do individuals of species in the subsequent descending order. The results also show that such differences among species increase with individual size, i.e., the larger/heavier the individuals, the larger the differences in the investment in shell material among gastropod species (Fig. 7).

Discussion

Differences among crab species in predation are attributed to their specific differences in size (weight) once there was no variation in the mean number of gastropods consumed and in the mean number of successful attempts among treatments (predator species) when crab weight was introduced to the linear model as a covariate. These results suggest that the four tested predator species have similar abilities to prey upon shells of *Cerithium*. Differences among species independent of their specific weight were recorded only for the number of unsuccessful attempts, which were higher for *Callinectes*. Thus, despite the higher absolute number of preys consumed by this species, its successful rate of predation upon shells of *Cerithium* was smaller than that of *Menippe*. This evidence the better adaptation of xanthid crabs, such as *Menippe*, than the portunid blue crabs, such as *Callinectes*, as a shell-breaking predator as argued by Vermeij (1978) and Yamada and Boulding (1998).

All predator species may prey upon the entire size range of the gastropods tested (except *Panopeus*). The effect each species will have in nature will depend on their abundance and microhabitat overlap with their preys. There are no estimates on the relative abundance of these crabs in the Araçá region but a clear microhabitat separation is evident. *Callinectes* occur almost exclusively in muddy substrates while the three other species are associated with cobbles and highly heterogeneous rocky shores. The components of the hermit crab ensemble in this region also show an evident habitat partitioning with *Pagurus criniticornis* occurring predominantly in mud and *Clibanarius antillensis*, *C. sclopetarius* and *C. vittatus* in coarse sand-gravel and hard substrates (Turra *et al.* 2000). The two former species use almost exclusively shells of *Cerithium* (Leite *et al.* 1998). On the other hand, *Cerithium* is a soft bottom gastropod that is frequently associated to hard substrates, algae and seagrasses (Denadai 2001). This feature may make this species available to predators from both habitats and, as a consequence, enhance its predation risk in relation to particular hermit crabs species.

Despite hermit crabs may present behavioral antipredator strategies (Bertness 1981b, Kuhlmann 1992, Scarratt and Godin 1992, Hazlett and Rittschof 2000), they are as susceptible as living gastropods to shell-breaking predators. Shell epibionts may help hermit crab protection against predators (Ross 1971, Partdrige 1980, Turra in press, but see Buckley and Ebersole 1994). In some costal areas, hermit crabs generally use shells in poorer condition (physical damages) than snails (A. Turra unpublished data), which can make them more vulnerable to predation (Vance 1972b, McClintock 1985, LaBarbera and Merz 1992). In addition, low shell adequacy and condition, which is also common to most intertidal species (Vance 1972a, Bertness 1980, Gilchrist and Abele 1984, Barnes 1999, Turra in press), may also enhance hermit crab vulnerability to predation (Angel 2000) in relation to gastropods. In this way, the preference of *Menippe* over *Cerithium* instead of *Pagurus* in the laboratory may be not occurring in nature due to shell constraints. In fact, even in laboratory experiments the preference of shell-breaking crabs may be highly variable. Rossi and Parisi (1973) showed that preference of *Eriphia verrucosa* on snails or hermit crabs using the same snail shell depended on the gastropod species but on average, is stronger for hermit crabs. On the other hand, Bertness and Cunningham (1981) demonstrated that the crabs *Ozius verreauxii* and *Eriphia squamata* did not show any preference between gastropods and hermit crabs. Despite *Pagurus* provides more energy intake to predators than did *Cerithium*, the preference of *Menippe* for the gastropod may be a consequence of the its lower mobility in relation to the hermit crab.

Differential architectural defenses of gastropods may explain the pattern of shell use (Bertness and Cunningham 1981) and selection (Borjesson and Szelistowski 1989) by hermit crabs. There are various shell characteristics that reduce predation risk such as thickened walls, narrow shell apertures, axial shell sculptures, short spires (Vermeij 1978, Bertness and Cunningham 1981, Lively 1988) and, in the case of hermit crabs, depth of withdrawn (Lively 1988). The three most common gastropod species in intertidal rocky shores in South-eastern Brazil have different investments in shell architectural defenses (*Morula*>*Tegula*>*Stramonita*) i.e., shell material. *Morula* has determinate growth, narrow and thickened apertures and axial ribbing (Magalhães 2000) while *Tegula* is a low-spire shell. The elongated shells of *Stramonita* have large apertures through which predators can introduce their claws. In addition, the energetic gain in relation to shell weight (crab effort) is higher in *Stramonita*. Such arguments explain the differences in the rate of prey consumption by *Menippe* and *Callinectes* between these three gastropod species. However, as Bertness (1981c) pointed out, there are conflicting advantages in relation to shell use by hermit crabs. Utilization of *Morula* may prevent predation but may also restrict growth, once this gastropod is on average smaller than *Tegula* and *Stramonita*, and may influence crab morphology (Markham 1968, Fotheringham 1976b, Blackstone 1985, Chapter 6). Utilization of relatively small shells may also reduce fecundity (Fotheringham 1976a) once it is directly related to crab size (Turra and Leite 1999, 2001). In this way, larger individuals are expected to use/prefer shells of *Tegula* or *Stramonita*. In areas with lower predation pressure, *Stramonita* would be preferred over *Tegula*, although relatively heavier shells (such as *Tegula*) may be preferred under high hydrodynamic conditions (Hahn 1998).

The effect of predation by crabs to shell availability of hermit crabs was shown to be largely dependent on the snail species (Vermeij 1978, Bertness and Cunningham 1981, Borjesson and Szelistowski 1989, but see Kuhlmann 1992), predator species (Bertness and Cunningham 1981), predator size and relative size of snail prey (Bertness and Cunningham 1981, but see Kuhlmann 1992). In general, relatively small shells are crushed while relatively larger ones are peeled as also shown by Bertness and Cunningham (1981). *Menippe* was shown to be a stronger and more efficient predator than *Callinectes* and generally crushed its preys. Large aperture shells (*Stramonita*) were more frequently preyed through the aperture than small aperture ones (*Tegula*, *Morula* and *Cerithium*). The shell condition after predation and its availability to hermit crabs also depended on the parameters cited above. *Menippe* generally crushed shells of *Cerithium* and produced empty shells with peeled apertures without both apex and high spires, used by medium-sized hermit crabs, and with only the shell apex, used only by recruits and small-sized individuals. Large hermit crabs use generally peeled shells, which were likely to be a consequence of predation events of *Callinectes* and *Menippe* over relatively larger shells. Crushing on shells of *Stramonita*, *Tegula* and *Morula* produce only shell fragments that can not be used by the hermit crabs hermit crabs as was also reported for *Nerita funiculata* (Bertness and Cunningham 1981). Only those relatively larger peeled or intact shells of the two former species can be further used. In general, crushers are considered a more specialized shell-breaking predator than peelers, dealing with gastropod defenses that avoid or support the attack of other crabs (Vermeij 1978). It would be expected a more marked widespread effect of peelers in gastropods and even hermit crabs ensembles due to consumption of a higher variety of shell types than crushers. Such species would be responsible for making quite different shell types available to hermit crabs. However, despite *Menippe* generally preyed upon its preys through crushing, this species was able to consume the same variety of gastropod species and may have similar effects on the types of shells becoming available to hermit crabs as the blue crab *Callinectes*, which generally peeled its preys.

In this way, the different species and strategies of shell-breaking predators produce a series of damages in the gastropod shells (Bertness and Cunningham 1981, McLean 1983, Lau 1987) that still enable their future use by hermit crabs. Bertness and Cunningham (1981) estimated that up to 63% of the shells used by hermit crabs have damages inflicted by predation attempts. In *Pagurus criniticornis* up to 68% of the shell of *Cerithium* used (74% of all shells used) have damages that evidence predation on previous shell occupants, either gastropods or hermit crabs. Such data support the important effect of shell-breaking crabs on shell availability to hermit crabs. *In situ* experiments showed that predation on hermit crabs can be as high as 100% in some situations and was shown to depend on gastropod species, shore height and season (Borjesson and Szelistowski 1989, but see Kuhlmann 1992). High values of mortality were also estimated for gastropods (Spight 1977, Bertness 1981b, Wilber and Herrnkind 1984) and positive relationships have been established between gastropod mortality rate and shell use by hermit crabs (Wilber and Herrnkind 1984). Utilization of intact shells by hermit crabs may be a consequence of predatory events of crabs on relatively large-sized preys through the aperture (see also Vermeij 1978), but may also be a consequence of stochastic mortality pulses such as the desiccation death of *Cerithium* recorded during low tides in summer of 2001 (A. Turra unpublished data).

General comments

In general, shell-breaking crabs prefer small-sized preys as a consequence of the mechanical costs of predation (Brown and Haight 1992, Juanes 1992). In fact, predation was stronger over small-sized individuals in the studied preys (costs) but relatively large-sized snails may be consumed through other strategies (peeling and consumption through the shell aperture). However, only the anterior part of the gastropods can be consumed in comparison to crushing. In this way, a higher number of preys are needed to furnish the energetic budget to the crabs. This would be a disadvantage of preying upon relatively large snails other than mechanical costs. Mechanical costs and energetic expenditures of crabs may inflate predation pressure over small-sized individuals, which would be consumed through crushing behaviors. This would enhance availability of tiny shell apex to recruits of hermit crabs, which, in association with the small-sized *Olivella minuta* are the only resource used by them in the studied area.

Predation has an important role in shell availability to hermit crabs and was demonstrated to be dependent on predator species (*Menippe>Callinectes*) and size, gastropod architectural defenses (*Morula>Cerithium>Tegula>Stramonita*) and on the relative predator-prey size, which will determine the predatory strategy and the damages inflicted in the shells.

Predation was demonstrated to be stronger over *Cerithium* than *Pagurus* in the laboratory. In nature this situation would result in a positive effect on hermit crabs due to enhancing shell availability without causing a reduction in hermit crab populations. However, if hermit crabs were using broken or poor fitting shells in nature, they may be preferred to gastropods. This would cause higher mortality rates in hermit crab populations, although also producing empty shells available to surviving crabs. Preference of shell-breaking crabs may also depend on the gastropod species and may be governed by the degree of overlap in their distribution patterns.

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Table 1. Number of each damage type caused in the shells of *Cerithium* consumed with (dead) or without (alive) success by the four crab predators tested.

Predator	Crushed		Peeled		Apex		Perforation	
	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
Callinectes	32	0	28	89	0	0	3	2
Panopeus	4	0	10	31	0	1	0	0
Eriphia	19	0	15	52	0	0	0	1
Menippe	101	0	4	41	0	2	1	1

Table 2. Comparison of the sizes (shell length, mm) of the largest peeled and largest crushed individuals of *Cerithium* by the four predators species. Only predator individuals that preyed through both peeling and crushing were used in this analysis. Mean prey size (±SD) for each predator species are shown in brackets.

Predator species and weight (g)	Largest peeled (mm)	Largest crushed (mm)
Callinectes (n=151, 23.93 ± 4.19 mm)		
85.40	27.85	28.20
89.00	27.80	18.70
62.90	32.80	20.20
77.30	28.85	25.85
73.10	30.55	29.50
39.30	30.60	25.45
Panopeus (n=46, 23.37 ± 4.34 mm)		
17.00	27.60	19.55
Eriphia (n=90, 24.67 ± 4.30 mm)		
23.00	27.60	22.20
25.20	28.75	23.30
46.20	32.00	26.40
24.00	28.85	23.20
19.40	23.40	29.20
24.90	24.15	26.20
Menippe (n=148, 24.45 ± 4.31 mm)		
99.50	32.10	26.20
70.10	29.40	30.45

Table 3. Comparison of the critical sizes (shell length, mm) of the largest peeled, largest crushed and largest intact individuals of *Stramonita haemastoma*, *Morula nodulosa* and *Tegula viridula* consumed by *Menippe* and *Callinectes* in the laboratory.*

Predator species and weight (g)	*Stramonita haemastoma*			*Morula nodulosa*			*Tegula viridula*		
	Peeled	Crushed	Intact	Peeled	Crushed	Intact	Peeled	Crushed	Intact
Callinectes									
101.40	27.35	23.15						7.10	
63.30	22.45								
43.30	25.85	11.45	31.30						
80.30	27.20	20.70							
58.40	21.80	17.05	29.50					3.80	
75.30	24.70		27.00				5.80		
65.20	26.15		36.50				6.00		
37.40	22.80		31.35				4.20		
Menippe									
122.00	26.40	28.65			19.70			14.30	
105.80		19.45	37.80					4.25	
295.10		32.00	44.65		19.65			16.75	
76.00	26.90	23.65						6.10	
149.20	25.60	25.75	35.90		18.30			14.70	
87.70	32.60	23.75						6.10	
82.20	44.75	26.50			12.40			6.10	
17.20	28.15								
39.80	24.65		26.55					4.85	

* Only predator individuals that preyed at least one gastropod were included in this table.

Table 4. Comparison of the critical sizes (weights of shells and soft parts, g) of the largest crushed individuals of *Stramonita haemastoma*, *Morula nodulosa* and *Tegula viridula* consumed by *Menippe* in the laboratory.

Menippe weight (g)	*Stramonita haemastoma*		*Morula nodulosa*		*Tegula viridula*	
	Shell weight	Flesh weight	Shell weight	Flesh weight	Shell weight	Flesh weight
122.00	4.966	0.262	0.954	0.030	3.030	0.166
105.80	1.425	0.070			0.191	0.009
295.10	7.092	0.381	0.948	0.030	4.342	0.244
76.00	2.677	0.137			0.436	0.021
149.20	3.521	0.182	0.782	0.024	3.226	0.178
87.70	2.713	0.139			0.436	0.021
82.20	3.862	0.201	0.273	0.008	0.436	0.021

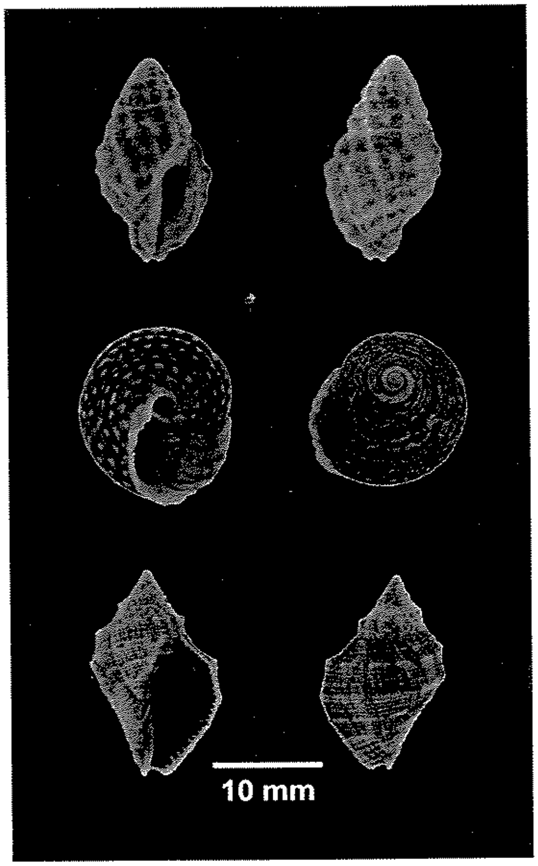

Fig. 1. Shells of *Morula nodulosa*, *Tegula viridula* and *Stramonita haemastoma* (from top to bottom) used in predation experiments.

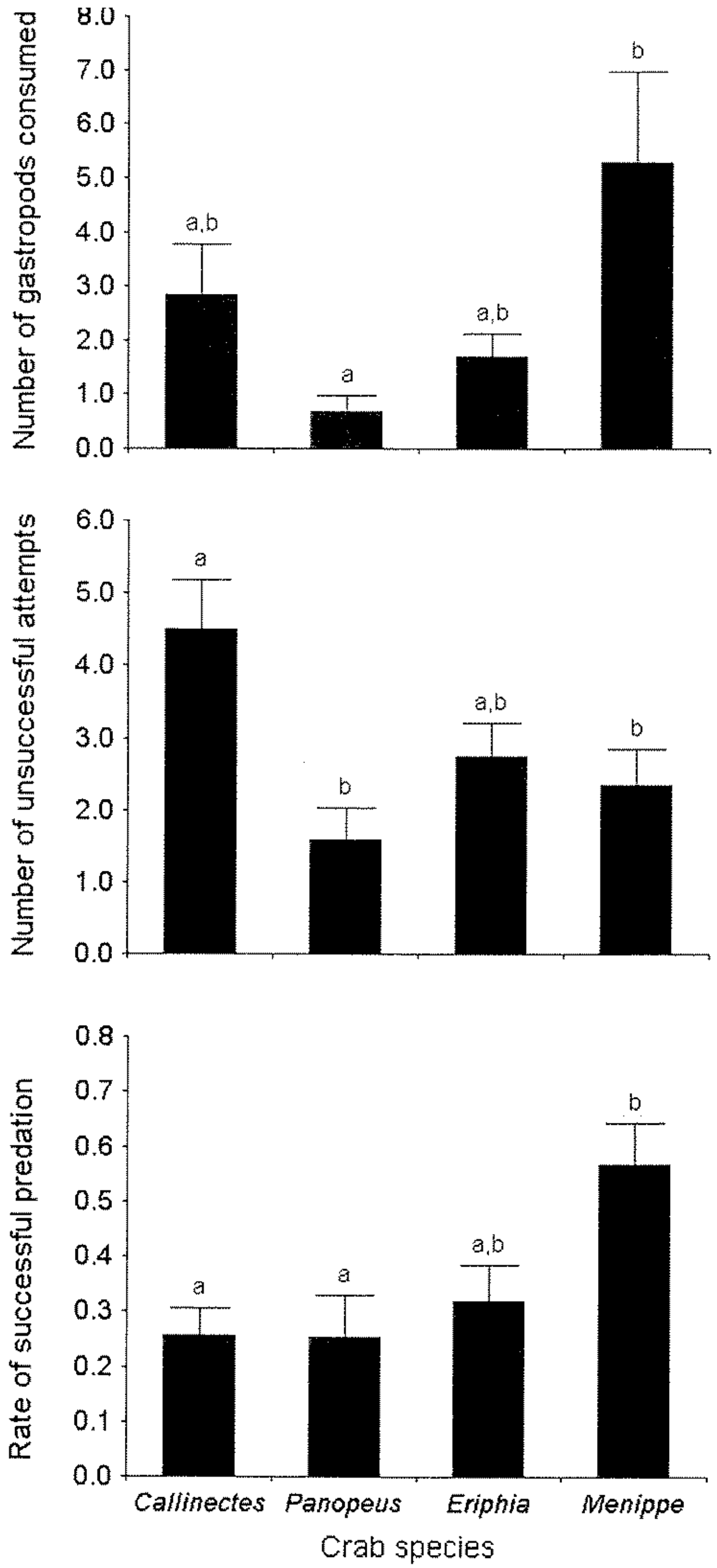

Fig. 2. Predation of *Cerithium atratum* by *Callinectes danae*, *Panopeus occidentalis*, *Eriphia gonagra* and *Menippe nodifrons*. Comparison of the mean (± SE) number of gastropods consumed (consumption rate), mean (± SE) number of unsuccessful attempts and mean (± SE) rate of successful predation (successful attempts/successful + unsuccessful attempts) during three subsequent days. Superscript labels indicate the result of the Tukey test for multiple comparisons.

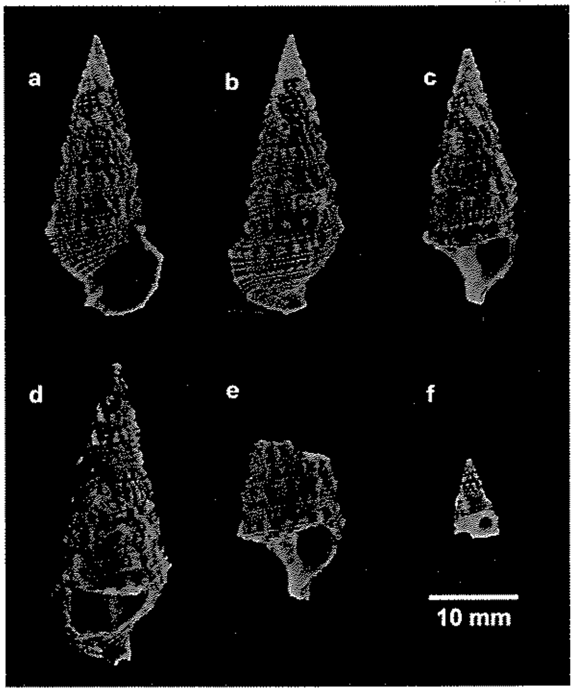

Fig. 3. Types of damage caused by successful predatory attempts on shell of *Cerithium atratum* in the laboratory. Labels: a, b – intact shell; c – peeled; d – perforation in the body whorl; e – crushed (pelled and without apex); f – crushed (only shell apex).

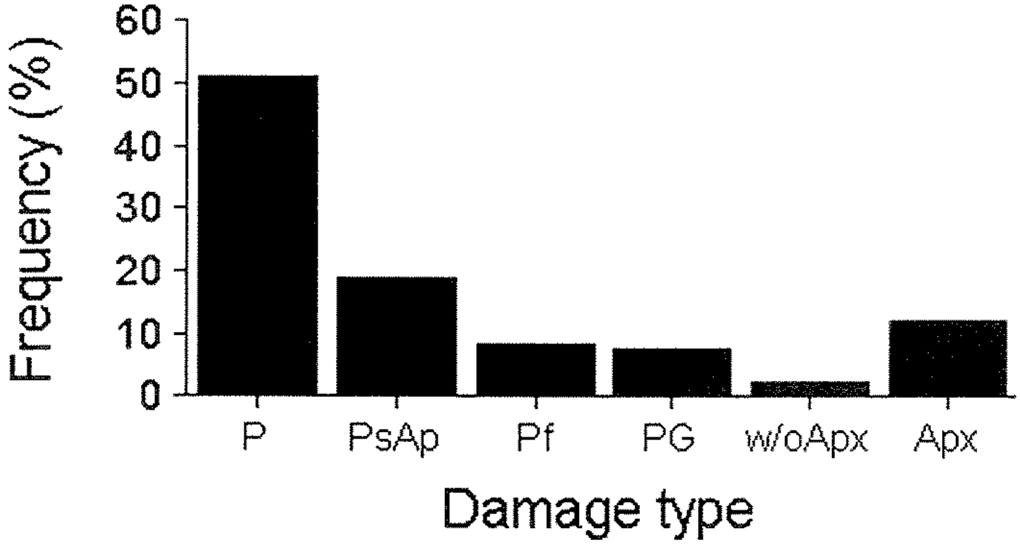

Fig. 4. Frequency of the types of damage recorded in the shells of *Cerithium atratum* used by *Pagurus criniticornis* in the Araçá region, São Sebastião Channel, South-eastern Brazil. Legend: P, peeled; PsAp, peeled and without both apex and high spires; Pf, perforation in shell body whorl; PG, perforation caused by a gastropod predator; w/oApx, without apex; Apx, only apex present.

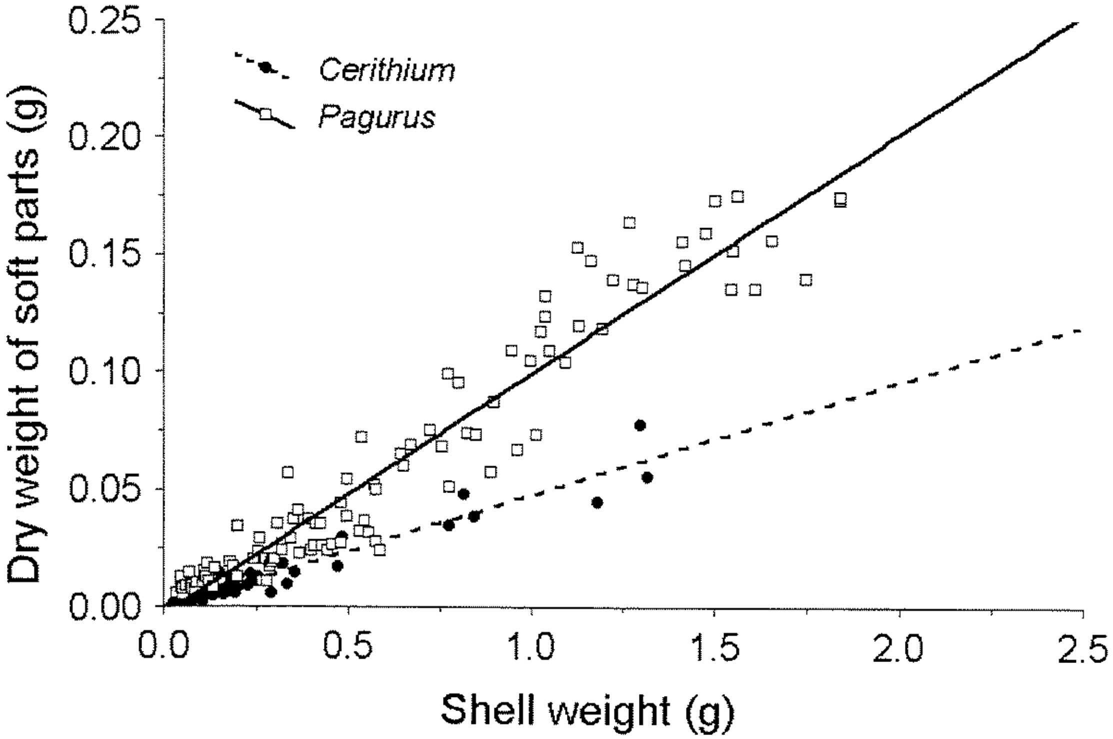

Fig. 5. Linear regressions between shell weight (g) and the dry weight of soft parts (g) of soft parts of the gastropod *Cerithium atratum* and the hermit crab *Pagurus criniticornis* in optimal shells of *Cerithium atratum*.

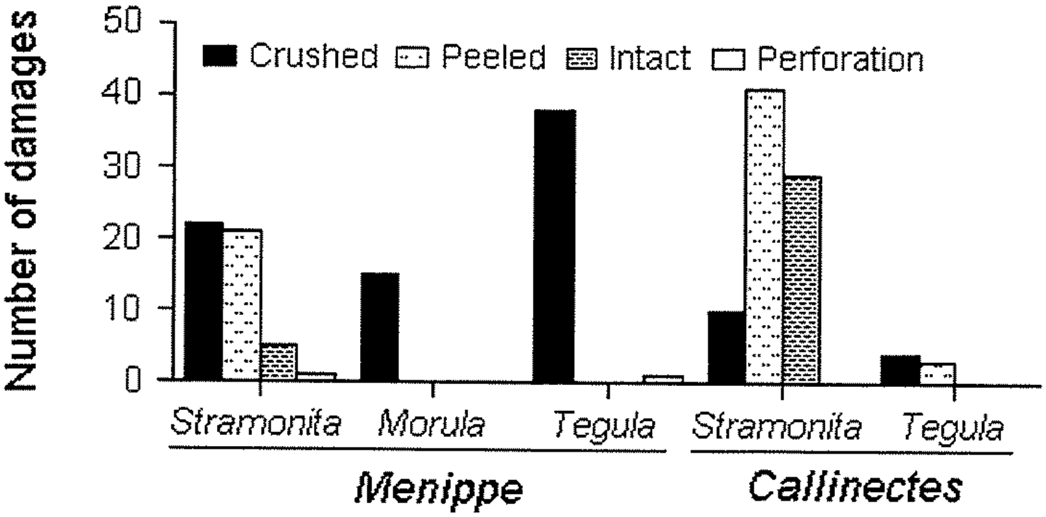

Fig. 6. Types of damage caused in the individuals of *Stramonita haemastoma, Morula nodulosa* and *Tegula viridula* (consumed + not consumed) by both *Menippe nodifrons* and *Callinectes danae* in the laboratory. Perforations are damages caused in the body whorl of the gastropod. Intact shells were those that were consumed but no damages were caused in the shell.

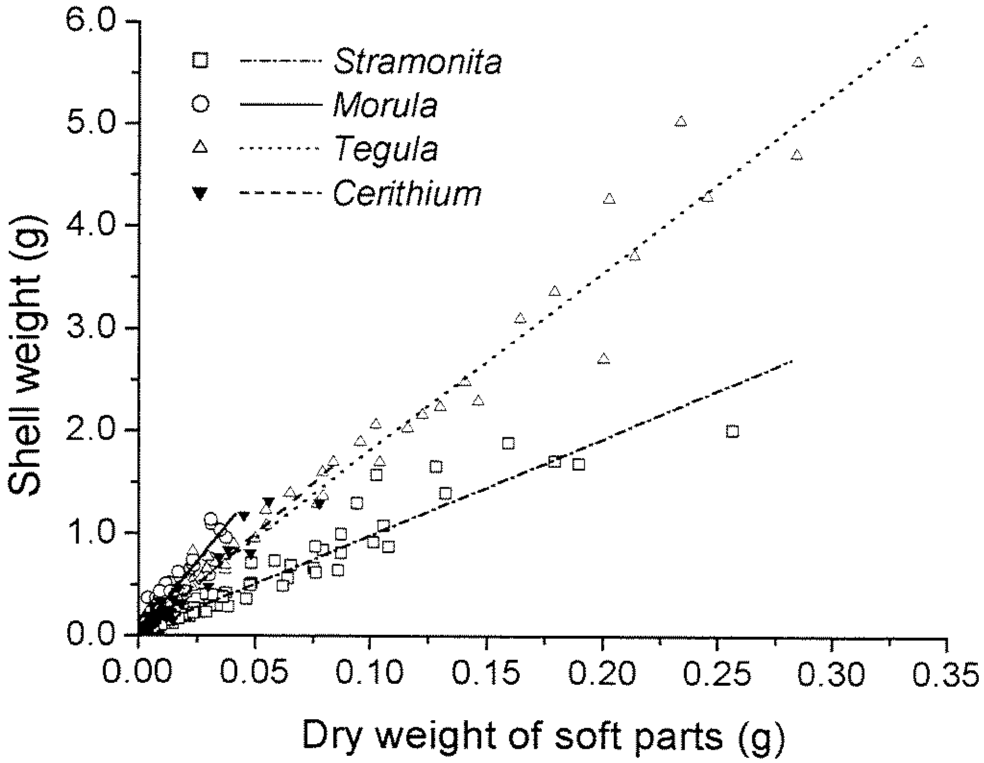

Fig. 7. Linear regressions between dry weight of soft parts (g) and shell weight (g) in the four studied gastropod species: *Stramonita haemastoma*, y=0.042 + 9.458x, N=64, r^2=0.917, p<0.001; *Morula nodulosa*, y=0.041 + 27.769x, N=77, r^2=0.909, p<0.001; *Tegula viridula*, y=0.088+17.373x, N=91, r^2=0.979, p<0.001; *Cerithium atratum*, y=0.009+19.8847, N=139, r^2=0.946, p<0.001.

Considerações finais

Embora muitos trabalhos tenham sido feitos e haja uma grande quantidade de informações sobre os ermitões, que aumentou substancialmente nas últimas duas décadas no mundo e no Brasil, ainda há lacunas no conhecimento deste grupo. O presente trabalho somou-se a este grande contingente de iniciativas para investigar a vida dos ermitões. Alguns dos estudos apresentados aqui são descrições pioneiras do comportamento e aspectos reprodutivos de espécies da costa brasileira e podem ter desdobramentos futuros, devendo ser encarados como base para se testar hipóteses mais específicas e conclusivas. Outros permitiram avanços significativos no conhecimento da história natural dos ermitões.

O estudo sobre o período de atividade dos ermitões confirmou a grande variação previamente observada entre espécies, gêneros e famílias e revelou que espécies simpátricas, sujeitas à influência das mesmas variáveis ambientais, podem apresentar padrões circadianos, circamareais e, até mesmo, ausência de padrão. Embora poucas interações reprodutivas tenham sido observadas neste estudo, pode-se verificar durante o estudo do comportamento reprodutivo que para as quatro espécies estudadas estas interações ocorrem durante todos os períodos do dia. O estudo do comportamento reprodutivo apresentou ainda descrições inéditas para *P. criniticornis* e permitiu uma avaliação mais aprofundada para *C. antillensis* e *C. sclopetarius*, pois as informações existentes na literatura para estas espécies eram baseadas em poucas observações. Este estudo permitiu a identificação de novos comportamentos, como a posição oblíqua de guarda e avaliação de machos em relação a fêmeas, mesmo para *C. vittatus*, cujo comportamento pré- e pós-copulatório havia sido detalhadamente descrito recentemente. Foi verificado para todas as espécies que os machos permanecem com as fêmeas durante um tempo após a cópula provavelmente para permitir a externalização dos ovos, após o qual eles podem deixá-las inativas e parcial ou totalmente enterradas no substrato. Por fim, este estudo possibilitou a descoberta de que indivíduos intersexo das três espécies de *Clibanarius* estudadas podem copular com sucesso como machos. Atenção especial foi dada a estes indivíduos, os quais apresentaram regressão dos poros genitais femininos. Esta informação foi utilizada em conjunto com dados comportamentais e ecológicos para discutir a possível relação entre intersexualidade e hermafroditismo protogínico em ermitões. Como não há registros deste tipo de hermafroditismo em decápodes, as poucas observações apresentadas aqui remetem a uma série de novos estudos comportamentais, morfológicos, histológicos e ecológicos para se testar esta hipótese.

Estes estudos comportamentais ainda forneceram informações que devem ser levadas em conta em experimentos futuros. Por exemplo, para espécies com ciclos diários (circadianos ou circamareais) os experimentos devem ter duração mínima de 24 h e estudos comportamentais visando comparações interespecíficas podem ter resultados mais precisos e exatos se realizados durante a noite, período de alta atividade para todas as espécies. Além disso, evidenciou-se que a busca por variações intraespecíficas no comportamento reprodutivo dos ermitões deve se basear em dados quantitativos e não apenas na presença ou ausência de comportamentos específicos. Porém, talvez fosse primordial lançar esforços no estudo inicial dos períodos de atividade e do comportamento reprodutivo de outras espécies presentes na costa brasileira, principalmente as de infralitoral, para as quais as informações são particularmente escassas.

Em vista da pequena quantidade de dados referentes ao desenvolvimento embrionário de ermitões, principalmente para as espécies brasileiras, e da importância destes para se compreender as variações na história de vida destes animais, a descrição macroscópica completa do desenvolvimento embrionário de *Clibanarius antillensis*, *C. sclopetarius*, *C. vittatus* e *Pagurus criniticornis* em conjunto com observações preliminares para *Pagurus brevidactylus* e *Paguristes tortugae* foram realizadas. Algumas diferenças morfológicas e no tempo de desenvolvimento foram encontradas entre espécies, as quais não impossibilitaram a identificação de estágios de desenvolvimento comuns a todas elas. Estas diferenças sugerem variações nas estratégias reprodutivas empregadas por estas espécies, porém é preciso haver descrições deste tipo para um maior número de espécies para que se possa ter um panorama geral das estratégias reprodutivas dos ermitões. Uma avaliação da composição e do modo como o vitelo é consumido nas diferentes espécies também pode contribuir para a compreensão destes padrões.

Dois estudos enfocando a questão da seleção de conchas por ermitões permitiram um avanço significativo na compreensão do modo como as conchas são selecionadas. Constatou-se que as relações entre dimensões de ermitões e conchas selecionadas apresentaram melhores ajustes que as relações obtidas com as conchas utilizadas na natureza, indicando que os ermitões estão utilizando conchas sub-ótimas no campo. Foi comprovado que as relações entre as dimensões de ermitões e conchas não dependem nem do tipo (arquitetura) da concha nem da espécie de ermitão. A única exceção ocorreu para as relações com a abertura e largura das aberturas das conchas, as quais mostraram uma maior relação com a arquitetura da conchas que outras variáveis. Comprovou-se também a hipótese do modelamento, que pressupõe a influência do tipo de concha utilizada (experiência prévia) na morfologia e nos padrões futuros de seleção de conchas dos ermitões. A alteração morfológica mais evidente foi verificada no grau de achatamento dorso-ventral dos ermitões, a qual esteve diretamente relacionada com a forma da abertura das conchas utilizadas. Assim, embora haja um componente inato e um de aprendizado na seleção de conchas por ermitões, ainda há um componente físico, ou seja, relativo às morfologias tanto de conchas quanto dos ermitões.

Na natureza, os ermitões se deparam com outros desafios relacionados às conchas que utilizam. Além de marcantes variações na disponibilidade de conchas entre ambientes muitas vezes próximos, algumas espécies de ermitões ocorrem em simpatria e acabam partilhando os recursos disponíveis. O modo como as espécies se relacionam e suas estratégias de obtenção do recurso muitas vezes determinam o modo como ele é partilhado. De fato, duas espécies comuns de ermitões no litoral brasileiro *Clibanarius antillensis* e *Pagurus criniticornis* utilizam basicamente conchas de *Cerithium atratum* na natureza, porém com pequenas diferenças entre elas. A utilização de conchas mais desgastadas e com mais danos físicos por *Pagurus criniticornis* em relação a *C. antillensis* pode ser explicada pelas diferenças nas estratégias competitivas empregadas por elas. *Pagurus criniticornis* foi demonstrado como um melhor explorador que *C. antillensis*, sendo atraído mais rapidamente por eventos de predação simulada de gastrópodes, enquanto *C. antillensis* mostrou-se um melhor competidor por interferência, subjugando *P. criniticornis* e roubando suas conchas. Desta forma, *P. criniticornis* tem um papel fundamental em aumentar a disponibilidade de conchas ainda que para outras espécies de ermitões com baixa habilidade em encontrar conchas novas, como *C. antillensis*. Outros estudos sobre interações competitivas entre espécies simpátricas de ermitões devem ser encorajados pois poderão ajudar a elucidar os padrões de uso de conchas na natureza.

Como exposto acima, os padrões de utilização de conchas por ermitões são influenciados por um grande conjunto de fatores como: a disponibilidade de conchas, o tamanho relativo de ermitões e conchas, da experiência prévia e aprendizado dos ermitões e das interações competitivas entre espécies. Em contrapartida, também foi demonstrado o efeito de predadores quebradores de conchas (caranguejos) na disponibilidade dessas para os ermitões. Constatou-se que esta influência é dependente do tipo (espécie) e tamanho do predador e do tipo de gastrópode. O tamanho relativo de predadores e gastrópodes determina quais estratégias de predação serão empregadas pelos predadores e, consequentemente, quais danos serão causados nas conchas.

Verificou-se que as diferenças estruturais entre espécies de gastrópodes proporcionam aos ermitões diferentes graus de proteção contra predadores. Porém, também ficou evidente que os ermitões levam em conta outros fatores relativos às conchas, como restrição ao crescimento e à fecundidade, em conjunto com sua capacidade protetora.