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ENVIRONMENTAL CONSTRAINTS ON POPULATION STRUCTURE AND CONDITION OF COASTAL AND ESTUARINE CRABS
(CRUSTACEA: BRACHYURA)

Valter Luís Morgado Amaral

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Tese orientada por
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This study was supported by:

Fundação para a Ciência e a Tecnologia (FCT), through a PhD fellowship co-financed by Science and Innovation Operational Programme 2010 (POCI 2010) and European Social Fund (FSE), and attributed to Valter Amaral (SFRH/BD/10471/2002)

Guia Marine Laboratory
Department of Animal Biology, Faculty of Sciences, University of Lisbon

Marine Biological Association of the UK

ProRecruit project (Shelf processes controlling recruitment to littoral populations in an eastern oceanic boundary: using barnacles and crabs as models), funded by FCT (No. POCTI/1999/BSE/36663)

PUMPSEA project (Peri-urban mangrove forests as filters and potential phytoremediators of domestic sewage in east Africa), funded by the European Comission (No. FP6, INCO-CT2004-510863)

This dissertation should be cited as: Amaral V (2008) Environmental constraints on population structure and condition of coastal and estuarine crabs (Crustacea: Brachyura). PhD Dissertation, University of Lisbon, Portugal

À minha família, em especial à minha avó Prazer e ao meu tio Carlos

À Rita

Aos meus amigos
To my friends

PRIOR NOTE

In accordance with Paragraph 1 of “Artigo 40, Capítulo V, do Regulamento de Estudos Pós-Graduados da Universidade de Lisboa, publicado no Diário da República – II Série No. 153, de 5 de Julho de 2003”, it is clarified that full scientific articles already published (2), in press (1) and submitted (4) for publication in peer-reviewed scientific journals, were used in the elaboration of this dissertation. Having such works been conducted in colaboration, the cadidate acknowledges that he was fully involved in the planning, sampling, in all laboratory processes, data analysis and discussion of the results of all the works, as well as in their preparation and submission for publication, with the exception of articles "RNA/DNA ratio of crabs as indicator of mangrove habitat quality" and "Effects of vegetation and sewage load on mangrove crab condition using experimental mesocosms", in which the candidate was not directly involved in the sampling.

CONTENTS

AGRADECIMENTOS/ACKNOWLEDGEMENTS

Muitos, e de várias formas, foram os que ao longo de pouco mais de cinco anos contribuíram para este trabalho, e para que ninguém seja esquecido, a todos apresento os mais sinceros agradecimentos. Este trabalho ficaria, com certeza, bastante mais pobre sem os conhecimentos, incentivos, entusiasmo e amizade de todos eles. No entanto, gostaria de agradecer com particular destaque:

Ao Professor Doutor José Paula pela oportunidade que me concedeu e toda a confiança que depositou em mim como seu aluno de doutoramento. Agradeço a ajuda, colaboração, apoio e orientação que me prestou, desde o início, no desenvolvimento deste projecto, e o esforço para disponibilizar as condições indispensáveis às investigações realizadas e à elaboração desta dissertação. Agradeço acima de tudo a amizade e bons momentos partilhados.

To Professor Stephen Hawkins for the opportunity to work at the Marine Biological Association (MBA) and to benefit from the contents of the National Marine Biological Library (NMBL). Thank you for all the support, trust, guidance and supervision provided since my first visit to the MBA. Thank you for the encouragement, for the extremely profitable and clarifying conversations and for the constant good mood.

To Professor Stuart Jenkins for co-supervise me while in the UK and for welcoming me in his work group at the MBA. Thank you for assuring all the necessary conditions for the work carried out in the UK, for all the assistance, counselling, guidance, training and constant availability throughout these years. Thank you for having me in your house, for letting me drive your car until its end and for all the unforgettable good moments. Above all, thank you for the friendship and all the faith in me.

À Fundação para a Ciência e Tecnologia pela concessão da bolsa de doutoramento que permitiu o desenvolvimento deste trabalho em colaboração e que culminou nesta dissertação, e pela cordialidade que sempre pautou a nossa relação.

To all co-authors of the papers included in this work for their valuable contributions, suggestions and improvements to the manuscripts.

A todas as pessoas do Laboratório Marítimo da Guia, desde a coordenação aos vários colaboradores com quem convivi ao longo destes anos. Obrigado por me por terem facultado as condições necessárias ao desenvolvimento dos meus trabalhos, terem criado um excelente ambiente de trabalho e companheirismo e por toda a ajuda e paciência que tiveram para comigo.

To all the staff of the MBA and NMBL, that made possible the development of this work, for all their constant and valuable assistance. To all staff and students that, by one way or another, helped me to conduct my experiments and made my visits to the UK a wonderful and learning experience. I'm particularly thankful to Georgina Budd, Kevin Atkins, Patricia Masterson and Pete Rendle for all their precious help with field and laboratory work.

Ao Professor Doutor Henrique Cabral pelo constante apoio ao longo destes anos, e pelo entusiasmante trabalho de colaboração. Obrigado por me ter sempre facultado espaço e condições de trabalho no seu laboratório, pelas críticas sempre construtivas e constante disponibilidade. Obrigado pela incondicional confiança em mim depositada, e acima tudo, pela forte amizade partilhada.

Ao Professor Doutor Luís Narciso por todo o apoio e intenso trabalho de colaboração, pelo seu constante optimismo boa disposição e, especialmente, pelos muitos momentos divertidos.

Ao Professor Doutor João Castro por me ter sempre facultado espaço de laboratório e alojamento no Laboratório de Ciências do Mar da Universidade de Évora (CIEMAR), indispensáveis a campanhas amostrais no Rio Mira. Obrigado a todo o pessoal do CIEMAR pelo seu apoio e amizade.

À Doutora Vanessa Fonseca por me ter introduzido às técnicas de análise de ácidos nucleicos e guiado durante os primeiros passos.

À Doutora Judite Alves por me ter recebido no seu laboratório, disponibilizado o material necessário à análise de ácidos nucleicos e por todo o seu interesse e entusiasmo.

À Inês Cardoso, Marisa Batista, Patrick dos Santos, Susana França e Susanne Tanner pelas correcções à versão final desta dissertação.

Aos caros colegas dos vários laboratórios com quem, ao longo destes anos, partilhei memoráveis momentos e com quem desenvolvi e aprofundei uma forte amizade e espírito de companheirismo. A todos eles expresso aqui a minha sincera gratidão. Agradeço em especial à Ana Pêgo, Ana Silva, Célia Teixeira, Inês Silva, Joana Marques, Pedro Ribeiro, Ricardo Mendes e Rita Vasconcelos por estarem sempre disponíveis para me ajudar.

To Laure Nöel, Pierre Helaouët and Frederic Verret for making me feel home while in the UK, for all the unforgettable good moments and friendship shared, and for the constant good mood, optimism and playful spirit.

A todos os meus amigos por todo o apoio, com quem partilho os melhores momentos e que tornam os menos bons mais fáceis de ultrapassar. Obrigado por me mostrarem que a amizade é essencial à vida. Obrigado acima de tudo por aceitarem as minhas falhas e por toda a paciência. Agradeço em especial ao José Carvalho toda a ajuda, esforço e dedicação que despendeu em intensas amostragens.

Ao David Sarmento, pela extraordinária e longa amizade e pelo incansável apoio, incentivo e companheirismo. Obrigado pela inesgotável paciência para ouvir os meus desabafos e por toda a ajuda e sacrifícios desenvolvidos em prol do trabalho aqui apresentado. Obrigado pelos inúmeros cafezitos ao longo de todos estes anos, indispensáveis para o meu equilíbrio e estamina emocionais.

À Rita, minha companheira e melhor amiga ao longo de todos estes anos. Obrigado pelo incondicional apoio, incentivo e confiança e pela incansável tolerância para comigo, especialmente nos momentos de fraqueza e mau humor. Obrigado pelas valiosas sugestões, críticas e correcções a todo este trabalho. Obrigado pelos inexcedíveis bons momentos que espero continuar a partilhar. Acima de tudo, agradeço a importância que tem na minha vida e o privilégio que tenho em ser feliz ao seu lado.

À minha família por todo o amor e carinho, especialmente à minha avó Laura, à minha irmã Carla, e aos meus pais, Isaura e José. Obrigado pelo constante apoio, incentivo e confiança e pela forma como me possibilitaram, mediante sacrifícios pessoais e familiares, a realização deste sonho. Agradeço o orgulho que sinto por serem sempre os meus modelos de integridade, honestidade, humanismo e sentido de justiça. Agradeço especialmente aos meus pais, responsáveis por tudo o que de bom existe em mim e a quem tudo devo. Não haverá nunca palavras suficientes que expressem a minha gratidão.

ABSTRACT

Crabs are amongst the most conspicuous and ecologically important organisms inhabiting nearshore environments. Their complex life cycle and bioindicator capacity makes them good models for the study of distribution and abundance patterns of marine fish and benthic invertebrate populations. Such knowledge is essential for explaining species occurrence and demographic patterns, predicting the success of biological indicators and controls, as well as for conservation and management purposes of exploited populations and ecosystems.

In this study, the impacts of specific environmental constraints at different life phases of estuarine and coastal crabs on population structure and physiological condition were evaluated and related to habitat quality. During settlement and recruitment events, the patchiness of artificial substrates significantly influenced settlement estimation, and strong water currents might have resulted in a decoupling pattern between supply and settlement. Estuarine sandy habitats presented higher quality for early recruits than seagrass ones. In fact, vegetated habitats were not of higher quality for crab populations than non-vegetated and rocky-shore ones. Adult-juvenile cannibalism showed potential to structure intertidal crab assemblages, especially under shortage of food and refuge. Interestingly, mangrove crabs were more affected by climatic season than by pollution, and coped well with several combinations of vegetation and sewage load.

Specific constraints of different life stages, individually and in combination, must be considered for full understanding distribution and abundance patterns of crab populations. Furthermore, the relative importance of a specific constraint is likely to change throughout ontogenesis, and the mechanisms by which it interacts with other environmental variables and processes, sometimes synergistically, have also to be considered. This study constitutes an extension to the knowledge on population structure and physiological condition of coastal and estuarine crabs and other marine invertebrate and fish species, and on their use as indicators of ecological status of habitats and ecosystems.

KEYWORDS: Brachyura, Cannibalism, Habitat, Northeast Atlantic, Pollution, RNA/DNA, Settlement, Western Indian Ocean

RESUMO

Os caranguejos encontram-se entre os organismos mais conspícuos e ecologicamente importantes presentes em ambientes costeiros e estuarinos, contribuindo para várias funções do ecossistema e para o seu bom funcionamento. Os caranguejos caracterizam-se por um ciclo de vida complexo, no qual os adultos produzem uma fase larvar pelágica e dispersiva, cujo desenvolvimento ocorre normalmente na zona costeira adjacente, e que precede o assentamento da fase juvenil no ambiente bentónico. Comunidades bentónicas, como as de caranguejos, são normalmente consideradas bons indicadores de variações ambientais integradas ao longo do tempo. Todas estas características conferem aos caranguejos o estatuto de bons organismos modelo para o estudo dos padrões de distribuição e abundância de populações marinhas, demográfica e geneticamente abertas, em ecossistemas costeiros e estuarinos, tais como as formadas por peixes e invertebrados bentónicos.

Apesar da sua importância ecológica e económica ser amplamente reconhecida, os ecossistemas costeiros e estuarinos sofrem de uma generalizada degradação devido às mudanças globais do clima, e consequente subida do nível do mar, e às tendências mundiais das actividades humanas. Como consequência, os padrões naturais de regulação das populações e funcionamento dos ecossistemas são afectados, colocando em sério risco a persistência ecológica e económica de tais ecossistemas e dos seus recursos. Em conformidade, os principais objectivos deste estudo foram avaliar os impactos provocados por constrangimentos ambientais, de origem natural e antropogénica, na estruturação e na condição fisiológica de populações intertidais de caranguejos presentes em ambientes costeiros e estuarinos heterogéneos, e relacionar tais respostas com a qualidade do habitat. Este conhecimento é extremamente relevante para o sucesso de programas, estratégias e politicas dirigidas à conservação, protecção, restauração e gestão sustentada dos ecossistemas costeiros e estuarinos.

Este estudo incorpora três partes: uma introdução geral (Secção I), sete investigações específicas (Secção II) e uma discussão geral considerando todas as investigações conduzidas e as suas principais conclusões (Secção III). As investigações realizadas resultaram em outros tantos artigos científicos (papers) que se encontram publicados (2), em publicação (1) ou na fase de revisão (4) em jornais científicos internacionais e indexados. Cada um destes artigos é apresentado sob a forma de um capítulo.

A introdução geral (Secção I) aborda a função dos constrangimentos ambientais na regulação e estruturação das comunidades e populações naturais, bem como o crescente interesse em tal conhecimento, no que respeita a populações marinhas de peixes e de invertebrados bentónicos. A relevância ecológica das comunidades de caranguejos para diversas funções do ecossistema e para o seu desempenho global, bem como o seu potencial e utilidade como bioindicadores da qualidade do habitat são explorados. Neste contexto, a importância relativa de diferentes constrangimentos ambientais em fases particulares do ciclo de vida dos caranguejos e no estabelecimento da estrutura das populações e da sua condição fisiológica são examinados. Simultaneamente, são apresentadas e exploradas as questões que fundamentaram as investigações conduzidas. No final da introdução, os objectivos e a importância deste estudo são discutidos e enquadrados num contexto de conservação e gestão, seguindo-se uma descrição da estrutura da dissertação.

Na parte dedicada às investigações realizadas (Secção II), a influência do tipo e forma de substratos artificiais na estimativa do assentamento de megalopas (a última fase larvar de caranguejos Brachyura) foi estudada, usando o caranguejo *Carcinus maenas* (L.) como organismo modelo (Capítulo 1). Diferentes medidas de assentamento foram estimadas a partir de colectores de "hog's hair" de vários tamanhos, e o potencial de superfícies de relva plástica para substituir aqueles colectores foi avaliado. Os padrões de assentamento variaram de acordo com a sua intensidade, mas em geral, uma maior razão perímetro:área, associada a uma maior área, resultou num menor número total de larvas e em taxas mais elevadas de assentamento por área. Estes padrões poderão ser o resultado dos colectores dos vários tamanhos terem maior ou menor superfície exposta às megalopas. As superfícies de relva plástica revelaram ser substitutos promissores dos colectores de “hog's hair” em estudos de assentamento.

A investigação das causas do desfasamento entre o fornecimento de megalopas de *C. maenas* em marés vivas e o assentamento em marés mortas, na parte inferior do estuário do Mira, Portugal, é apresentada no Capítulo 2. Para avaliar os processos tidais envolvidos no recrutamento larvar, foram realizadas amostragens exaustivas do fornecimento, fluxo, disponibilidade planctónica a cada hora e do assentamento larvar. Os resultados indicaram que o desfasamento não resultou de comportamentos larvares inesperados, nem de sub-estimativas dos processos de recrutamento. Constrangimentos de ordem física, nomeadamente elevados níveis de turbulência durante as marés vivas, podem ter limitado o assentamento larvar na parte inferior do estuário e anulado a inibição natatória das larvas devida à intensidade luminosa.

No estudo apresentado no Capítulo 3, coortes sucessivas das fases juvenis iniciais de *C. maenas*, foram estudadas em períodos consecutivos de marés mortas durante a época de recrutamento em habitats de areia e de *Zostera noltii* Hornemann, 1832. As diferentes coortes foram identificadas pelo método de Bhattacharya. O crescimento, estrutura de tamanhos e condição fisiológica (razão RNA/DNA) das populações foram estimadas em cada habitat, e as suas implicações foram discutidas em termos do potencial de sobrevivência dos indivíduos na população e da qualidade do habitat. Um crescimento mais lento, uma condição fisiológica mais baixa e uma frequência mais elevada de indivíduos de menores dimensões, caracterizaram o habitat vegetado, relativamente ao arenoso. Estes padrões podem resultar de uma maior competição por alimento e espaço no habitat vegetado, devido a uma maior densidade de indivíduos, e também a dispersão secundária dos juvenis do habitat vegetado para o arenoso.

O Capítulo 4 é dedicado à importância e qualidade relativas de habitats estuarinos e costeiros para populações de *C. maenas*, sendo habitat de boa qualidade definido como aquele onde o crescimento e a sobrevivência de indivíduos e populações são potenciados relativamente a outros. A densidade, estrutura de tamanhos e condição fisiológica das populações foram avaliadas e comparadas em duas áreas do Atlântico Norte, perfazendo cinco habitats diferentes: uma zona mais a sul (SW de Portugal), com os habitats *Z. noltii* e lama argilosa; e uma mais a norte (SW de Inglaterra), com os habitats *Fucus* spp., lama grosseira e praia rochosa. O habitat praia rochosa na área mais a norte apresentou a qualidade mais elevada, sugerindo que habitats estuarinos vegetados não são necessariamente os de maior qualidade. Ambos os tipos de habitats estuarinos parecem ter uma qualidade semelhante para as populações de ambas as áreas, apesar de diferenças na sua importância relativa ao longo do ciclo de vida, e do facto de os habitats vegetados suportarem um maior numero de indivíduos, podendo favorecer níveis mais elevados de competição intraespecífica.

O Capítulo 5 é dedicado à investigação das interacções canibalistas e interespecíficas, avaliadas como perda de apêndices, mortalidade das presas e consumo de presas, nos caranguejos *Cancer pagurus* (L.) e *Porcellana platycheles* (Pennant, 1777). Apesar de ocorrerem no mesmo ambiente, estas espécies apresentam padrões comportamentais e alimentares bastante diferentes. Foram conduzidas várias experiências laboratoriais em simultâneo, onde foram manipuladas as densidades de presas e canibais, o fornecimento de alimento e de presas alternativas, bem como o tipo de habitat. Os resultados sugerem que *P. platycheles* é uma espécie não agressiva que pode constituir uma presa preferencial de *C. pagurus*. As interacções canibalistas entre diferentes classes de tamanho podem estruturar populações intertidais de *C. pagurus*, apesar de serem significativamente reduzidas na presença alimento, presas alternativas e refúgios apropriados. No habitat de *Fucus* spp., o fornecimento de comida resultou em 100 % de sobrevivência dos juvenis.

O potencial da condição fisiológica dos caranguejos de mangal, nomeadamente Ocypodidae e Grapsidae, como indicador bioquímico da qualidade do habitat em termos da poluição e de variações sazonais, foi investigado no Capítulo 6. A razão RNA/DNA de *Uca annulipes* (H. Milne Edwards, 1837) e *Perisesarma guttatum* (A. Milne Edwards, 1869) foi comparada em dois mangais em condições relativamente pristinas e um mangal peri-urbano. Apenas a condição fisiológica de *U. annulipes*, Ocypodidae, revelou potencial como bioindicador, sendo significativamente mais baixa no mangal peri-urbano. Os hábitos alimentares de ambas as espécies podem estar envolvidos nas suas respostas fisiológicas à poluição. Ambas as espécies foram mais afectadas por variações sazonais do que pela poluição, tendo apresentado melhor condição na estação seca. Foi detectado um efeito sinergístico da variação sazonal e da poluição, potenciando o efeito dos impactos causados por um possível aumento adicional das elevadas temperaturas que caracterizam os sistemas de mangal.

O Capítulo 7 diz respeito à investigação dos efeitos da vegetação e de diferentes cargas de esgoto urbano em *U. annulipes* e *Uca inversa* (Hoffman, 1874), em mesocosmos experimentais. A condição fisiológica de ambas as espécies foi estimada em diferentes condições de esgoto urbano e vegetação (*Avicennia marina* [Forsk.], *Rhizophora mucronata* Lamk. 1804 e substrato sem vegetação) ao longo de 12 meses e relacionada com a qualidade do habitat. Os resultados mostraram que o sistema de mesocosmos, filtrando eficazmente o esgoto urbano, proporcionou uma boa condição fisiológica de ambas as espécies e em todas as condições. À semelhança de sistemas naturais de mangal, a condição fisiológica dos caranguejos foi mais afectada por variações sazonais do que pela poluição. As condições que mais se assemelharam às naturais foram obtidas com *A. marina* que, juntamente com caranguejos do género *Uca*, pode contribuir para uma boa qualidade do habitat e um bom desempenho ecológico do sistema.

Finalmente, na Secção III, todas as investigações conduzidas são integradas numa discussão geral onde a importância relativa de diferentes constrangimentos ambientais, que impactam fases específicas da vida de caranguejos costeiros e estuarinos, é reconhecida. Este estudo apresenta evidências de que mais do que algum constrangimento ambiental *per se*, a interacção entre todos em conjunto e os efeitos daí resultantes, devem ser considerados para uma compreensão efectiva dos padrões de distribuição e abundância de organismos e populações em ambientes naturais heterogéneos. Este estudo constitui uma extensão ao conhecimento da condição fisiológica e estrutura populacional de caranguejos costeiros e estuarinos, e de outras espécies de peixes e invertebrados marinhos, e que pode ser empregue em programas de conservação e gestão de tais organismos e dos seus habitats.

PALAVRAS CHAVE: Assentamento e pós-assentamento, Brachyura, Canibalismo, Nordeste Atlântico, Oceano Índico Oeste, Poluição, Qualidade do habitat, RNA/DNA

LIST OF PAPERS

This dissertation comprises the following scientific manuscripts:

Amaral V, Paula J (2007) *Carcinus maenas* (Crustacea: Brachyura): influence of artificial substrate type and patchiness on estimation of megalopae settlement. Journal of Experimental Marine Biology and Ecology 346:21-27

Amaral V, Queiroga H, Skov M, Paula J (2007) Planktonic availability and settlement of *Carcinus maenas* (L.) megalopae at different time scales in the lower Mira Estuary (SW Portugal). Marine Ecology Progress Series 348:239-248

Amaral V, Cabral HN, Paula J (in review) Implications of habitat-specific growth, size-structure and physiological condition on early juvenile crab populations. Marine and Freshwater Research

Amaral V, Cabral HN, Jenkins S, Hawkins S, Paula J (in press) Quality of estuarine and nearshore marine native habitats: the *Carcinus maenas* case. Marine Ecology Progress Series

Amaral V, Paula J, Hawkins S, Jenkins S (in review) Cannibalistic interactions in two sympatric decapod species: effects of density, food alternative prey and habitat. Journal of Experimental Marine Biology and Ecology

Amaral V, Penha-Lopes G, Paula J (in review) RNA/DNA ratio of crabs as indicator of mangrove habitat quality. Aquatic Conservation: Marine and Freshwater Ecosystems

Amaral V, Penha-Lopes G, Paula J (in review) Effects of vegetation and sewage load on mangrove crab condition using experimental mesocosms. Estuarine Coastal and Shelf Science

SECTION I

GENERAL INTRODUCTION

Opening remarks

Understanding the factors and processes regulating the distribution and abundance of organisms and populations in heterogeneous environments, lies at the heart of ecology. This regulation is thought to be fundamental for the persistence of natural populations of many species, despite the underlying mechanisms often having eluded identification (Murdoch 1994, Hixon et al. 2002). Knowledge on the mechanisms of regulation is essential for explaining species occurrence and demographic patterns, predicting the success of biological indicators and controls, as well as for management of exploited populations and communities. As human activities interfere with natural regulatory processes of populations of many species, ultimately driving them towards extinction, the preservation of those processes are also crucial for conservation (Hixon et al. 2002). This study addressed these issues using crabs as a focal model system.

The following section of this introduction provides an overview of population biology, focusing on the effects of environmental factors on population ecology. Afterwards, a review on the biology of crabs, the chosen model organism, is presented in the context of the main study sites used. Finally, the aims and objectives of this study are outlined, and the rationale driving to the investigations conducted is explained.

Population biology

Regulation of populations requires the occurrence of negative density-dependent feedback mechanisms whereby the population has a propensity to increase when small and decrease when large (Murdoch 1994, Hixon et al. 2002). Due to both abiotic and biotic constraints, populations often fluctuate in affinity with the environment at levels below their potential demographic carrying capacity (Hixon et al. 2002, Berryman 2004). Density-dependent processes usually arise from biotic constraints such as diseases, parasites, competition for resources (e.g. food, refuge, breeding and settlement grounds) and predation, including cannibalism (Murdoch 1994, Hixon et al. 2002, Berryman 2004). The impact of these constraints on the population is more intense at enhanced organism densities, affecting a higher proportion of individuals. On the other hand, density independent processes are usually caused by abiotic factors such as physical disturbances, harsh climatic conditions and pollution, that are capable of obliterating part or all the population, irrespective of organism density (Murdoch 1994, Hixon et al. 2002, Berryman 2004).

Understanding the mechanisms by which all such environmental processes affect organisms and populations is of increasing importance not only to researchers, but also to local communities and policy-makers, as they have ecological, conservation and economic implications. Interest in determining the physiological condition of organisms in a natural context, resulting from recognized environmental variation has been escalating (e.g. Wagner et al. 1998, Dahlhoff 2004, Fonseca & Cabral 2007). Physiological changes in organisms, as a response to environmental stresses, usually occur before external indicators, such as growth rates, become discernable, and may thus help in the early detection of the effects of stressors. The physiological state of organisms under natural environmental conditions, have thus become central to integrative studies evaluating organism and population health and their respective implications to processes structuring populations and affecting ecosystem performance (Dahlhoff 2004, Gilliers et al. 2004, Fonseca & Cabral 2007).

Most early theoretical and empirical studies on the impact of environmental constraints were based on terrestrial systems, where mechanisms characteristic of closed populations prevail. Therefore, interest has been rising on the structure and condition of open marine populations i.e., those whose local recruitment is uncoupled from local reproduction and where gene flow and migratory movements can openly occur. This vagility is characteristic of most fish and marine benthic invertebrate species with a two-phase life cycle, in which adults produce a pelagic dispersive larval stage that usually develops in coastal waters before recruitment (addition of new individuals to successive phases of the life cycle within the population, *sensu* Caley et al. [1996]). The transition from the pelagic to a more benthic existence is called settlement, and is a crucial event in the recruitment process. Population structure and physiological condition of species with such a two-phase life cycle can, at least theoretically, be affected during only one or both phases of the life cycle by abiotic and biotic constraints, making the understanding of population abundance and distribution patterns more challenging (Roughgarden et al. 1988, Caley et al. 1996).

The relative importance of recruitment and post-settlement constraints in structuring populations of those species has generated some debate among ecologists

(Roughgarden et al. 1988, Ólafsson et al. 1994, Caley et al. 1996). Almost six decades ago, Thorson (1950) observed that the majority of marine invertebrate species with such a life cycle, have larger abundance fluctuations than those of one-phase life cycle, suggesting that this resulted from the hazards of planktonic existence that enhance temporal and spatial variability in recruitment. The recruitment limitation hypothesis arising from this rationale, implicated that abundance and structure of adult populations were directly dependent on the rate of larval availability, rather than being dictated by abiotic and biotic constraints after this ontogenetic stage.

The acknowledgement that levels of recruitment are capable of structuring populations led to a reassessment of previous models of population regulation, which relied almost exclusively on post-settlement factors and processes operating over juvenile and adult stages (e.g. Menge & Sutherland 1987, Roughgarden et al. 1988). For many researchers, the hypothesis proposed by Thorson was the key to understanding the distribution and abundance of open populations, especially those from marine and estuarine environments. However, if populations or settlement habitats are saturated by organisms, or if settlement is geographically limited, even large fluctuations in larval availability will have little impact on the population distribution and abundance patterns (Roughgarden et al. 1988, Hyder et al. 2001).

Biotic processes generating post-settlement competition for resources, as well as those of an abiotic nature limiting the performance and persistence of organisms and populations, have also been recognized as capable of affecting population size and structure, as well as the physiological status of fish and marine benthic invertebrate species (Roughgarden et al. 1988, Ólafsson et al. 1994, Caley et al. 1996, Hunt & Scheibling 1997, Dahlhoff 2004). In particular, the early post-settlement period is recognized as one of the most vulnerable phases throughout the ontogeny of organisms, both due to the relatively small body size and the high energetic effort spent during settlement and metamorphosis to the juvenile stage (Ólafsson et al. 1994, Diehl & Ekloev 1995, Gosselin & Qian 1997, Wagner et al. 2001).

In addition to all natural abiotic and biotic factors, human activities, together with climate change and consequent sea-level rise, pose a different set of constraints to both organisms and populations (Walther et al. 2002, Kaiser et al. 2005, Kathiresan & Qaim 2005, Hogarth 2007). Among all human induced constraints, pollution and habitat destruction are amongst the most widespread and harmful to organisms and populations, acting synergistically in many occasions, and especially impacting environments influenced by man, such as estuarine, coastal and other nearshore ecosystems (e.g. Eshky et al. 1996, Walther et al. 2002).

The majority of marine fish and benthic invertebrate species use a wide range of different recruitment, nursery and/or reproduction habitats throughout ontogenesis, such as rocky and sandy shores, estuarine seagrass meadows and different estuarine non-vegetated areas, increasing the diversity within communities and assemblages (e.g. Feldman et al. 1997, Moksnes et al. 1998, Parslow-Williams et al. 2001, Paula et al. 2006). These different habitats likely present distinct conditions and features, such as structural complexity, animal density, physical and chemical characteristics, etc (e.g. Reid et al. 2006), which contribute to the high heterogeneity of coastal and estuarine environments. This heterogeneity, and the associated variability of habitat characteristics, must thus be considered for a full understanding of the effects of environmental processes acting in different habitats.

Only the recognition of the relative importance of the different constraints acting throughout ontogenesis of organisms, and the assessment of their consequences on the structure and condition of populations, will allow the understanding of the distribution and abundance patterns of populations of fish and marine benthic invertebrate species in heterogeneous environments. This has been the approach adopted in this study.

Crabs (Crustacea: Brachyura) as the case study

Among decapod crustaceans, crabs form the infra-order Brachyura, dating back from the Jurassic period (Spears et al. 1992). Brachyuran crabs have undergone extensive radiation, resulting in a group of organisms extremely diverse, both morphologically and ecologically (Spears et al. 1992). Their evolution resulted in an increasing robustness of the body and a reduction of the abdomen, which is folded beneath the cephalothorax, and inserted between the pereiopods or in a special cavity. Crab species inhabit all types of marine environments from the tropics to polar seas, being perhaps the most ubiquitous organisms of rocky shores and intertidal areas of wetland ecosystems (Kritzer & Sale 2006, Hogarth 2007). Several crab species also support longstanding important fisheries, which have provided powerful incentives for the scientific study of their ecology and population dynamics.

Crabs are particular important organisms of intertidal environments, namely rocky shore and estuarine systems, where they have a vital importance in the structure and functioning of the ecosystem (Kaiser et al. 2005, Kathiresan & Qaim 2005, Hogarth 2007). Their burrowing and feeding activities improve the aeration of the substrate, enhance the recycling of soil sediments and the regeneration of organic nutrients, increase the decomposition rates of plant and animal debris and also create microhabitats and favour the growth of micro-organisms in the substrate (Smith et al. 1991, Kaiser et al. 2005, Hogarth 2007). Crabs also constitute a fundamental link between primary producers and secondary consumers, while contributing to secondary production (Kaiser et al. 2005, Kathiresan & Qaim 2005, Hogarth 2007).

Macrofaunal benthic assemblages, including crab communities, have been considered to be amongst the most sensitive to changes in environmental variables, reflecting the conditions integrated on the benthos over time, rather than just those at the time of sampling (Smith et al. 1991, Guerra-Garcia & Garcia-Gomez 2004, Elias et al. 2005, Pagliosa & Barbosa 2006). Consequently, an increasing number of studies have used crab individuals, populations and assemblages as bioindicators of habitat quality and ecosystem health, both in relation to natural and anthropogenic factors (e.g. Bamber & Depledge 1997, Mayrand et al. 2000, Bosire et al. 2004, Martin-Diaz et al. 2005).

In addition to the two-stage life cycle, populations of most crab species of marine and estuarine environments exhibit well-defined spatial structuring linked to habitat preferences and requirements (Kaiser et al. 2005, Hogarth 2007). These characteristics of crab assemblages, together with their ecological importance and usefulness as bioindicators of several ecosystem parameters, makes them particularly valuable models for the study of environmental constraints affecting the structure and condition of open marine populations.

After hatching, larvae of estuarine and coastal crabs are usually dispersed to nearshore waters where they develop through several stages for days or months, depending on the species and environmental conditions (Dawirs 1985, Nagaraj 1993, Queiroga 1996, Flores et al. 2007). Most commonly, brachyuran crabs undergo indirect development, advancing throughout zoea and megalopae larval stages to the juvenile stage. Those different larval stages can be characterized by their swimming activity, namely if it is dependent on thoracic or abdominal appendages, respectively (Bliss 1982). There is some controversy in the use of the term post-larvae, since it has been defined both as the last larval stage (in this case, megalopae) and as the first stage after the larval phase i.e., the first juvenile (Bliss 1982). To avoid misunderstandings, the term post-larvae will not be used in this study, and megalopae will refer to the last larval stage i.e., that before the first juvenile stage, which will be referred as such.

Near the end of planktonic development, larvae must reach shallow estuarine and coastal habitats where settlement may occur with success before metamorphosis of megalopae to the juvenile stage (Little & Epifanio 1991, Bilton et al. 2002, Tankersley et al. 2002, Queiroga et al. 2006). Settlement is a critical event during the life of many crabs, but also of several fish and invertebrate species, as it is dependent on environmental factors, as well as on larval physiological and behavioural adaptations, constituting a critical phase in the population biology of such species. The quality of settlement habitats is thought to be particularly crucial for the survival success of megalopae and early juveniles, and the size and shape, as well as the physical and chemical characteristics of such habitats are known to be involved in their active selection by settling megalopae (Pawlik 1992, Eggleston et al. 1998, Jenkins et al. 2002, Moksnes 2002, Moksnes & Heck 2006).

Settlement studies usually involve the use of artificial settlement substrates (collectors) that enhance the thigmotactic response of the cryptic megalopae and juvenile stages, by simulating the required settlement needs (Phillips & Booth 1994, Eggleston et al. 1998). The surface material most common and broadly used in artificial collectors of various designs is the hog's hair filter, whose structural complexity fulfils many of the required characteristics of quality settlement grounds, especially for brachyuran crabs (Phillips & Booth 1994, Phillips et al. 2001, Paula et al. 2003, Paula et al. 2006). For the use of these collectors to be effective, beyond the acquisition of individuals for aquaculture purposes, the settlement patterns and rates obtained need to be well correlated with those of natural settlement.

A recent study revealed that the interaction between the structural complexity of the collectors and the surrounding deployment environment, might significantly affect the settlement rates obtained from such collectors (Paula et al. 2006). Furthermore, the effects of size and shape of hog's hair collectors on settlement estimation are largely unknown. More specifically, is not well understood whether a larger perimeter:area ratio of the collector surface will yield higher or lower settlement rates, and if such response is dependent on settlement intensity. Such knowledge will help the validation and understanding of settlement patterns and rates in relation to those of natural settlement, as well as facilitate comparisons among studies using different collector designs.

Despite the generalized recognition of their usefulness, the use of hog’s hair collectors poses several problems, and there is a need for an effective substitute. The high structural complexity provided by the hog’s hair fibres might restrict the movement of settled organisms, masking real settlement patterns by preventing the return of megalopae to the water column. In addition, the retrieval of settled organisms is always associated with mechanical stress that can cause the death of such organisms, and the cost and availability of hog’s hair material is becoming limiting. Regular plastic grass surfaces are characterized by a high structural complexity, conferred by numerous small plastic blades, being also cheaper and easier to obtain. Furthermore, the resistance and flexibility provided by the plastic material might be advantageous in both the field and laboratory. All together, this suggests that plastic grass surfaces may have the potential to be a substitute for hog’s hair ones, but their efficiency needs to be tested.

Settlement events are usually preceded by the supply of megalopae to nearshore waters. In mesotidal systems, megalopae are transported in upstream direction by travelling near the surface during flood tides and remaining near the bottom during ebb tides, and settle by the end of flood tide (Little & Epifanio 1991, Queiroga 1998, Tankersley et al. 2002, Paula et al. 2004). Both upstream transport and settlement usually occur during darkness, as the swimming activity of megalopae is inhibited by light (Queiroga 1998, Forward et al. 2004).

Interestingly, in the Mira Estuary, Portugal, settlement of *Carcinus maenas* (L.) occurs around neap tides, when co-occurrence of flood tide and darkness is minimal, which is clearly decoupled from periods of maximum larval supply occurring around spring tides (Paula et al. 2006, Queiroga et al. 2006). Several explanations of both abiotic and biotic nature have been suggested for the occurrence of such patterns (Queiroga et al. 2006). However, all of them are yet to be tested, as data available on *C. maenas* recruitment is restricted to daily or lower frequencies, and presents possible underestimations of larval supply and settlement due to incomplete sampling designs.

In order to assess the tidal processes involved in larval transport and settlement in mesotidal systems, an exhaustive sampling of all aspects of *C. maenas* recruitment, performed at high temporal resolution, is necessary to address the decoupling between supply and settlement events in the Mira Estuary. Specifically, such sampling effort should be able to answer whether megalopae entering the estuary remain there throughout, whether settled megalopae return to the water column due to crowding, whether settlement has been previously underestimated, and whether the reported pattern is due to deviations from the conceptual model of reinvasion and settlement for estuarine brachyuran crabs.

Several fish and benthic invertebrate species, including brachyuran crabs, use a variety of different settlement and nursery habitats, with deferring structural complexity, yielding specific growth and survival conditions to the individuals (Hyder et al. 2001, Gilliers et al. 2004, Paula et al. 2006, Reid et al. 2006). Newly metamorphosed juveniles are particularly vulnerable to the surrounding environment, and individuals with higher growth rates and better physiological condition will have enhanced survival potential within the population (Suthers 1998, Fonseca et al. 2006).

Habitats of higher structural complexity, usually characterized by the presence of macrophytes (e.g. seagrass meadows), are thought to constitute quality habitats for early juvenile stages, as they provide higher availability of food and refuge from predation and physical stresses, when compared to less structurally complex environments (Orth & van Montfrans 1990, Feldman et al. 1997, Beck et al. 2001, Reid et al. 2006). Furthermore, seasonal environmental conditions are also likely to have repercussions on juvenile growth and condition in several marine species (Jarrett 2003, Fonseca et al. 2006). Although enhanced growth and survival may be expected in vegetated habitats, there is little empirical evidence that clearly supports higher growth rates and better physiological conditions of early juvenile stages in such habitats, in comparison with others of lower structural complexity. Particularly, knowledge is lacking on whether juvenile crab physiological condition is in fact higher in seagrass meadows than in sandy areas, whether such difference is reflected on the size-structure of the juvenile population, and whether it is dependent on a period during the settlement season.

Vegetated habitats are also generally characterized by larger populations than adjacent non-vegetated habitats (Polte et al. 2005, Fonseca et al. 2006, Paula et al. 2006). However, non-estuarine and non-vegetated habitats can also represent quality settlement and nursery habitats for a variety of marine fish and invertebrate species (Beck et al. 2001, Glancy et al. 2003, Moksnes & Heck 2006), even when adjacent to vegetated habitats (Diehl & Ekloev 1995, Parslow-Williams et al. 2001). Furthermore, the relative quality of different habitats, and thus their contribution to the ecosystem functioning, may change throughout ontogenesis, especially in mobile species such as brachyuran crabs (Werner & Gilliam 1984, Gosselin & Qian 1997), but also both geographically, due to climate regime and species pool, and locally, due to geophysical characteristics (Orth & van Montfrans 1990, Beck et al. 2001, Bas et al. 2005).

Habitat quality, together with availability of resources, can thus regulate the distribution and abundance patterns of populations, and their assessment is a valuable tool for conservation, management and exploitation of populations and ecosystems. However, knowledge is lacking on whether estuarine vegetated habitats are in fact of higher quality for crab populations as a whole than adjacent non-vegetated ones, including rocky shores, not only in terms of population density and size-structure, but also in terms of physiological condition of the individuals. There is also few empirical data on whether such habitat quality persists at different latitudes, especially when considering native distribution ranges.

Settlement and nursery habitats may create favourable conditions resulting in the accumulation of individuals at high densities and, hence, high levels of intraspecific competition (Caley et al. 1996, Hunt & Scheibling 1997). Competition was the first process to be recognized as capable of regulating populations (Volterra 1928, Lotka 1932) (see Hixon et al. 2002). However, the importance of cannibalism, an outcome of aggressive intraspecific competition, on population structure and synchrony and in stock-recruitment relationships, has only been acknowledged in the last decades (Fox 1975, Polis 1981, Moksnes 2004, Wise 2006).

Cannibalism is a common behaviour in populations of several crab species (Lovrich & Sainte-Marie 1997, Luppi et al. 2001, Moksnes 2004), but its intensity and repercussions may be considerably different both within and among species, due to specific behaviour and variation in environmental conditions (Fox 1975, Polis 1981). Prey and predator densities, respective size ratio, food availability, alternative prey and habitat type, all influence cannibalistic behaviour (Fox 1975, Polis 1981, Wise 2006). Nevertheless, most of our understanding of such behaviour results from the sum of different studies, each usually focusing on only a few of such factors.

Understanding of cannibalistic interactions and associated factors as a whole, is essential for the success of conservation and management plans, and has also gained importance due to the increase in significance of aquaculture, where an objective is the mass production of single species. For such comprehension, it will be necessary to evaluate whether intrasize class cannibalism is determined by density, food supply or the absence of alternative prey, what are the effects of prey and predator densities, food supply, alternative prey and habitat type on intersize class cannibalism, and whether any or both intra and intersize class cannibalism are capable of population regulation.

Unfortunately, and despite the above stated recognized importance of estuarine and nearshore marine habitats to a variety of vital ecological functions, including the persistence of fish and benthic invertebrate species, their loss and deterioration due to increasing anthropogenic pressure, and all associated impacts, continues at alarming rates (Gibson 1994, Costanza et al. 1997, Kaiser et al. 2005, Airoldi & Beck 2007, Duke et al. 2007). Among those impacts, pollution, and particularly sewage discharges, are amongst the most harmful anthropogenic induced constraints on such natural ecosystems (Hogarth 2007). This is of particular concern in developing countries where resources and technology for appropriate wastewater treatment are scarce (Boonsong et al. 2003, Kathiresan & Qaim 2005, Hogarth 2007). Furthermore, and in addition to the long term-trends of global climate change, several of such countries are characterized by natural elevated temperatures, which may act synergistically with stressors such as pollution.

Mangroves are characteristic wetland ecosystems common in many tropical countries, whose vital and broadly recognized support to many ecological and economical functions are at serious risk due to several pollution issues (Costanza et al. 1997, Alongi 2002, Duke et al. 2007). Brachyuran crabs are conspicuous organisms of mangrove systems, and besides constituting perhaps the most important macrofauna taxa on such environments, they also play an indispensable role in the system functioning through their feeding and burrowing activities (Smith et al. 1991, Kathiresan & Qaim 2005, Hogarth 2007). However, despite its recognized importance, knowledge on the effects of pollution on mangrove crabs is greatly lacking, especially when those are associated with seasonal climatic fluctuations. In particularly, the bioindicator potential of the physiological condition of mangrove crabs regarding habitat quality, as affected by pollution and climatic season, has been highly neglected.

Furthermore, the need for such empirical information has been rising due to the study and use of constructed mangrove wetlands as successful alternative sewage treatment areas (e.g. Ye & Tam 2002, Boonsong et al. 2003). The introduction of crabs in those areas may constitute an important contribution for a healthy ecological performance of the system. However, knowledge is lacking on which sewage load and type of mangrove vegetation, if any, are more appropriate to yield favourable conditions for mangrove crabs and, ultimately, for a good sewage filtering capacity and ecological system functioning.

Aims and importance of this study

This study aimed to evaluate the impacts of important environmental constraints acting on particular life phases of crab populations living in intertidal communities, within estuarine and coastal heterogeneous environments. Patterns of abundance, distribution and physiological condition of organisms and populations, were examined and their implications were related to habitat quality.

More specifically, an objective of this study was to evaluate the importance of habitat patchiness on the estimation of settlement rates of crab megalopae, when using artificial collectors. The efficiency of a new type of collector, plastic grass surfaces, was tested in the estimation of daily settlement rates, when compared to those obtained from hog's hair collectors, with the intent of their future use as substitutes of the previous ones. Another objective was to investigate the mechanisms and processes responsible for the decoupling between larval supply and settlement events of shore crab megalopae reported for the lower Mira Estuary. This study further intended to understand the physiological condition and growth patterns of shore crab early recruits of on successive cohorts during the recruitment season, in different estuarine nursery habitats: a seagrass meadow and a sandy beach. Another goal was to assess the relative quality of different estuarine and coastal native habitats for shore crab populations in terms of density, size-structure and physiological condition, and to relate such quality with latitudinal differences. The understanding of cannibalistic and interspecific interactions between two co-occurring crab species with different feeding and behavioural patterns, considering concurrently several important environmental constraints, constituted another aim of this study. Finally, this study intended to evaluate the bioindicator potential of the physiological condition of mangrove crabs, in terms of pollution and seasonal fluctuation, to address the effects of mangrove vegetation and sewage load on such physiological condition, and to relate it with habitat quality and ecological performance of the system.

Both marine fish and benthic invertebrate populations, are an essential component of estuarine and coastal ecosystems, and support several economically important fisheries and aquaculture operations. The generalized clearance and deterioration of nearshore ecosystems, together with global climate change and associated sea-level rise, are jeopardizing the ecological and economical persistence of such populations, putting at risk the stability and functioning of ecosystems and that of local communities (Costanza et al. 1997, Walther et al. 2002, Hogarth 2007). Therefore, there is an urgent need for effective conservation, protection, restoration and sustainable managing programmes for such ecosystems and resources (Hixon et al. 2002, Duke et al. 2007).

The success of such integrative programmes will depend on intelligent, responsible and informed policies, strategies and actions based on available comprehension on the environmental mechanisms constraining the structure and condition of marine fish and benthic invertebrate populations. Crabs are good model organisms of such populations, and thus the knowledge of their ecology constitutes a valuable tool to be used in the efforts to guarantee the persistence of wetland ecosystems and all its constituents.

Structure of the dissertation

In order to accomplish the objectives of this study, seven specific investigations were conducted and each resulted in a scientific manuscript (paper). This dissertation incorporates these papers, each constituting a full chapter (Section II), being either published (2), in press (1) or under review (4) in peer refereed international scientific journals.

Several issues are posed by the use of hog’s hair collectors in the estimation of settlement parameters. Dealing with such issues, the study of the effects of size and shape of hog’s hair collectors, and the potential of plastic grass surfaces as their substitutes, on estimation of megalopae settlement of *Carcinus maenas* (L.) is presented in Chapter 1, using *Carcinus maenas* (L.). The interesting pattern of decoupling between supply and settlement events of *C. maenas* in the Mira Estuary, Portugal, was addressed by evaluation of planktonic availability and settlement of megalopae at different time scales. Such investigation is the subject of Chapter 2.

Immediately following settlement, habitat characteristics have a major influence on the survival of young recruits. The implications of habitat-specific growth, size-structure and physiological condition on early juvenile crab populations of *C. maenas* are the subject of Chapter 3. Furthermore, the quality of estuarine and nearshore native habitats for local populations as a whole, was investigated also using *C. maenas* as a test organism (Chapter 4).

A constraint resulting from strategies adopted at settlement and during early juvenile life is the accumulation of individuals at high densities in natural habitats, which might create favourable conditions for aggressive intraspecific competition. In this sense, intra and intersize class cannibalism, as well as interspecific interactions in two co-occurring crab species, *Cancer pagurus* (L.) and *Porcellana platycheles* (Pennant, 1777), were studied in simultaneous experiments manipulating prey and predator densities, food supply, alternative prey and habitat type. This investigation is presented in Chapter 5.

Despite its global coverage and harmful impacts, the effects of pollution on crab populations are largely unknown, especially in wetlands of developing countries, where such issues are, unfortunately, magnified by the lack of appropriate resources and technology. The potential bioindicator of habitat quality of the physiological condition of mangrove brachyuran crabs, regarding pollution stressors in different climatic seasons, is the subject of Chapter 6. Based on the results of such evaluation, the effects of mangrove vegetation and sewage load on crab condition were studied in constructed mesocosms, and the respective data are presented and discussed in Chapter 7.

A general discussion considering all the investigations conducted, the main conclusions drawn from this study and the contributions made to the understanding of the ecology of marine open populations are the contents of Section III, together with the issues that have arose and on which further research is necessary.

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SECTION II

CHAPTER 1

Carcinus maenas (Crustacea: Brachyura): influence of artificial substrate type and patchiness on estimation of megalopae settlement

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J Exp Mar Biol Ecol (2007) 346:21-27

Carcinus maenas (Crustacea: Brachyura): influence of artificial substrate type and patchiness on estimation of megalopae settlement

ABSTRACT

Settlement patterns of decapod crustaceans are influenced by size, shape and nature of substrates, as well as by species-specific behaviour patterns. Hog's hair filter material is one of the most commonly used artificial substrates in the study of settlement rates. However, the use of hog's hair collectors in settlement studies poses several problems: movement restriction of settled animals, relatively long and complicated laboratory processing time, and cost and decreasing availability. Despite widely used, no consistent investigation has focused on the influence of size and shape of hog's hair collectors on rates of benthic settlement. A first experiment was set to investigate the effects of collector patchiness on settlement abundances of *Carcinus maenas* megalopae. Benthic hog's hair collectors of different sizes were deployed intertidally in the lower Mira Estuary. Settlement was addressed as ind.collector^{-1} and ind.m^{-2} in relation to collector' surface area and perimeter:area ratio. Results showed that collector patchiness significantly influenced settlement response of *C. maenas* megalopae, which differed according to settlement intensity and measure. Megalopae settlement responded to lower scales of habitat patchiness at high than at low intensity. Settlement as ind.collector^{-1} generally increased, while as ind.m^{-2} generally decreased, with increasing collector' surface area (decreasing perimeter: area ratio). A second experiment investigated the efficiency of plastic grass, as a new type of artificial surface, in estimating settlement patterns of *C. maenas* megalopae, and compared it with that of hog's hair. Collectors of both types were deployed daily and intertidally in the lower Mira Estuary. Settlement showed the same pattern and intensity on both collector types. Plastic grass collectors constitute a good alternative to those of hog's hair, possessing several advantages over them.

KEYWORDS: *Carcinus maenas*, Hog's hair, Mira Estuary, Patchiness, Plastic grass, Settlement

INTRODUCTION

The life cycle of many coastal and estuarine decapod crustaceans includes the transition of a planktonic larval phase to a benthic juvenile existence. This transition frequently produces bottleneck effects on the number of individuals, affecting the dynamics of juvenile and adult populations, with special relevance for fisheries and aquaculture management (e.g. Rabalais et al. 1995, Moksnes 2004). The cryptic nature of megalopa and juvenile stages usually makes the study of respective recruitment and settlement patterns difficult. To overcome this constraint, several different types of artificial collectors have been developed in the recent decades specifically for certain organisms, and then adapted for several others. See Phillips and Booth (1994) and Phillips et al. (2001) for reviews of collectors' designs. The function of all collector types is to enhance the thigmotactic response of megalopa and juvenile stages by simulating their required settlement needs, allowing the assessment of settlement patterns and providing animals for aquaculture purposes.

Hog's hair filter material has been the most broadly used settlement surface in different collector designs, especially for spiny lobsters and brachyuran crabs: Hunt's collectors, a current version of Witham collectors, have been used for spiny lobsters *Panulirus* spp. (Phillips et al. 2005); cylindrical floating collectors have been used for the blue crab *Callinectes sapidus* (e.g. Olmi et al. 1990, Rabalais et al. 1995), *Carcinus maenas* (e.g. Moksnes & Wennhage 2001, Moksnes et al. 2003) and other brachyuran species (Paula et al. 2001, 2003); flat collectors have been deployed in the water column (e.g. van Montfrans et al. 1990, Boylan & Wenner 1993) or in the benthic zone for several brachyuran species (e.g. Paula et al. 2003, 2006).

Several studies have emphasized the importance of using artificial substrates to indicate the correlation between recruitment and settlement patterns of decapod crustaceans, when compared with those that are natural (e.g. Phillips & Booth 1994, Metcalf et al. 1995, Eggleston et al. 1998). However, the physical and chemical nature of artificial substrates, as well as its interactions with the surrounding areas of deployment and with local biota, may in fact enhance or decrease propensity to settle (Goodrich et al. 1989, Moksnes & Wennhage 2001, Paula et al. 2006). Furthermore, size, shape and complexity of settlement grounds may also have significant effects on recruitment intensity of crustacean decapods as well as other marine organisms. Studies in seagrass and other structured marine substrates have revealed that several individual small substrate patches may increase overall colonization rates when compared to larger patches with smaller perimeter:area ratios (e.g. Keough 1984, McNeill & Fairweather 1993, Pineda & Caswell 1997, Eggleston et al. 1998, Bologna & Heck 2002, Hovel & Lipcius 2002). However, for other marine organisms, recruitment and settlement rates vary positively with surface area (Attrill et al. 2000, Lee et al. 2001, Jenkins et al. 2002, Atilla et al. 2005). Nevertheless, and despite the generalized use of hog's hair collectors to assess settlement and recruitment patterns of brachyuran crabs, no specific study has addressed the influence of collector size and shape on such patterns.

The use of hog's hair collectors presents several problems. The development of most brachyuran crabs includes a settling megalopa stage with strong abdominal swimming pleopods, and the structural design of hog's hair fibres may restrict swimming movements of pleopods and trap spinulate pereiopods. This can mask real settlement patterns of species in which megalopae settlement may not be final. *Carcinus maenas* and *C. sapidus* megalopae settle only temporarily if substrates are not suitable and metamorphose is not imminent (Zeng & Naylor 1996, Welch et al. 1999, Tankersley et al. 2002, Moksnes et al. 2003). The necessary time taken to retrieve megalopae from hog's hair fibres may also become a handicap in high sampling intensity experiments. Furthermore, hog's hair material is becoming expensive and difficult to assess, as also noted by Phillips et al. (2005). Consequently, there is a demand for an alternative type of collector that allows free movement of settled megalopae, with easier and faster laboratory processing and economical viability.

This study was therefore designed with two objectives. The first was to evaluate the influence of hog's hair collector size on settlement rates of *C. maenas* megalopae. The second objective was to test the efficiency of a new type of collector, plastic grass surfaces, in estimating daily settlement rates of *C. maenas* megalopae, when compared to that on hog's hair collectors. In order to accomplish the first and second objective, hog's hair collectors of different sizes in surface area and perimeter:area ratio were deployed intertidally during different settlement intensity periods, and plastic grass and hog's hair collectors were deployed daily also in the intertidal zone of the lower Mira Estuary, respectively.

MATERIALS AND METHODS

Larval biology of *C. maenas*

Carcinus maenas inhabits both hard and soft coastal and estuarine shallow habitats. After release, the planktonic zoeal phase is exported to coastal waters (Queiroga et al. 1994, Queiroga 1996), where eventually the fourth zoeal stage undergoes metamorphosis to the megalopae phase. It is the megalopae that then reinvade estuarine waters by selective tidal stream transport and actively select their settlement grounds (Zeng & Naylor 1996, Moksnes et al. 1998, Queiroga 1998). Megalopae prefer relatively structured habitats, where they begin a benthic existence with metamorphosis to first crab stage (Hedvall et al. 1998, Moksnes 2002). During spring and early summer in the Mira Estuary, settlement of *C. maenas* megalopae occurs with a semilunar periodicity during neap tides (around quarter moons), and variability in the intensity of settlement events also characterizes this settlement pattern (Paula et al. 2006, Queiroga et al. 2006).

Area description

All experiments were conducted on a sandy beach, located ~1 km inside the Mira Estuary. The Mira Estuary is a small mesotidal system located on the southwestern Portuguese mainland coast (37°40′N, 8°40′W). It presents a single river channel, with 400 m maximum width and average depth of 5 to 10 m. The tidal regime is typically semidiurnal, with tidal amplitude ranging between 1 and 3 m during neap and spring tides, respectively, and tidal influence extending to ~40 km inland (Paula et al. 2006). During neap tides, tidal penetration reaches 2.5 km and water column stratification occurs. During spring tides, tidal penetration reaches 7.5 km and homogenization of the water column occurs due to water turbulence (Paula 1998, Blanton & Andrade 2001). The estuary has a low, seasonal and limited freshwater input (due to Santa Clara dam located 60 km upstream), with the lower section presenting a marine dominance, characterized intertidally by extensive meadows of *Zostera noltii*, bare sandy areas and muddy substrates with boulders and pebbles. Bordering salt-marches occur as far as 20 km upstream.

Methods

Experiment 1

To assess the influence of collector size on the settlement rates of *C. maenas* megalopae, benthic hog's hair artificial substrates varying in surface area and perimeter:area ratio were used. Collectors (n = 4) of four different sizes were deployed on two days on each of two neap tide periods during the recruitment season (8, 10 and 23, 25 May, 2003). All collectors were 2.5 cm thick, and were attached to the substrate with J-shaped metal stakes. The choice of collector sizes was based on a range of dimensions feasible for routine operation and these varied between half and double the size of the recommended standard surface area of 0.2 m^2 for benthic hog's hair artificial substrates

(Paula et al. 2003, Paula et al. 2006). Dimensions, surface area and perimeter:area ratio of the four collector sizes were respectively: size I — 0.25 × 0.40 m, 0.1 m^2, 13.0; size II — 0.50 × 0.40 m, 0.2 m^2, 9.0; size III —0.75 × 0.40 m, 0.3 m^2, 7.7; size IV — 0.5 × 0.80 m, 0.4 m^2, 6.5 (an increase of 0.1 m^2). Significantly lower settlement intensity was recorded during the first than in the second neap tide period, with means of 11.4 and 83.3 ind.m^{-2}, respectively ($t = -12.36$, $p < 0.001$). Accordingly, the first period was considered of low settlement intensity and the second period of high settlement intensity, and days inside each period were pooled together by collector size, in order to assess possible influences of settlement intensity. The effects of collector size on settlement rates during each period were evaluated by one-way analysis of variances (ANOVA), considering two settlement measures: ind.$collector^{-1}$ and ind.m^{-2}. In all analyses, Cochran's tests revealed homoscedasticity of variances, and Shapiro–Wilk's tests revealed normality of residuals. *A posteriori* comparisons between different collector sizes were performed by tests of Tukey's honest significant differences (HSD). To estimate the collector size that would have maximized settlement during each intensity period, a polynomial function was fitted to the numbers of ind.$collector^{-1}$, using Least Mean Square comparisons.

Experiment 2

The efficiency of a new type of artificial surface, plastic grass, was tested and compared with hog's hair collectors, in estimating daily benthic settlement of *C. maenas* megalopae. Collectors of both types (n = 4) were deployed attached to similar metal frames to prevent movement caused by water currents (as in Paula et al. 2006), on 20 consecutive days during the recruitment season. The new collectors were 0.40 × 0.32 m in surface area and made from polyethylene green grass mats, composed of vertical 1 × 2 × 25 mm blades (6.8 blades.cm^{-2}, Velcoc™, ref. 8000771610455). The vertical design of the blades allows free movement of megalopae, while conferring high complexity to the structure of the collector' surface area. Hog's hair collectors were 0.50 × 0.40 m in surface area and as described previously. Megalopae settlement was measured as ind.m^{-2} and comparisons of daily and overall settlement on both collector types were performed by Student's *t*-tests.

All collectors where conditioned prior to use by immersion in seawater for one week. Collectors were deployed randomly on the intertidal zone, lying 2 to 4 m apart from each other, at the time of the diurnal low tide and recovered after 2 tidal cycles (25 h). After recovery, collectors were soaked in freshwater for 5 min, rinsed with freshwater jets through a 500 µm sieve, and crab megalopae and juveniles were retrieved.

RESULTS

Experiment 1

Settlement of *C. maenas* megalopae showed significant effects of collector size during both low and high intensity periods, irrespective of settlement measure (ind.collector^{-1} or ind.m^{-2}), being more significant during the high intensity period (Table 1.1). These effects were somehow different according with settlement intensity. During the high intensity period, settlement as ind.collector^{-1} increased with increasing collector' surface area and decreasing perimeter:area ratio (Fig. 1.1A). Collector sizes III and IV presented the same and significantly higher values than those of sizes I and II ($p < 0.001$ and $p < 0.05$, respectively).

Table 1.1. Results of one-way ANOVA analyses for settlement of *Carcinus maenas* megalopae on overall hog's hair collectors of different sizes, by settlement intensity period and settlement measure (ind.collector^{-1} and ind.m^{-2}). Significant p-values in bold.

Settlement intensity	Measure	Source	df	MS	*F*	p
Low	ind.collector^{-1}	Size	3	10.79	7	**< 0.05**
		Error		28	1.55	
	ind.m^{-2}	Size	3	180.06	6	**< 0.05**
		Error		28	31.38	
High	ind.collector^{-1}	Size	3	207.26	11	**< 0.001**
		Error		28	19.58	
	ind.m^{-2}	Size	3	6273.70	13	**< 0.001**
		Error		28	475.30	

The best polynomial fit to these data was a linear function that revealed that settlement maximization was achieved with the size IV collectors (Fig. 1.2A, $F = 28.5$, $p < 0.001$). Conversely, settlement as ind.m^{-2} showed a general decrease with increasing collector' surface area and decreasing perimeter:area ratio (Fig. 1.1B). However, and despite size I collectors had caught significantly more megalopae than those of other sizes ($p < 0.05$, $p < 0.001$ and $p < 0.001$ for collector size II, III and IV respectively), none of these showed significant differences between each other.

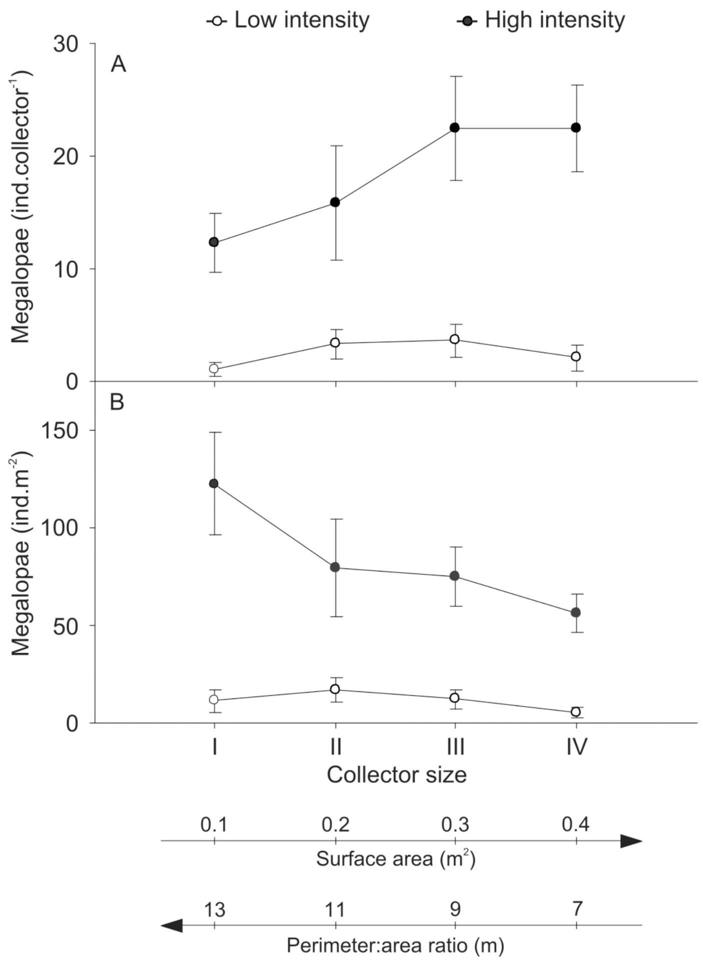

Fig. 1.1. *Carcinus maenas*. Average settlement of megalopae on the different size' collectors at low and high settlement intensity periods (A) as ind.collector^{-1} and (B) as ind.m^{-2}. Arrows represent increasing collector' surface area and decreasing perimeter:area ratio with increasing collector size. Error bars: ±SE.

In the low intensity period, settlement as ind.collector^{-1} was significantly lower on size I than on size II and III collectors ($p < 0.01$ and $p < 0.05$, respectively), not being different between any other collector sizes (Fig. 1.1A). The best polynomial fit to these data was a quadratic function revealing a collector size between sizes II and III (0.27 m^2 in surface area) as the one that would have maximized settlement (Fig. 1.2B, $F = 18.1$, $p < 0.001$). Settlement as ind.m^{-2} decreased significantly with increasing surface area (decreasing perimeter:area ratio) between size II to size IV collectors ($p < 0.05$), not showing significant differences between any other collector sizes (Fig. 1.1B).

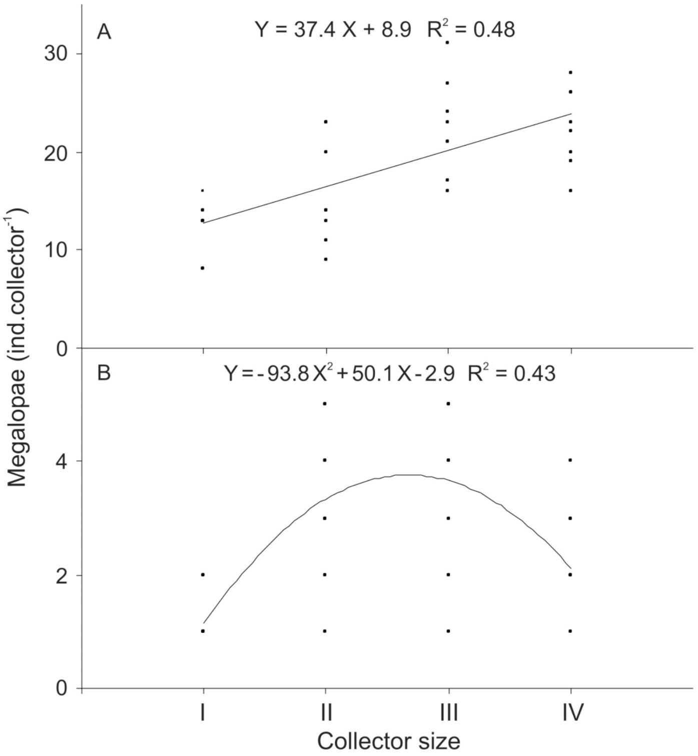

Fig. 1.2. *Carcinus maenas*. Fitness of a polynomial model to megalopae settlement, as ind.collector^{-1}, on the different size' collectors at (A) high and (B) low settlement intensity.

Experiment 2

Settlement of *C. maenas* megalopae on plastic grass collectors showed the same pattern that on those of hog's hair throughout all sampling: high settlement intensity from the 3rd to the 6th and from the 13th to the 18th days, and low values outside these periods (Fig. 1.3). Maximum settlement occurred in the same days for both collector types, being higher on those of hog's hair on the 1st period, and on those of plastic grass on the 2nd period (Fig. 1.3). Overall mean daily settlement on plastic grass collectors was not significantly different from that on those of hog's hair (9.3 ± 5.3 and 11.0 ± 4.6 ind.m^2, respectively; t = -0.888, p = 0.376). Student's t tests did not reveal any significant difference on settlement intensity between both collector types for any of the sampled days.

Laboratory processing of plastic grass collectors was considerably easier and less time consuming than that of hog's hair collectors. Less soaking time and use of freshwater jets rinsing were necessary to remove megalopae. Average processing time of a plastic grass collector was half of that of a hog's hair one, i.e. 6–7 min. Second and third rinsing did not retrieve more megalopae from plastic grass collectors, while it sometimes did (1–3 megalopae) from hog's hair ones. Furthermore, immediate observation of retrieved megalopae, revealed that less damaged and higher rates of survived animals were obtained from plastic grass than from hog's hair collectors.

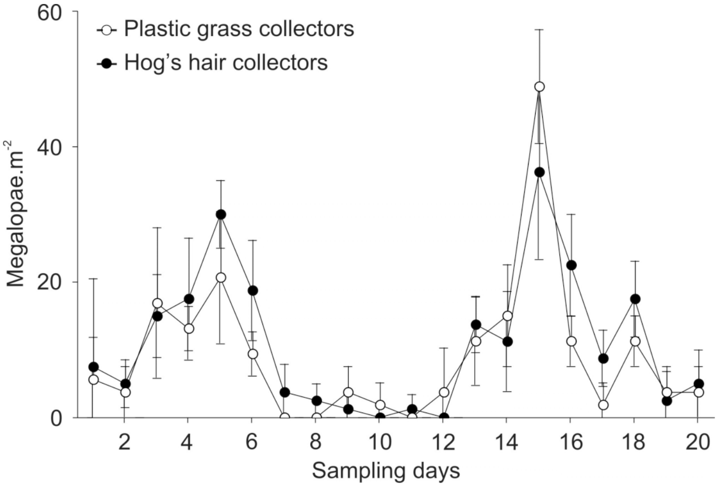

Fig. 1.3. *Carcinus maenas*. Average daily settlement of megalopae on plastic grass and hog's hair collectors. Error bars: ±SE.

DISCUSSION

Influence of collector patchiness

According with Phillips et al. (2001) collector testing is only practical at times of high settlement intensity, since at other times results may be unreliable due to high variability in catch numbers. In this study, however, variability in settlement numbers was similar at high and low intensities, being also similar to those of other studies conducted recently in the same area (Paula et al. 2006, Queiroga et al. 2006), supporting the meaningfulness of results. However, caution must be taken when analyzing the present results, in the sense that it was not possible to dissociate the effect of collector' surface area from collector' perimeter:area ratio. In order to dissociate between these two effects, collectors varying in surface area but preserving perimeter:area ratio, and vice versa, should be investigated.

Abundances of *C. maenas* megalopae on hog's hair collectors were significantly influenced by collector patchiness at scales between 0.1 and 0.4 m^2 in surface area, irrespective of settlement intensity and settlement measure (ind.collector^{-1}, ind.m^{-2}). However, responses were different with different settlement intensities and measures. At both high and low intensity settlement as ind.collector^{-1} generally increased with increasing surface area (decreasing perimeter:area ratio) between size I and III collectors, stabilizing and decreasing on those of size IV, at high and low intensity, respectively. These results seem to indicate that a collector size proximate to that of size III (0.3 m^2 in surface area) would have maximized settlement numbers irrespective of settlement intensity. This is reinforced by fitness of polynomial functions to these data that revealed collector sizes of 0.4 and 0.27 m^2 in surface area as those maximizing settlement at high and low settlement intensity, respectively.

Considering settlement as ind.m^{-2}, settlement generally tended to decrease with increasing collector' surface area (decreasing perimeter:area ratio), with exception to size I collectors at low settlement intensity. However, this tendency differed with settlement intensity: settlement responded to lower scales of habitat patchiness at high than at low intensity, when only the highest and lowest values were significantly different. Different responses to habitat patchiness according to organism density, and also body size, have been previously reported in recruitment of grass shrimps *Palaemonetes* spp. and blue crab *C. sapidus* (Eggleston et al. 1998). Nevertheless, the results generally agree with those of previous studies on several marine organisms that found higher settlement and recruitment densities on smaller natural patches when compared to larger patches, with higher and lower perimeter:area ratios, respectively (Keough 1984, McNeill & Fairweather 1993, Eggleston et al. 1998, Bologna & Heck 2002, Hovel & Lipcius 2002). Higher perimeter:area ratios usually enhance the probability of encounter rates by organisms, since more patch edge is exposed. This gains special relevance for organisms that possess a swimming capacity, as it is the case of *C. maenas* megalopae that actively search for suitable settlement substrates, usually with a certain degree of complexity (Hedvall et al. 1998, Moksnes 2002). In addition to what has hitherto been discussed, it is generally accepted that a more or less strong indissociable biological component is also involved in recruitment and settlement responses of marine organisms to habitat patchiness, further suggesting specificity in those responses (Eggleston et al. 1998, Jenkins et al. 2002, Atilla et al. 2005).

Influence of collector type

Plastic grass surfaces efficiently estimated benthic settlement of *C. maenas* megalopae when compared to that on hog's hair collectors, presenting the same pattern, similar settlement densities and variability. As pointed out by Phillips et al. (2005), hog's hair filter material is becoming difficult to access, and an appropriate substitute should allow comparisons with results from previous studies. The good homology between daily catches on the two types of collectors suggests that a conversion factor could be achieved with a proper sampling design. Besides a good settlement estimation, laboratory processing including the condition of retrieved animals, handling procedures and logistic requirements, overall weight and size, strength and durability, need for replacement, cost and effort of construction/acquisition are also important characteristics that may invalidate the use of a certain collector type. Laboratory processing of plastic grass collectors revealed to be easier and less time consuming (~half the time) than that of hog's hair ones. Megalopae retrieval was considerably more rapid and effective, resulting in less mechanical stress suffered by megalopae and higher percentage of live specimens being retrieved. Plastic grass collectors are entirely made of light flexible polyethylene, resulting in easy to handle and transportable structures, resistant to wave action and sea exposure. This provides them with extreme durability and stability of surface area complexity when compared to those of hog's hair, diminishing considerably the necessity for replacement. In fact, there is a continuous erosion of surface area complexity in hog's hair collectors, since fibres are easily lost with wave action. Polyethylene green grass mats are widely available, being easier and cheaper to obtain than hog's hair filters. We thus consider that plastic grass collectors may constitute a good alternative to those of hog's hair, especially when there is a demand for high sampling intensities and/or live megalopae.

In summary, this research indicates that settlement of *C. maenas* megalopae responded to patchiness of benthic hog's hair collectors (0.1 to 0.4 m^2 in surface area), as a function of either the collectors' surface area or perimeter:area ratio, irrespective of settlement intensity. However this response was contrasting with distinct settlement measures. Megalopae numbers would have been maximized with collector' surface areas around 0.3 and 0.4 m^2 at low and high settlement intensities, respectively. The choice of hog's hair collector' size should thus be cautious and made according with the purpose of the study and the expected settlement intensity. Plastic grass collectors may constitute a good alternative to those of hog's hair.

ACKNOWLEDGEMENTS

We thank A. R. Amaral for helping with the fieldwork, Dr. J. Castro (CIEMAR — Laboratório de Ciências do Mar da Universidade de Évora) for providing laboratory and other logistic facilities and two anonymous referees for their critical comments that greatly improved the manuscript. We acknowledge a PhD grant to V.A. (SFRH/ BD/10471/2002) funded by Fundação para a Ciência e a Tecnologia (FCT). This work is part of the research project “ProRecruit — Shelf processes controlling recruitment to littoral populations in an eastern oceanic boundary: using barnacles and crabs as models”, also funded by FCT (No. POCTI/1999/BSE/36663). Financial support was allocated by FCT under the Support Community Framework III, Operational Programme Science, Technology and Innovation.

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CHAPTER 2

Planktonic availability and settlement of *Carcinus maenas* (L.) megalopae at different time scales in the lower Mira Estuary (SW Portugal)

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Mar Ecol Prog Ser (2007) 348:239-248

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ABSTRACT

The current conceptual model of reinvasion and settlement for estuarine brachyuran crabs in mesotidal systems is that megalopae undergo selective tidal stream transport, in an upstream direction, and settle by the end of the flood tide. Recent studies on recruitment processes of *Carcinus maenas* (L.) have reported a clear decoupling between supply, as larval influx, and settlement events in the lower Mira Estuary (SW Portugal). We investigated, at high temporal resolution, whether overestimation of planktonic abundances and/or deviations of megalopal responses from the conceptual model were responsible for such decoupling. Daily settlement of megalopae was analyzed using regression and spectral analyses to identify periodicities and correlated with tidal amplitude. Hydrological parameters and supply, net flux and hourly concentration of megalopae in the plankton were addressed as factors influencing short, intermediate and long-term settlement, through 50 h sampling series. Supply and surface net flux levels of megalopae were similar and clearly decoupled from settlement on bottom-deployed collectors. Both hourly planktonic concentration and settlement patterns of megalopae fitted the conceptual model at high temporal resolution, with some deviations in terms of light intensity influences. Furthermore, our results suggest that processes preventing settlement and capable of overriding the influence of light intensities - such as turbulence - may explain the decoupling between supply and settlement of shore crab megalopae in the lower Mira Estuary.

KEYWORDS: Bottom-deployed collectors, *Carcinus maenas*, Decoupling, Megalopae, Net flux, Settlement, Supply, Tidal and semilunar rhythms

INTRODUCTION

Newly hatched larvae of many estuarine decapod crustaceans are exported to coastal waters where they may remain for days or months. By the end of planktonic development, larvae must return to habitats where benthic settlement takes place. These are critical events that involve larval behavioural and physiological adaptations, as well as environmental processes.

The conceptual model of reinvasion and settlement for estuarine brachyuran crabs in mesotidal systems suggests that megalopae are transported upstream during flood tide by selective tidal stream transport (STST) (Little & Epifanio 1991, Zeng & Naylor 1996, Queiroga 1998, Forward & Tankersley 2001), and settle by the end of the flood tide (Zeng et al. 1997, Tankersley et al. 2002). Megalopae undergoing STST ascend in the water column in response to increasing salinity associated with the flood tide and are stimulated to swim by high turbulence levels (De Vries et al. 1994, Tankersley et al. 1995, Welch et al. 1999, Welch & Forward 2001). After mid-flood tide, megalopae are cued to descend and settle by decreasing turbulence and current speed, being inhibited from swimming during the ebb tide by decreasing salinity (Welch & Forward 2001, Tankersley et al. 2002). Swimming is also inhibited by light, causing STST and settlement to occur mainly during darkness (Tankersley et al. 1995, Zeng & Naylor 1996, Queiroga 1998, Forward et al. 2004).

Hatching in the portunid shore crab *Carcinus maenas* (L.) occurs on nocturnal ebb tides during spring and early summer, with larvae being exported to adjacent shelf waters (Paula 1989, Zeng & Naylor 1997). Within 4 to 10 wk, depending on temperature, larvae develop through 4 zoeal stages (before metamorphosing into megalopae) and reinvade estuarine waters (Dawirs 1985, Nagaraj 1993). In Portuguese coastal waters, larval release occurs during neap tides (quarter moon periods) (Paula 1989, Queiroga et al. 1994), while supply, as larval influx back to estuarine waters, occurs during spring tides (new and full moon periods) (Almeida & Queiroga 2003, Queiroga et al. 2006).

One of the most interesting findings on shore crab recruitment processes in the Mira Estuary (SW Portugal) is that maximum settlement occurs around neap tides, clearly decoupled from supply (Paula et al. 2006, Queiroga et al. 2006). Queiroga et al. (2006) proposed that this could result from (1) density-dependent secondary dispersal by settled megalopae, i.e. a large number of settling megalopae are offset by those leaving after settlement because of crowding, (2) supply from a pool of larvae in an early developmental stage, and/or (3) higher turbulence levels preventing megalopae from settling during spring tides. However, these explanations are yet to be tested, since data available on shore crab recruitment have been obtained with daily or lower frequencies, and higher temporal resolution is required to examine tidal processes. In addition, supply is a measure of megalopal invasion of estuarine waters, which does not consider the possibility of megalopae being carried out on ebb tides (Zeng & Naylor 1996). If significant numbers of megalopae are carried out, especially with the stronger currents of spring tides, supply patterns might not correctly describe the real abundance levels of megalopae in the Mira Estuary, and thus bear no relationship with settlement events. In this case, net flux, as the balance between megalopae entering and leaving the estuary, would be a more accurate measure of planktonic abundance of megalopae. Furthermore, in the Mira Estuary settlement has been assessed only on intertidal collectors, without considering the possibility of this being a mainly subtidal event, especially during spring tides. In this case, the decoupling pattern would be an artefact caused by underestimation of subtidal settlement intensity during spring tides.

This study was set up to investigate the decoupling between supply and settlement of shore crab megalopae in the lower Mira Estuary. We assessed such events at high temporal resolution and their relationship to hydrological parameters and lunar, diel and tidal cycles. Our aim was to determine whether upstream movement and settlement of megalopae conform to the conceptual model for brachyuran crabs during spring and neap tides, or whether deviations from the model are responsible for the decoupling pattern. Specifically, our hypotheses were as follows: (1) supply levels of megalopae are similar to net flux levels during spring and neap tides, i.e. the majority of megalopae entering the estuary remain there; (2) the decoupling pattern is an artefact attributable to underestimation of settlement intensity, resulting from density-dependent secondary dispersal of settled megalopae during spring tides; (3) the decoupling pattern is an artefact attributable to using only intertidal collectors when settlement is mainly subtidal during spring tides; (4) both upstream movement and settlement of megalopae follow the conceptual model during spring and neap tides. In order to test these hypotheses, abundances of shore crab megalopae, from a combination of planktonic passive nets and tows and bottom-deployed collectors with different immersion periods and locations on the shore, were obtained and related to hydrological parameters and environmental cycles.

MATERIALS AND METHODS

Sampling

Sampling was conducted on a sandy beach, approximately 1 km inside the Mira Estuary (37°40’ N, 8°40’ W) (Fig. 2.1). The Mira Estuary is a single-channel system with a semidiurnal tidal regime of 12.4 h and ca. 3.5 m maximum amplitude; tidal influence extends for 40 km inland. On average, tidal penetration ranges from 2.5 to 7.5 km during neap and spring tides, respectively (Paula 1989). Water column structure varies from vertically homogeneous to slightly stratified during spring and neap tides, respectively

(Blanton & Andrade 2001). Lunar and daily settlement patterns of *Carcinus maenas* megalopae were assessed from 7 March to 8 June, 2003, on artificial hog's hair collectors (0.4 × 0.5 × 0.02 m, see Amaral & Paula [2007]). Collectors (n = 4) were bottom-deployed on the intertidal zone every day at low tide and sampled after two tidal cycles (daily series).

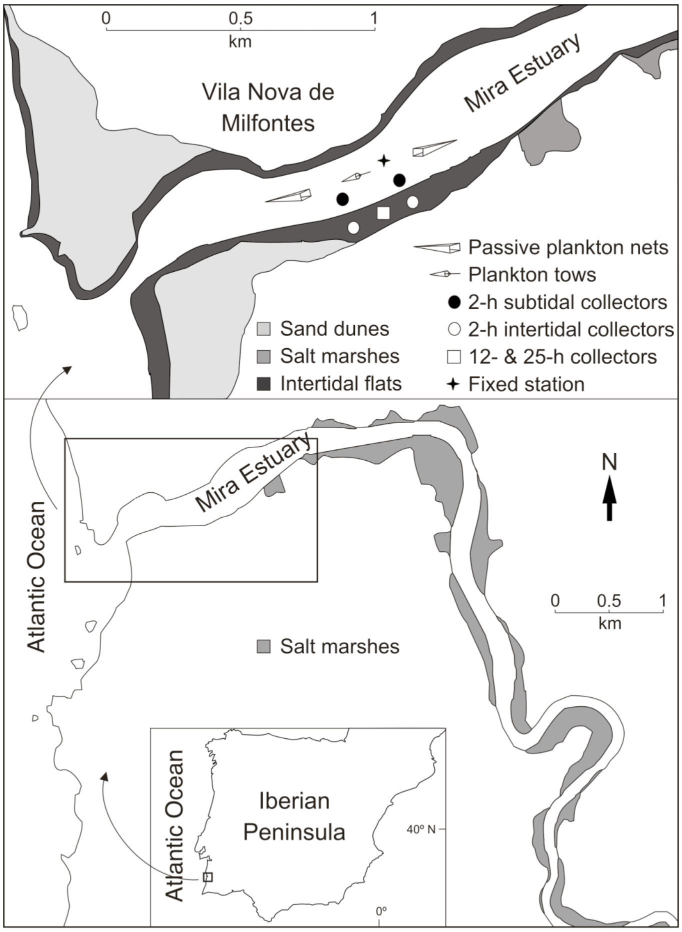

Fig. 2.1. Sampling area, Mira Estuary, Iberian Peninsula and deployment of plankton nets and collectors.

We designed 50 h (4 tidal cycles) sampling series to study the effects of diel and tidal cycles on planktonic availability and settlement patterns of megalopae at high temporal resolution. Two spring and two neap tide sampling periods were planned. Unfortunately, due to relatively low settlement intensity, only one settlement event of proximate magnitude to those reported by Queiroga et al. (2006) was identified beforehand throughout the daily series. Thus, only one period of each type was sampled. The effects of spring and neap tides were examined from 19 to 21 March and 6 to 8 June, 2003, respectively. In both periods, planktonic availability of megalopae was estimated as supply, net flux and hourly concentration, and these were addressed as factors influencing settlement. Supply is a measure of total megalopal invasion of the estuary during flood tides, while net flux further integrates megalopae that are carried out on ebb tides, thus measuring the population remaining within estuarine boundaries. Both supply and net flux were measured using passive plankton nets, which were specifically designed for sampling only during tidal flows that they face. Two passive plankton nets, each facing a tide direction, were deployed just below the surface and ca. 0.2 m above the bottom and were sampled every two tidal cycles (Fig. 2.1). These nets were 2 m long, with a 0.10 m^2 mouth opening and 500 µm mesh; an internal funnel collapses during opposite tidal flows to prevent collected material escaping (see Queiroga et al. 2006 for details). Supply was assessed from the contents of nets facing flood tides, and net flux from the balance between contents of nets facing flood and ebb tides, at each water depth. Hourly concentration was assessed with surface plankton tows (5 min) performed each hour along the sampling area (Fig. 2.1). A conical, 330 µm mesh net, with 0.05 m^2 mouth opening equipped with a flowmeter, was used. Settlement was assessed on bottom-deployed collectors (n = 4) on short, intermediate and daily time frames. Short-term collectors (2 h) were deployed both intertidally and subtidally and sampled every 2 h. Intermediate-term (12 h) and daily (25 h) collectors were deployed only intertidally and sampled every tidal and every two tidal cycles, respectively (Fig. 2.1). To assess cumulative settlement, 12 h collectors were always deployed in the same place. Megalopae captures were related to hourly measurements of water level, salinity, temperature and current speed conducted at a fixed station at three depths: just below the surface, in the middle of the water column and above the bottom (Fig. 2.1).

Collectors of each set were deployed randomly, 2 to 4 m apart from each other, in similar metal frames to prevent movement caused by water currents (Amaral & Paula 2007). Crab megalopae and juveniles were recovered by immersing the collectors in freshwater, rinsing them with freshwater jets through a 0.5 mm sieve and sorting out the animals from the debris. Passive plankton net and tow samples were immediately preserved in 4% formalin, and later sorted for crab megalopae and juveniles.

Statistical analysis

Periodic regression analysis was performed to estimate the period and amplitude of daily settlement in relation to the lunar cycle. A correction of the sinusoidal model for sharply peaked sinusoidal oscillations, evidenced by visual inspection of the data, was applied using nonlinear least-squares regression analyses (Batschelet 1981):

$$Y = M + A\cos\left[\left(\frac{2\pi t}{\lambda} - \theta\right) + \nu\sin\left(\frac{2\pi t}{\lambda} - \theta\right)\right] + e$$

where Y is the dependent variable, M is the mean level of Y, A is the Y amplitude, t is the time when the sample was collected, λ is the period length, θ is the acrophase angle, ν is the peakedness parameter and e is the random error term. One problem with this correction is the appearance of undesired oscillations when $-1.05 \le \nu \le 1.05$. No attempt was made to adjust the model to diel differences or asymmetries in the amplitude of peaks. The regression coefficient (R^2) provides an estimate of the proportion of variation in the dependent variable accounted for by the sinusoidal model. Daily settlement values were log(x + 1)-transformed prior to analysis to down-weight large numbers. Spectral analysis (Chatfield 1996) was used to investigate settlement periodicities related to differences between waxing and waning, and new and full moon periods. Kolmogorov-Smirnov *d* statistic was used to assess the significance level of spectral analysis. The relation between daily settlement and tidal amplitude was determined by a non-parametric Spearman correlation analysis.

Periodic regression analysis was also performed to estimate period and amplitude of hourly concentration of megalopae and settlement on 2 h collectors, in relation to the tidal cycle. Such analyses were made using λ obtained for water level data of each respective 50 h sampling period. For both periods, supply was compared with megalopae captures on passive nets facing ebb tides using Student's *t*-tests. Net flux of megalopae was calculated for each depth, and also compared using Student's *t*-tests. These analyses were conducted on the number of megalopae captured by each net type, since standardization by volume would be subject to a large error (Queiroga et al. 2006). Settlement on 25 h and cumulative settlement on 12 h collectors were compared using Student's *t*-tests.

Regression analysis confirmed that megalopae abundances were consistently higher during flood tides, and thus light vs. dark comparisons were only performed on such data. Although flood tides occurring during dark hours were entirely nocturnal during the spring tide period and only crepuscular during the neap tide period, both kinds were defined as night-influenced tides. Light vs. dark comparisons were performed on hourly concentration of megalopae and settlement on 2 and 12 h collectors by Student's *t*-tests.

RESULTS

Daily series

Settlement of shore crab megalopae was clearly more intense around quarter moons during neap tides (Fig. 2.2B). This semilunar periodicity was confirmed by periodic regression and correlation analyses. Periodic regression of the settlement series showed a period of 14.7 d ($R^2 = 0.34$), and correlation analysis indicated a strong relationship of settlement with days of lowest tidal amplitude ($r = -0.614$, $p < 0.001$) (Fig. 2.2A). Relatively high settlement was recorded in spring tides from Days 74 to 76, when the sampling area remained submerged for 3 d, due to a sea storm. Spectral analysis detected two major peaks at periods of 94.0 and 15.7 d, and three other minor peaks ($d = 0.439$, $n = 94$, $p < 0.005$) (Fig. 2.3). The first peak is an artefact of the statistic (it is equal to the number of samples: 94). The 15.7 d period of the second peak further supports the semilunar settlement periodicity. No periodicity related to differences between waxing and waning or new and full moon periods (ca. 29.5 d) was detected.

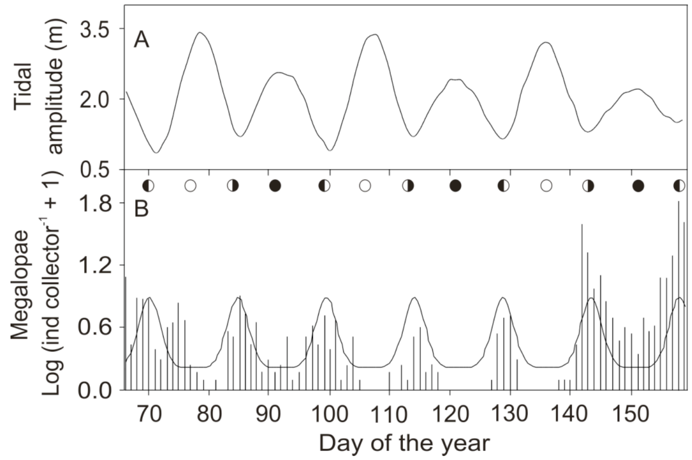

Fig. 2.2. *Carcinus maenas*. (A) Tidal amplitude. (B) Log(x + 1) mean daily settlement of megalopae on collectors. Full, new, waxing and waning moons are represented by open, closed, right-opened and left-opened circles, respectively. Curve represents fitting of a sinusoidal model using nonlinear least-squares regression analysis (see Table 2.1).

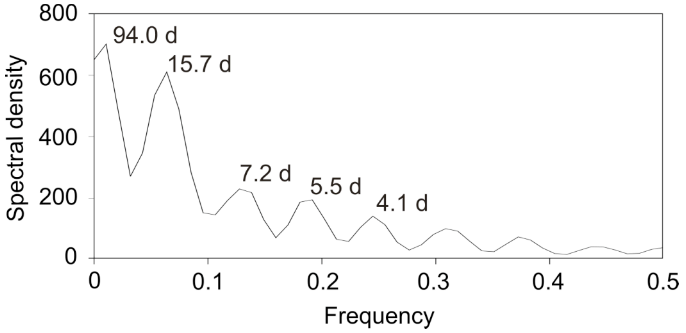

Fig. 2.3. *Carcinus maenas*. Spectral analysis of mean daily number of megalopae on collectors. Numbers above peaks represent the period of oscillation.

50 h series

Hydrological parameters

Water level showed the expected semilunar and semidiurnal tidal regimes, varying from 4.3 to 8.7 m and 5.2 to 7.3 m during the spring and neap tide periods, respectively (Table 2.1, Figs. 2.4A & 2.5A). Salinity values were similar during both periods. However, some stratification occurred during the last tidal cycle in the spring tide period, most probably due to heavy rainfall, but also in the neap tide period, especially during ebb tides (Figs. 2.4A & 2.5A).

Table 2.1. Results of periodic regression analyses for water level and for *Carcinus maenas* megalopae captures on plankton tows, and on 2-h intertidal and subtidal collectors, during the spring and neap tide periods. Period length estimates (λ; in h) for water level were used to fit the remaining sinusoidal model parameters (θ - acrophase; *M* - mean level; *A* - amplitude; R^2 - regression coefficient) for megalopae captures on plankton tows and on 2-h intertidal and subtidal collectors for the respective sampling period. Acrophase values are expressed relatively to water level, and therefore represent time (in h) to high tide.

	Spring tide period					Neap tide period				
Sampling effort	λ (h)	θ (h)	*M*	*A*	R^2	λ (h)	θ (h)	*M*	*A*	R^2
Water level	12.05	—	6.71	1.50	0.88	12.48	—	6.34	0.76	0.84
Plankton tows	—	-1.94	0.15	0.14	0.36	—	-4.01	0.17	0.16	0.41
2-h intertidal collectors	—	-2.78	0.18	0.14	0.19	—	-2.93	3.90	2.95	0.61
2-h subtidal collectors	—	-4.51	0.29	0.44	0.39	—	-1.80	0.87	0.37	0.09

Temperature stratification patterns were similar to those recorded for salinity, but amplitude and absolute values were higher during the neap tide period (Figs. 2.4B & 2.5B). Water current speed varied from 0.0 m.s^{-1} above the bottom, to 1.14 and 0.71 m.s^{-1} at the surface, during the spring and neap tide periods, respectively. During both periods, current speeds were generally higher near the surface, especially during mid-tidal phases, when peaks were recorded at all depths (Figs. 2.4C & 2.5C). These peaks, and respective amplitude changes, were of much higher intensity during the spring tide period.

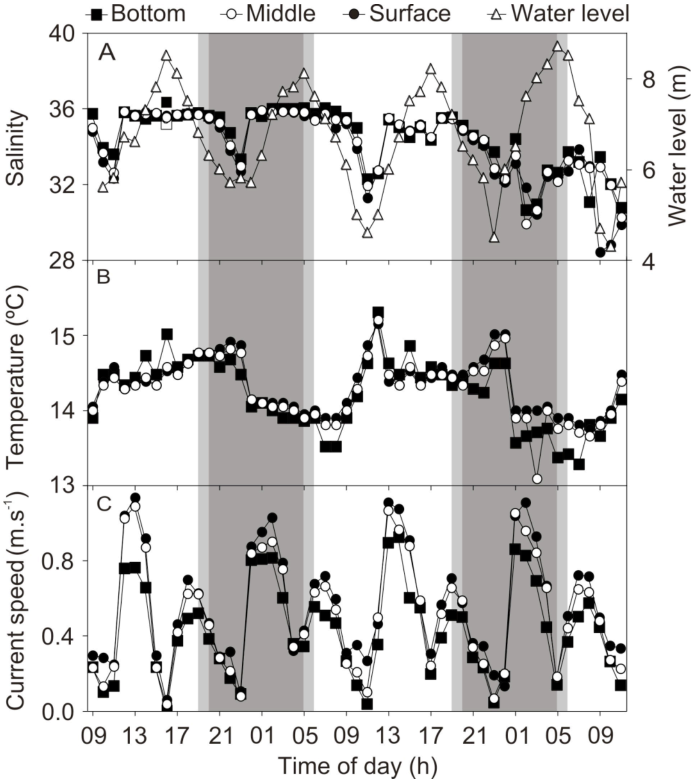

Fig. 2.4. Hydrological parameters during the spring tide period. (A) Salinity and water level, (B) temperature, and (C) current speed. Dark grey, light grey and unshaded areas represent dark, crepuscular and daylight hours, respectively.

Planktonic availability

Supply was clearly more intense (by a factor of about 2) during the spring tide than during the neap tide period (Table 2.2). The fact that considerably fewer megalopae were captured on the surface net facing flood tides during the second day of the neap tide period is reflected in the larger SEs obtained, explaining the lack of significant differences found in this period (Table 2.2). Caution should thus be taken in analyzing these results. Megalopal captures on passive nets facing ebb tides were much lower than those on nets facing flood tides during both periods, suggesting that the majority of megalopae remained inside the estuary. Accordingly, supply was similar to surface net flux of megalopae during both periods (Table 2.2). Megalopal net flux was higher at the surface than at the bottom during both periods, although.

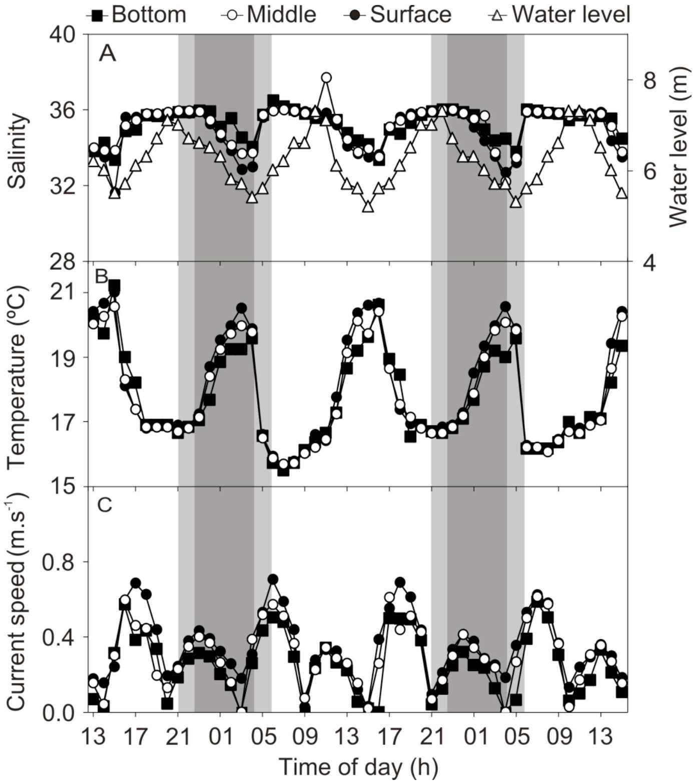

Fig. 2.5. Hydrological parameters during the neap tide period. (A) Salinity and water level, (B) temperature, (C) current speed. Dark grey, light grey and unshaded areas represent dark, crepuscular and daylight hours, respectively.

Table 2.2. *Carcinus maenas*. Mean daily captures of megalopae in passive plankton nets and on 12-h and 24-h collectors (ind.collector^{-1}.d^{-1}). Captures in passive nets are presented for nets facing flood (supply) and ebb tides (ind.d^{-1}), and as net flux (ind.net^{-1}.d^{-1}) at the water surface and bottom. Captures on 12-h collectors are presented for cumulative, light and dark periods. Student's *t*-test comparisons were made between captures in flood and ebb-facing nets, surface and bottom net fluxes, 24-h and cumulative 12-h collectors, and light and dark 12-h collectors: * $p < 0.05$, *** $p < 0.001$. Significant results in bold.

	Spring tide period		Neap tide period	
Sampling effort	Mean	SE	Mean	SE
Passive nets				
Flood (Supply)	1028.5	**184.5***	555	434
Ebb	43.5	31.5	11	5
Surface net flux	974	**218***	412	377
Bottom net flux	11	2	132	52
12-h collectors				
Light	1.88	1.45	11.52	**8.57*****
Dark	0.63	0.70	36.03	25.19
Cumulative	2.50	**1.50***	59.63	13.10
24-h collectors	0.13	0.33	53.38	18.72

Hourly concentration of megalopae was similar during both sampling periods. Periodic regression analyses also revealed strong tidal periodicities of hourly concentration of megalopae during both periods: during flood tide, concentration increased with increasing salinity and decreased concurrently with current speed, in agreement with upstream movement by STST (Table 2.1, Figs. 2.4, 2.5, 2.6B & 2.7B). Despite the similar patterns, significantly higher abundances were detected on night-influenced flood tides during the neap tide period ($t = -2.948$, $p < 0.01$), but not during the spring tide period ($t = -0.299$, $p = 0.767$).

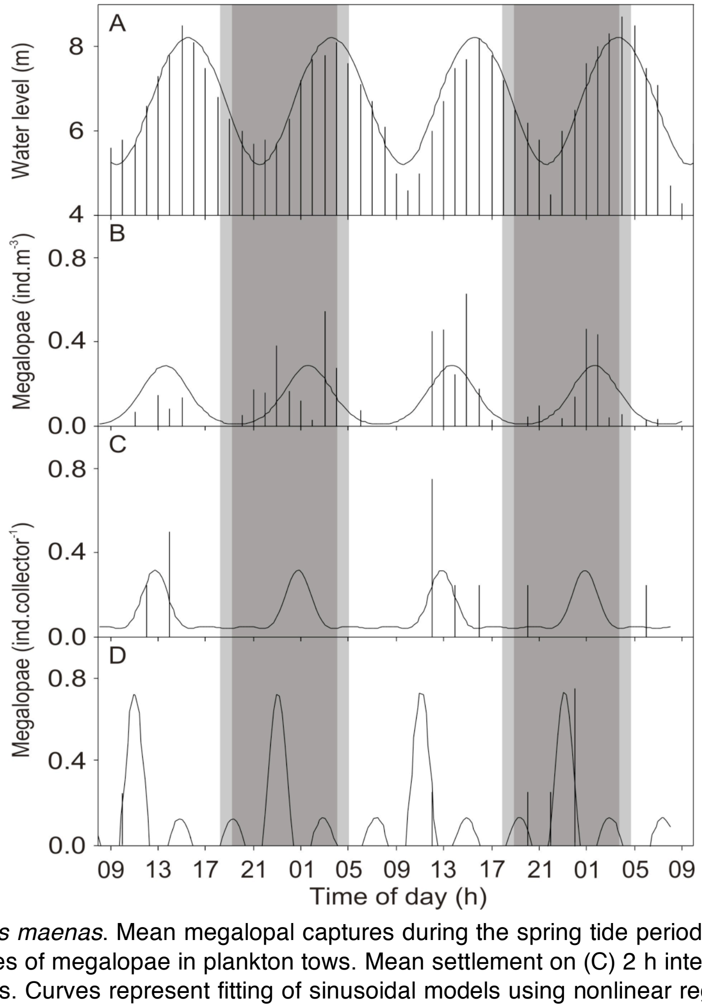

Fig. 2.6. *Carcinus maenas*. Mean megalopal captures during the spring tide period. (A) Water level. (B) Mean captures of megalopae in plankton tows. Mean settlement on (C) 2 h intertidal and (D) 2 h subtidal collectors. Curves represent fitting of sinusoidal models using nonlinear regression analysis (see Table 2.1). Dark grey, light grey and unshaded areas represent dark, crepuscular and daylight hours, respectively.

Settlement

Following the semilunar pattern, settlement was higher during the neap than during the spring tide period. In fact, settlement on 2 h collectors was extremely low throughout the spring tide period: only in 2 out of 7 settlement events on intertidal collectors, and in 1 out of 5 on subtidal collectors, were more than 1 megalopae collected (Table 2.1, Fig. 2.6C,D).

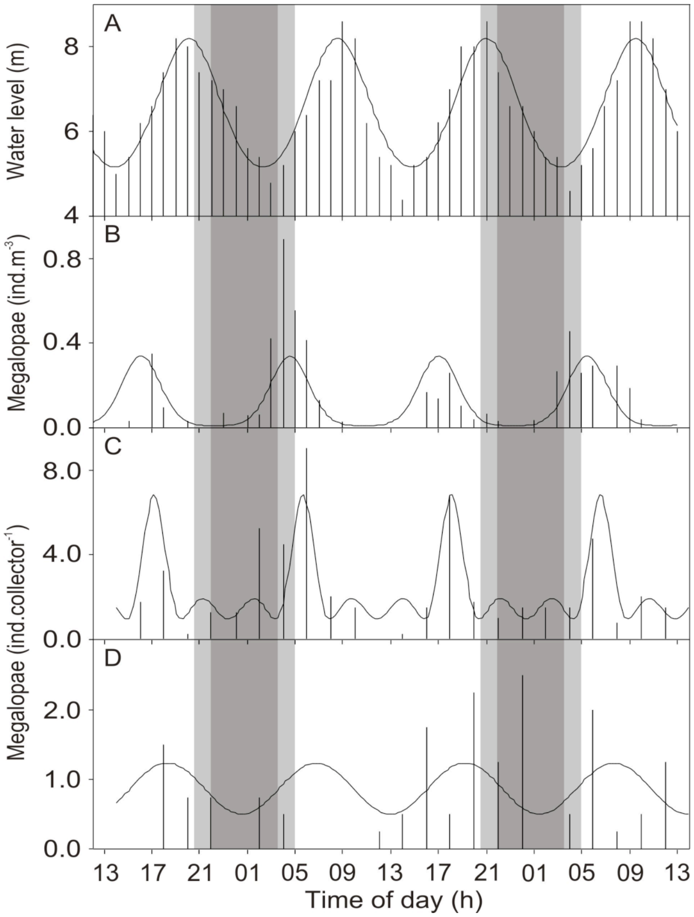

Fig. 2.7. *Carcinus maenas*. Mean megalopae captures during the neap tide period. (A) Water level. (B) Mean captures of megalopae in plankton tows. Mean settlement on (C) 2 h intertidal and (D) 2 h subtidal collectors. Curves represent fitting of sinusoidal models using nonlinear regression analysis (see Table 2.1). Dark grey, light grey and unshaded areas represent dark, crepuscular and daylight hours, respectively.

Settlement was also very low on 12 and 25 h collectors and, despite the outcome of statistical analysis, no reliable conclusions can be drawn from such data. Nevertheless, cumulative settlement on 12 h collectors was significantly higher than settlement on 25 h collectors (*t* = 19.000, $p < 0.05$), without any significant light vs. dark difference (*t* = 2.053, p = 0.059) (Table 2.2).

The settlement patterns obtained during the neap tide period are in agreement with the conceptual model. Settlement on 2 h intertidal collectors was generally higher than on subtidal ones, with an average of 2.4 megalopae.collector^{-1}.h^{-1}. Periodic regression analyses only revealed a tidal periodicity on intertidal collectors (Table 2.1, Fig. 2.7). Settlement peaks occurred soon after mid-flood tides, following hourly concentration maxima of megalopae in the plankton, with a peak lag of ca. 1 h. By these times, salinity was high, temperature was low and current speed had started to decrease (Fig. 2.5). Significant light vs. dark settlement differences were not detected on intertidal (*t* = -0.780, p = 0.454) or subtidal (*t* = 0.067, p = 0.948) 2 h collectors. However, significantly more megalopae settled on 12 h collectors sampling night-influenced flood tides, suggesting that light inhibition was only detectable with a cumulative effect (*t* = -4.757, $p < 0.001$) (Table 2.2). Cumulative settlement on 12 h collectors equalled settlement on 25 h collectors, suggesting that post-settlement secondary dispersal in less than 25 h was unlikely to occur during the neap tide period (Table 2.2).

DISCUSSION

Frequent sampling of available planktonic and settled megalopae requires high occurrences of both for these data to be meaningful. In order to deal with this constraint, we attempted to identify periods of high occurrence of megalopae beforehand and extended to 4 consecutive tidal cycles the effort of each sampling period. However, the low occurrences recorded only allowed for one spring and one neap tide periods to be sampled. Nevertheless, the results presented in this study support hypotheses (1) and (4) under test: the majority of megalopae entering the estuary remained inside, and both upstream movement and settlement conformed to the conceptual model for brachyuran crabs. Density-dependent secondary dispersal of settled megalopae was not detected at high temporal resolution, and settlement was mainly intertidal, refuting hypotheses (2) and (3), respectively.

Planktonic availability

Supply levels of megalopae were similar to those of surface net flux during both spring and neap tide periods, which indicates that both measures correctly described megalopal abundances in the plankton. This supports the idea that the vast majority of megalopae that entered the estuary with flood tides remained within estuarine boundaries after ebb tides, as indicated by the low captures on passive nets facing ebb tides. Furthermore, both megalopal supply and surface net flux were significantly higher (by a factor of approximately 2) during the spring tide period, in accordance with the semilunar supply pattern proposed by Queiroga et al. (2006) for the Mira Estuary. This suggests that the decoupling between supply and settlement of shore crab megalopae previously reported for the Mira Estuary (Paula et al. 2006, Queiroga et al. 2006), and confirmed in the present study, is not an artefact caused by overestimation of planktonic abundances of megalopae during the spring tide period, as overestimation did not occur in these studies.

Hourly concentration of megalopae showed similar levels in both periods, which implies that the higher megalopal supply and net flux of the spring tide period did not result from higher concentrations of megalopae, but possibly from the higher water flux entering the estuary during that period. Patterns of hourly concentration of megalopae were similar during both periods and conformed to upstream movement of megalopae by STST. However, only during the neap tide period did megalopae occur in the water column preferentially during night-influenced flood tides, in complete agreement with the conceptual model. The higher amplitude changes in current speed of the spring tide period may have cued megalopae to swim also during daylight flood tides, overriding the swimming inhibition by light. Regarding upstream movement of megalopae, these results suggest that no deviations from the conceptual model that could explain the decoupling between supply and settlement of shore crab megalopae occurred during the spring and neap tide periods.

Settlement

Settlement of shore crab megalopae on the collectors showed a marked semilunar periodicity throughout the daily series, with maxima during neap tides, on days of waxing and waning moons. Despite the relatively lower settlement levels recorded, these results are in accordance with recent studies conducted in the same estuary (Paula et al. 2006, Queiroga et al. 2006); interannual fluctuations in larval recruitment and settlement levels are common in marine invertebrate species (e.g. Roughgarden et al. 1988). The general settlement pattern reported by all three studies conducted in the Mira Estuary deviates from that of the conceptual model for brachyuran crabs in the sense that settlement was not maximized when flood currents occurred in darkness. During this study, flood currents occurring in darkness mainly took place at spring tides. In fact, settlement maxima occurred during neap tides, when such co-occurrence was minimal. This suggests that processes other than light inhibition might be responsible for the settlement pattern of the shore crab in the Mira Estuary. Furthermore, the high correlation found between tidal amplitude and settlement suggests that tidal-related processes (e.g. turbulence) may be involved in such deviation from the conceptual model.

Settlement on collectors of higher temporal resolution followed the reported semilunar settlement pattern, thus being clearly decoupled from megalopal supply and surface net flux. These results confirm, at higher temporal resolution, the decoupling between supply and settlement of the shore crab recently reported for the Mira Estuary (Paula et al. 2006, Queiroga et al. 2006). Also in agreement with previous studies, settlement was higher on intertidal than on subtidal short-term collectors, supporting the idea that it mainly occurred high on the shore, where juvenile shore crabs are most abundant (Zeng et al. 1997, 1999, Silva et al. 2006). Although these results were predictable, based on the literature, they were also essential to refute the hypothesis that settlement occurs subtidally, especially during spring tides, and thus masks the settlement patterns hitherto reported for the Mira Estuary. This indicates that the low settlement recorded during the spring tide period did not result from density-dependent secondary dispersal of settled megalopae, as virtually no megalopae initially settled, intertidally or subtidally. Therefore, the reported decoupling between supply and settlement did not result from failure in detecting settlement during the spring tide period, suggesting that it resulted instead from processes preventing such settlement.

On the other hand, during the neap tide period, settlement occurred consistently on 2 h intertidal collectors, showing marked tidal and diel periodicities, in strict accordance with the conceptual model. Settlement peaks occurred approximately 1 h after peaks of planktonic availability of megalopae. These patterns seem not to have been influenced by secondary dispersal of settled megalopae, as supported by the parallelism of cumulative settlement on 12 h collectors with that on 25 h collectors. This suggests that the decoupling pattern did not result also from unexpected behavioural settlement responses of megalopae to hydrological parameters during the neap tide period.

In summary, our results seem to corroborate, at higher temporal resolution, that supply of shore crab megalopae is decoupled from settlement in the lower Mira Estuary.

Furthermore, they suggest that the observed decoupling did not result from incorrect estimation of megalopal abundances in the plankton, or from deviations from the conceptual model of reinvasion and settlement for brachyuran crabs in mesotidal systems. In fact, the results indicate that processes preventing settlement and capable of overriding megalopal responses to light intensities during spring tides may explain such decoupling. Queiroga et al. (2006) proposed that the competence state of megalopae might explain the decoupling between supply and settlement in the Mira Estuary. They suggested that supply from a pool of megalopae in an earlier stage of development during spring tides could result in a delay between supply and settlement. Marked and consistent semilunar differences in development stages of megalopae are hard to conceive due to the heterogeneous nature of coastal waters where larvae develop (Pineda 2000). Furthermore, we conducted a pilot study in the Mira Estuary, which revealed that such differences do not exist. In that study, one surface and one bottom passive plankton nets were deployed facing flood tides and sampled every 24 h for 3 d at each moon phase from 27 April to 5 June, 2002. Ten megalopae were randomly sampled from each depth and moon phase and checked for development stage according to Gonzalez-Gordillo et al. (2004). Maxilliped stages 2 to 6 were identified during both spring and neap tides, with no different occurrences between such periods ($\chi^2 = 2.292$, $p < 0.01$). Another explanation proposed by Queiroga et al. (2006) was that higher turbulence levels during spring tides might prevent megalopae from settling in the lower Mira Estuary. Megalopae are known to respond to turbulence changes rather than absolute values (Welch et al. 1999, Pineda 2000, Welch & Forward 2001). The higher amplitude changes in current speed values (an indirect measure of turbulence) registered during the spring tide period might have prevented megalopae from settling. This hypothesis is further supported by the high correlation found between daily settlement and days of lowest tidal amplitude, associated with lower turbulence levels (neap tides). Higher turbulences during the spring tide period may thus have cued megalopae to swim also during daylight flood tides, which prevented settlement and governed upstream movement and settlement events in the lower Mira Estuary during this study. This discussion further raises the question of what happens to most of the megalopae coming in as the peak supply during spring tides. We can speculate that they may be transported on consecutive flood tides and colonize more upstream areas with lower turbulence levels. Future studies should focus on megalopal responses to turbulence levels, associated with tidal amplitude, and its interactions with light inhibition pressures, for a better understanding of recruitment processes in mesotidal systems.

ACKNOWLEDGEMENTS

We are indebted to S. Geist and K. Kreutz for field and laboratory help and for creating a nice working atmosphere. We thank Mr. I. Gomes da Silva and I. Silva for their help with fieldwork, and 3 anonymous referees for critical comments that greatly improved the manuscript. We acknowledge a PhD grant to V.A. (SFRH/BD/10471/2002) funded by Fundação para a Ciência e a Tecnologia (FCT). This work is part of the “ProRecruit - Shelf processes controlling recruitment to littoral populations in an eastern oceanic boundary: using barnacles and crabs as models” research project, also funded by FCT (No. POCTI/1999/BSE/36663). Financial support was allocated by FCT under the Support Community Framework III, Operational Programme for Science, Technology and Innovation.

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CHAPTER 3

Implications of habitat-specific growth, size-structure and physiological condition on early juvenile crab populations

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Implications of habitat-specific growth, size-structure and physiological condition on early juvenile crab populations

ABSTRACT

Post-settlement processes are usually body size-dependent and can regulate size and structure of marine fish and benthic invertebrate populations. Higher growth rates and better physiological condition generally increase survival potential, being usually associated with habitats of higher structural complexity. Successive cohorts of early juvenile *Carcinus maenas* were followed in sandy and *Zostera noltii* habitats during the recruitment season to estimate juvenile population growth, size-structure and physiological condition (evaluated by RNA/DNA ratio). Mean cohort growth was similar in both habitats. However, in the sandy habitat, population size-structure progressed to cohorts of larger carapace width (CW) and juvenile physiological condition was always higher than in the *Z. noltii* habitat. In the *Z. noltii* habitat, size-structure was characterized by low CW cohorts throughout and juvenile condition only increased after 4.5 to 5.0 CW. High juvenile densities associated with more structurally complex habitats, may restrain growth and condition as a result of higher competition for resources and predation pressures, and lead to secondary dispersal to less populated habitats. Smaller juveniles showed the worst condition and higher sensitivity to temporal and spatial variability, while lager ones were characterized by the highest condition. Environmental conditions early in the recruitment season seem to favour juvenile growth.

KEYWORDS: Estuarine, Habitat-quality, NanoDrop, Nucleic acids, Population dynamics

INTRODUCTION

Most fish and marine benthic invertebrate species present a two-phase life cycle, in which adults produce a larval phase that usually develops in coastal waters before recruitment to local populations (addition of new individuals to successive stages of the life cycle within populations, *sensu* Caley et al. [1996]). More than five decades ago, Thorson (1950) noted that such species have larger abundance fluctuations than those of one-phase life cycle, suggesting that population size is directly dependent on larval recruitment. However, if a population and/or recruitment habitat is saturated, or if settlement is geographically limited, settlement levels will have little impact on population dynamics (Hyder et al. 2001). Post-settlement processes dictating competition for resources are usually size-dependent, and have also been recognized as capable of regulate population size and structure of fish and marine benthic invertebrate species

(Roughgarden et al. 1988, Ólafsson et al. 1994, Caley et al. 1996). Individuals with a rapid growth and better physiological condition will have an increased survival potential within the population, as their capacities to feed, escape predators and support environmental stress are enhanced (e.g. Suthers 1998). Knowledge on growth rates and physiological condition of new recruits is, therefore, essential to understand demographic patterns, and ultimately, population dynamics.

Growth and physiological condition of individuals and populations generally present a degree of temporal and spatial variability throughout ontogenetic life, especially during early stages, with environmental and habitat conditions playing key roles on the survival success of new recruits (Hyder et al. 2001, Reid et al. 2006). Different recruitment and nursery habitats have been identified as vital areas for the ecological success of several marine fish and benthic invertebrate populations in both coastal and estuarine environments (Orth & van Montfrans 1990, Feldman et al. 1997). In fact, several marine species use a range of different recruitment and nursery habitats or niches, usually varying in structural complexity, increasing diversity within communities and assemblages (e.g, flatfishes [Gilliers et al. 2004], lobsters [Parslow-Williams et al. 2001], crabs [Moksnes et al. 1998, Paula et al. 2006] and shrimps [Feldman et al. 1997]). Different habitats commonly present different food availabilities, predation pressures and physical disturbances, inducing specific growth rates and physiological levels on individuals (Reid et al. 2006). Generally, habitats with higher structural complexity, such as seagrass meadows, constitute higher quality settlement and nursery areas in relation to less structured habitats, such as sandy areas (Feldman et al. 1997, Fonseca et al. 2006). Over the last decades, several studies have focused in assessing such differences through different measures of individual and population growth and condition (e.g. Buckley et al. 1999, Gilliers et al. 2004). The RNA/DNA ratio is among the most used indices to infer habitat quality by evaluating the physiological condition of individuals, and has been used for marine invertebrate and fish species (Parslow-Williams et al. 2001, Gilliers et al. 2004, Norkko & Thrush 2006).

To date, few studies have addressed juvenile physiological condition during the early post-settlement life at short time intervals, especially on mobile species. This results, in part, from the difficulty in identifying successive cohorts, especially in species with cryptic juveniles, such as marine benthic invertebrates, which are known by their plasticity in the use of different recruitment and nursery habitats. Nevertheless, the early post-settlement period is potentially one of the most important phases in the life of fishes and marine benthic invertebrates with pelagic larvae, as processes acting on these life stages may play a significant role in population regulation and community organization (Ólafsson et al. 1994, Caley et al. 1996, Gosselin & Qian 1997, Hunt & Scheibling 1997).

The shore crab *Carcinus maenas* (L.) (Brachyura: Portunidae) is a cosmopolitan species inhabiting both hard and soft coastal and estuarine shallow habitats. After hatching, larvae develop through 4 zoeal stages, and metamorphose into megalopae, in adjacent shelf waters (Paula 1989, Queiroga 1998). Megalopae reinvade estuarine inlets, actively selecting settlement grounds (Moksnes et al. 1998, Paula et al. 2006, Queiroga et al. 2006). In the lower Mira Estuary (SW Portugal), settlement is decoupled from supply, occurring mainly during neap tides (Queiroga et al. 2006, Amaral et al. 2007). Two different recruitment habitats have been reported in the lower Mira Estuary: sandy areas with cobbles and meadows of *Zostera noltii* Hornemann, 1832 (Paula et al. 2006). Despite the knowledge gathered on settlement and other aspects of adult *C. maenas* populations, little is known about the processes in between, i.e. growth rates and physiological condition of early juveniles. This study was thus designed to evaluate the physiological condition of *C. maenas* early recruits on successive cohorts during the recruitment season in different habitats in the lower Mira Estuary. It was hypothesized that juvenile physiological condition should be higher in *Z. noltii* meadows than on sandy areas. We further hypothesized that such a difference should be reflected on population size-structure in both habitats. In order to test these hypotheses, size-structure and RNA/DNA ratio of early juvenile *C. maenas* populations were assessed and followed on successive settlement events during the recruitment season.

MATERIALS AND METHODS

Sampling

Sampling was conducted in the lower Mira Estuary, a small mesotidal system located on the southwestern Portuguese mainland coast (37°40’ N, 8°40’ W, Fig. 3.1). It extends for 40 km inland and is characterized by a single river channel with 400 m maximum width in the lower part and average depth of 5 to 10 m. Maximum tidal amplitude is ~3.5 m, and tidal penetration ranges from 2.5 to 7.5 km during neap and spring tides, respectively (Paula 1989). Water column structure varies from vertically homogeneous to slightly stratified during spring and neap tides, respectively (Amaral et al.

2007). The estuary has a low and seasonal freshwater input, with the lower section presenting a marine character. In such section, extensive meadows of *Z. noltii*, salt marshes and areas of sandy and muddy substrates with cobbles and pebbles characterize the intertidal zone. A dense cover of *Zostera marina* L. occurs subtidally adjacent to *Z. noltii* meadows. The middle part of the estuary is bordered by sedimentary formations dominated by salt-marsh vegetation, as far as 20 km upstream (Amaral & Paula 2007).

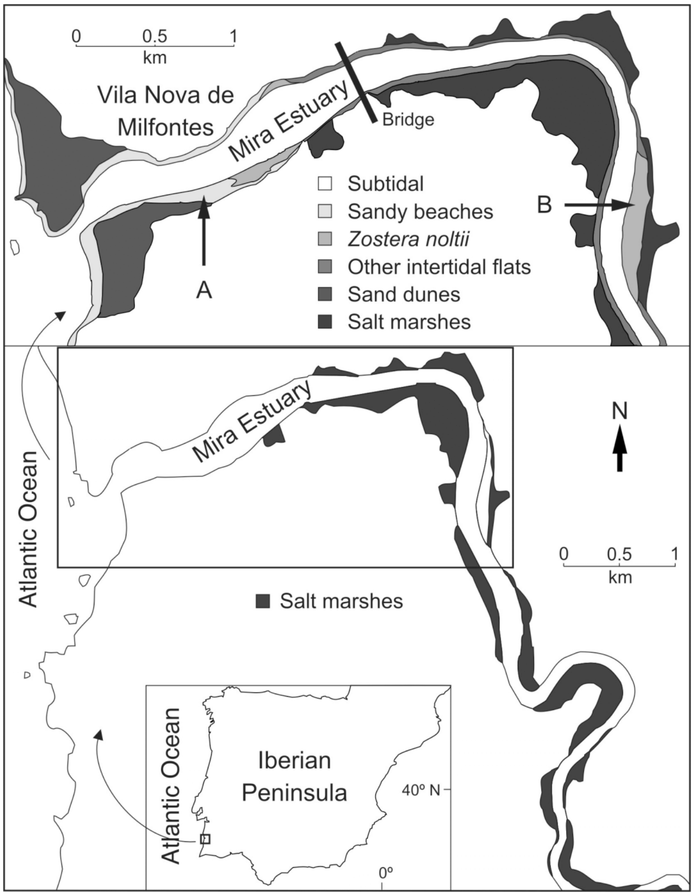

Fig. 3.1. Location of Mira Estuary and sampling areas: (A) sandy and (B) *Zostera noltii* habitats.

The lower section of the estuary, where the sampling took place, is characterized by similar temperature, salinity and water circulation regimes (Andrade 1986). Two different habitats were considered in this study: a sandy beach with cobbles; and a *Z. noltii* meadow (Fig. 3.1). High and low densities of juvenile shore crabs characterize *Z. noltii* and sandy habitats, respectively (Paula et al. 2006). From March to July, 2005, both habitats were sampled intertidally during the morning low tide on consecutive neap tides, on the day of the quarter moon. Nine and seven of these periods were sampled in the sandy and *Z. noltii* habitats, respectively. For each sampling, 200 to 300 juvenile *Carcinus maenas* were collected by hand from randomly selected areas of the high intertidal, were juveniles seek refuge (Silva et al. 2006). All juveniles with <10 mm carapace width (CW) present in the surveyed areas were considered. Concurrently, measurements of water temperature and salinity were conducted, adjacent to sampling sites, at water surface (~1.5 m above the bottom) at beginning, halfway through and end of juvenile crab collection. Salinity values are based on the Practical Salinity Scale of 1978 (PSS 78). After collection, individuals were immediately frozen at -20 °C. At the laboratory, all individuals were identified, enumerated and measured to the nearest 0.1 mm (maximum CW). Selected individuals (see below) were stored individually and kept sealed in 1.5 ml vials at -80 ºC until biochemical analyses were conducted.

Statistical analysis

Data on crab juvenile size were arranged in CW-frequency distributions of 0.25 mm size class increments for each sampling date, in each habitat. Cohorts were identified through modal progression analysis of CW distributions, based on the Bhattacharya method, using FISAT II software, version 1.1.2 (FAO 2002). Mean ± SE cohort CW was the size range considered for each cohort, without overlap between cohorts. To study the effect of seasonality on crab juvenile condition, three periods were considered in the recruitment period: beginning, from March to April; middle, from May to mid June; and end, from mid June to July. The effects of recruitment period, habitat and size class were evaluated by three-way analysis of variance (ANOVA), using the software STATISTICA version 6 (StatSoft). Cochran's tests revealed homoscedasticity of variances, and Shapiro-Wilk's tests revealed normality of residuals. *A posteriori* comparisons were performed by Tukey's HSD tests.

Biochemical analysis

Based on mean cohort CW, three different size classes (2.3 - 3.3, 4.3 - 5.3 and 6.4 - 7.4 mm CW) and three cohorts, attempting equivalence between habitats, were selected for nucleic acids quantification. For each habitat, and in each sampling date, seven haphazardly chosen individuals of each CW size class and cohort were chosen for biochemical analyses. Nucleic acids were quantified in whole-animal tissue following the Schmidt-Thannhauser method, according with the procedures of Buckley & Bulow (1987). Some modifications were introduced to adapt the method to the species and equipment used. All animals were analyzed individually and reagent volumes were adjusted according with the animal size (Table 3.1).

Table 3.1. Reagents volumes (µl) used for RNA/DNA ratio determination, according with juvenile *Carcinus maenas* carapace width.

	Carapace width (mm)		
	< 4.0	4.0 – 6.5	> 6.5
0.2 N PCA homogenization	300	300	375
0.2 N PCA wash	100	150	200
0.3 N NaOH	60	100	120
2.0 N PCA	15	25	30
0.6 N PCA	30	70	100

All reagent solutions were used at ice-cold temperature. Whole-animal tissue was weighted to the nearest 0.1 mg (fresh weight) and immediately homogenized in 0.2 N $HClO4$ (PCA) with a hand driven homogenizer. Samples were incubated in ice for 15 min and centrifuged (all centrifugations were at 6000 x g, at 0 – 4 °C, for 13 min), and the supernatant was discarded. The pellet was washed twice with 0.2 N PCA, by resuspending the pellet with a motor driven pellet pestle (Sigma), centrifuging and discarding the supernatant after each centrifugation. The pellet was resuspended in 0.3 N NaOH, vortexed and incubated at 37 °C for 1 h. PCA at 2.0 N was added, the sample was vortexed, incubated in ice for 30 min and centrifuged. The supernatant, containing the RNA fraction, was warmed to room temperature and measured for its absorbance at 260 nm. The pellet was washed twice with 0.2 N PCA as described above and the supernatant discarded. PCA at 0.6 N was added and the sample was vortexed and incubated at 70 °C for 30 min. The sample was vortexed, incubated in ice for 15 min and centrifuged. The supernatant, containing the DNA fraction, was warmed to room temperature and measured for its absorbance at 260 nm. Absorbance was measured with a NanoDrop® ND-1000 full spectrum spectrophotometer (NanoDrop, USA), which enables highly accurate analyses of 1 µl samples with high reproducibility (detection limits 2 - 3000, 3700 ng/µl for RNA and double stranded DNA, respectively), allowing analyses of individual juvenile shore crabs as small as 1.5 mm CW. Its full spectrum feature allowed inspection of nucleic acid contamination concurrently to samples analyses by estimation of 260/280 and 260/230 nm ratio. Three readings of each nucleic acid were performed per sample. All plastic and glassware was autoclaved prior to use.

RESULTS

During the sampling period, water temperature and salinity varied from 17.7 to 22.9 ºC and 34.5 to 36, respectively. Totals of 2061 and 2274 juvenile crabs were collected in the sandy and *Z. noltii* habitats, respectively. Several cohorts were identified and values of mean CW were linked tentatively between consecutive dates to follow modal progression (Fig. 3.2). In both habitats, minima and maxima numbers of cohorts were identified in the beginning and middle of the sampling period, respectively. Cohorts of new recruits surged with relative low frequencies, progressively increasing their relative importance in the population. In the sandy habitat, the size-structure of the juvenile population progressed from low and mid to mid and high cohort CW. In the *Z. noltii* habitat, cohorts of low and mid CW characterized population structure throughout the sampling period. Overall mean cohort CW increment per sampling date was similar in the sandy (0.53 mm) and *Z. noltii* (0.48 mm) habitats (two-tailed *t*-test, $t = 0.630$, df = 17, $p = 0.537$).

In the sandy habitat, RNA/DNA ratio increased with juvenile size until ~4.5 mm CW, after which it remained high and more or less stable, only decreasing after ~8.5 mm CW. Starting at 4.2 mm CW, cohort 1 presented constant and highest RNA/DNA ratio until ~8.5 mm CW (regression, $b = -0.16$ $R^2 = 0.19$, $p < 0.001$) (Fig. 3.3). For cohorts 2 and 3, RNA/DNA ratio increased throughout, with a significantly lower (two-tailed *t*-test, $t = 3.907$, df = 108, $p < 0.001$) regression slope for cohort 2 ($b = 0.50$, $R^2 = 0.75$, $p < 0.001$) than for cohort 3 ($b = 0.75$, $R^2 = 0.81$, $p < 0.001$). Both these cohorts presented significantly higher regression slopes than cohort 1 (two-tailed *t*-test, $t = 11.670$, df = 122, $p < 0.001$, and $t = 13.354$, df = 108, $p < 0.001$, respectively).

In the *Z. noltii* habitat, RNA/DNA ratio was always lower at a size than in the sandy habitat (Fig. 3.3). RNA/DNA ratio remained low until ~5.0 mm CW, increasing afterwards to values around 3.0 until ~8.0 mm CW. Starting at 4.4 mm CW, cohort 1 presented an increase in RNA/DNA ratio after 5.0 mm CW (regression, $b = 0.56$, $R^2 = 0.73$, $p < 0.001$). Cohorts 2 and 3 showed a low RNA/DNA ratio throughout, and presented regression slopes similar to zero ($b = 0.01$, $R^2 = 0.001$, $p = 0.852$, and $b = -0.07$, $R^2 = 0.02$, $p = 0.352$, respectively) and significantly lower than that of cohort 1 (two-tailed *t*-test, $t = 8.355$, df = 94, $p < 0.001$, and $t = 7.185$, df = 87, $p < 0.001$, respectively).

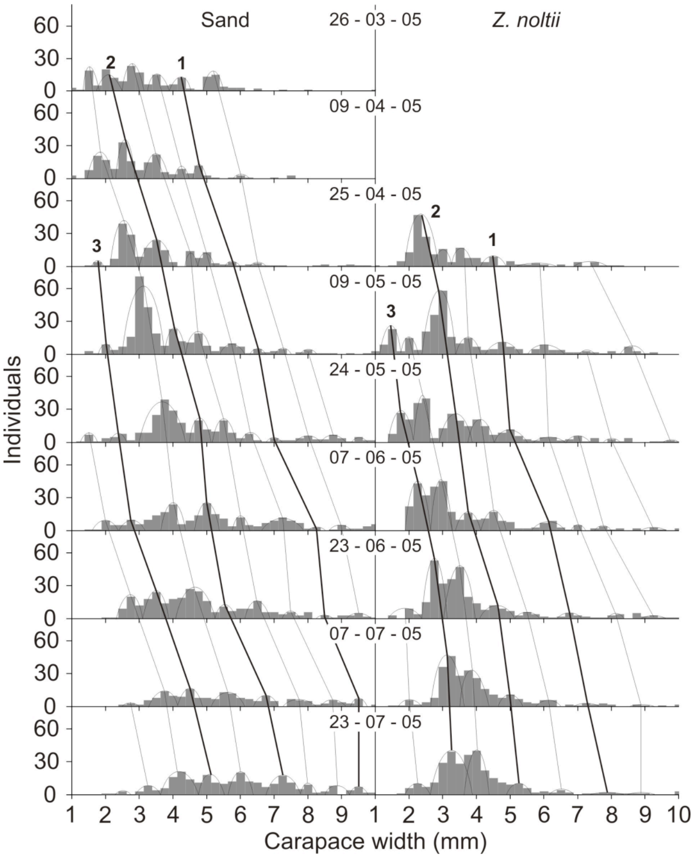

Fig. 3.2. Frequency distribution of carapace width of juvenile *Carcinus maenas* on successive neap tide periods in the sandy and *Zostera noltii* habitats, during the recruitment season. Curve lines represent cohorts identified by the Bhattacharya method. Cohorts were tentatively connected to indicate modal progression. Bold lines and numbers indicate the three cohorts (numbered chronologically) chosen for acid nucleic quantification.

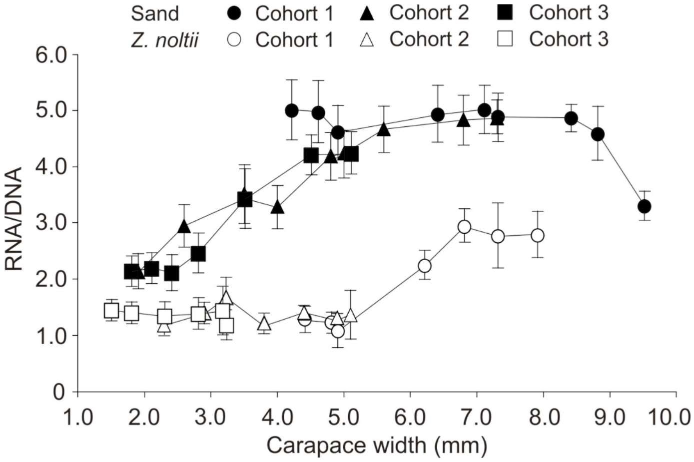

Fig. 3.3. Mean RNA/DNA ratio of three *Carcinus maenas* juvenile cohorts in the sandy and *Zostera noltii* habitats, during the recruitment season. Error bars: ±SE.

Three-way ANOVA showed significant effects of habitat, recruitment period and size class, as well interaction on RNA/DNA ratio (Fig. 3.4, Table 3.2). Overall, RNA/DNA ratio was significantly higher in the sandy than in the *Z. noltii* habitat, and decreased significantly during the recruitment period and increased significantly with size class ($p < 0.001$ in all cases). Overall, RNA/DNA ratio of the smallest and intermediate size classes decreased from the 1st to the 2nd period, but not from then on, while that of the largest size class was similar in the 1st and 2nd periods, but lower in the 3rd ($p < 0.001$ in all cases showing differences).

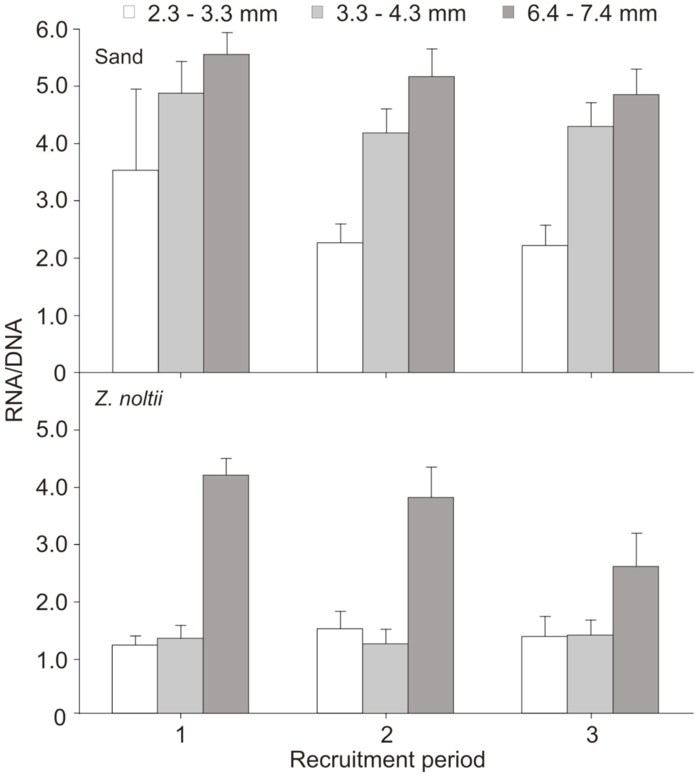

Fig. 3.4. Mean RNA/DNA ratio of *Carcinus maenas* juvenile size classes in the sandy and *Zostera noltii* habitats, during the recruitment season: (1) March to April; (2) May to mid June; and (3) mid June to July. Error bars: SE.

In the sandy habitat, RNA/DNA ratio of both smallest and intermediate size classes decreased from the 1st to the 2nd period ($p < 0.001$), but not further on, with no differences in the largest size class throughout (Fig. 3.4). While the smallest size class showed always the lowest RNA/DNA ratio ($p < 0.001$ in all cases), only in the 2nd period was the ratio of the intermediate size class lower than that of the largest ($p < 0.001$). Nevertheless, overall RNA/DNA ratio increased significantly with size class ($p < 0.001$ in all cases).

In the *Z. noltii* habitat, RNA/DNA ratio was similar during the three recruitment periods for each size class, with exception to the largest class with a lower ratio in the 3rd period ($p < 0.001$) (Fig. 3.4). At all times, the smallest and intermediate size classes showed similar RNA/DNA ratio, being lower than that of the largest size class ($p < 0.001$ in all cases).

Table 3.2. Results of three-way ANOVA analyses on the effect of habitat, recruitment period and size class on RNA/DNA ratio of *Carcinus maenas* early juveniles. Significant p-values in bold.

Source of variation	df	MS	*F*	p
Habitat	1	266.03	858.89	**< 0.001**
Period	2	8.11	26.20	**< 0.001**
Size class	2	113.31	365.82	**< 0.001**
Habitat x Period	2	2.53	8.18	**< 0.001**
Habitat x Size class	2	21.64	69.88	**< 0.001**
Period x Size class	4	2.41	7.80	**< 0.001**
Habitat x Period x Size class	4	2.25	7.26	**< 0.001**
Error		297	0.31	**< 0.001**

DISCUSSION

While estuarine sandy habitats are characteristic of more exposed areas, vegetated habitats usually occur in places of low hydrodynamic, where plant roots can establish on the sediment. These habitats are thus characterized by specific geographic occurrences inside estuaries. Unfortunately, due to the small dimension of the Mira Estuary, it was not possible to perform a desirable geographic replication of such habitats. Furthermore, the choice of the sampling locations had in consideration the migratory behaviour of juvenile *C. maenas* (Moksnes 2004), and avoided contiguous areas of both habitat types. Nevertheless, we feel that the conducted sampling plan, especially its frequency, together with the similar physico-chemical regimes characterizing both sampling areas, support the ecological validation of our results and allow us to address the hypotheses under test. Although cohort number and overall growth were similar in both habitats, size-structure in the sandy habitat progressed to cohorts of higher CW and juvenile physiological condition was higher. Both habitats seem to be important recruitment areas, and the observed differences in size-structure and condition of juvenile crabs may result from environmental factors specific of each habitat.

Seagrass meadows, and other highly structured habitats, function as nursery or feeding areas for several marine fish and benthic invertebrates, accumulating high organism densities (e.g. Moksnes 2004, Fonseca et al. 2006, Paula et al. 2006). When densities exceed a critical level, inter- and intra-specific density-dependent processes, such as predation and competition for resources, may shape population size and structure, by acting on early post-settlement life stages, more vulnerable to abiotic and biotic factors due to their relative small size and energy levels (Ólafsson et al. 1994, Diehl & Ekloev 1995, Moksnes 2004). *Carcinus maenas* juveniles presented a lower condition at any cohort CW and time in the *Z. noltii* than in the sandy habitat. In fact, even 1st instar juveniles (< 2 mm CW) presented lower RNA/DNA ratio in this habitat. As nucleic acids are known to quickly respond to changes in the environment at which the organism is exposed (Buckley et al. 1999), these patterns may already be due to habitat influence on juvenile physiological condition. Another possible explanation is that megalopae settling in that habitat may already have a lower condition. Cohorts of 1st instar juveniles were recorded in this study with relatively low frequencies, contrasting with a previous study conducted in the same estuary that reported relatively higher frequencies (Silva et al. 2006). In fact, most marine benthic invertebrate and fish species with similar life-history strategies are characterized by the arrival of new recruits in large numbers (Roughgarden et al. 1988). The relatively low frequencies may have resulted from low recruitment levels (Amaral & Paula 2007, Amaral et al. 2007) or high mortality of recently settled juveniles, prior to sampling. High mortality in the first hours and days after settlement have been reported on 1st instar juvenile shore crabs (Moksnes et al. 1998) and on several other invertebrate species (Gosselin & Qian 1997).

While in the sandy habitat, a progression in cohort CW characterized juvenile population, in the *Z. noltii* habitat, small and mid CW cohorts prevailed throughout. Furthermore, juvenile physiological condition increased significantly with size in the sandy habitat, stabilizing with maximum RNA/DNA ratio after ~4.5 - 5.0 mm CW. In the *Z. noltii* habitat, condition remained very low, only increasing after reaching that CW. These results suggest that smaller juveniles are more sensitive to habitat characteristics than larger ones. Despite mean cohort CW increment had been similar in both habitats, CW increment was lower for smaller size classes in the vegetated habitat. Density-dependent post-settlement processes characteristic of habitats of high structural complexity and intra-specific competition, food availability, space limitation and physical interference, have been suggested to limit growth and condition of individuals and populations of fishes and marine benthic invertebrates (Ólafsson et al. 1994, Diehl & Ekloev 1995, Moksnes 2004).

As crustacean growth is based on moults, growth increments in early juvenile stages are a low proportion of total size, which can explain the similarity of growth rates in both habitats, despite the differences in physiological condition.

The relatively low frequencies of larger individuals on the vegetated habitat, likely did not result from predation, as it is usually lower on habitats of higher structural complexity, such as seagrass meadows (Gosselin & Qian 1997, Hunt & Scheibling 1997), suggesting that other processes may be involved in structuring juvenile populations in the *Z. noltii* habitat. Furthermore, juvenile shore crabs larger than 5 mm CW have been reported to attain a size refuge from predation by juvenile conspecifics, which are their major predators in seagrass meadows (Moksnes et al. 1998). High densities of recently settled juveniles in seagrass habitats can result in competition for space and mutual interference, leading to secondary dispersal of juveniles to habitats with lower animal density, such as the sandy habitat (Moksnes 2004). This would lower density-dependent competition levels for non-migrant siblings, enhancing their physiological condition and growth rates. Many aquatic animals shift their diet and habitat at some point during the juvenile phase (e.g. Werner & Gilliam 1984). In fact, benthic juvenile invertebrate are considered to be ecologically distinct from later stages in their use of habitat and food resources and in vulnerability to mortality factors (Gosselin & Qian 1997). On the other hand, the observed size-structure, growth and condition patterns may be a "secondary effect" of natural selection. This is, selection pressure may be stronger in the sandy habitat, eliminating animals in worse physiological condition. In the *Z. noltii* habitat, where densities are generally much higher, animals in a better condition would be relatively less represented, at least until a certain size.

During the sampled period, smaller juvenile shore crabs showed lower RNA/DNA ratio and larger juveniles presented higher ratio. The ontogenetic pattern of RNA/DNA ratio in crustaceans generally agrees with those found in both habitats, especially in the sandy habitat, with levels reaching minimum values after metamorphosis to 1st instar juveniles, and gradually increasing with animal size (e.g. Wagner et al. 2001, Lemos et al. 2002). Our results indicate that the physiological condition of juvenile shore crabs is best during the first half of the recruitment season, suggesting that 1st instar juveniles have in this period an enhanced probability of survival than those later in the season. This suggestion is under the hypotheses "bigger is better", in the sense that a better condition translates in faster growth rates, leading to higher food consumption rates and lower vulnerability to predators (Suthers 1998). The same interpretation was proposed for flatfishes in a study conducted in the Tagus Estuary, on which juvenile condition was also evaluated by RNA/DNA ratio (Fonseca et al. 2006).

Summarizing, less structured habitats, such as a sandy beach with cobbles, may present several advantages over habitats of higher structural complexity, enhancing the physiological condition of juvenile crabs, and thus their survival probability during the early post-settlement phase. Density-dependent post-settlement processes may decrease the physiological condition and growth rates of early juvenile shore crabs. Such processes influence the strength of juvenile cohorts and the survival probabilities within a cohort. This study further suggests that early juveniles may have enhanced growth rates during the first half of the recruitment season in the lower Mira Estuary. Future research should focus on whether the higher condition of early juvenile *C. maenas* on sandy habitats is maintained in larger individuals, in order to understand the ecological role and effect of different recruitment habitats on the regulation and structure of crab populations.

ACKNOWLEDGEMENTS

We thank V. F. Fonseca for her help with nucleic acid analyses. V.A. acknowledges a PhD grant (SFRH/BD/10471/2002) funded by Fundação para a Ciência e a Tecnologia. All work complied with Portuguese government laws.

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CHAPTER 4

Quality of estuarine and nearshore marine native habitats: the *Carcinus maenas* case

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Mar Ecol Prog Ser (in press)

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ABSTRACT

Quality habitats are those where individual and population growth, survival and reproductive potential are enhanced. Habitat quality is only valid on a comparative basis and can be evaluated through bioindicators, especially of a biochemical nature. The European shore crab *Carcinus maenas* (L.) inhabits a variety of coastal, estuarine and lagoonal habitats. The quality for shore crabs of five different habitats in a southern (SW Portugal) and a northern (SW UK) area, within its native distribution region, was evaluated and compared in terms of individual physiological condition (RNA/DNA ratio) and population density and structure indices. While density and condition showed some similarities between habitats, population structure was exclusively habitat specific. In the southern area, the *Zostera noltii* Hornemann, 1832 habitat showed higher quality than the unvegetated habitat (clay mud), which may be important for bigger individuals. In the northern area, the rocky shore habitat showed higher quality than both vegetated (*Fucus* spp.) and non-vegetated (coarse mud) estuarine habitats, whose quality was similar. Habitat quality was not necessarily higher for *C. maenas* in estuarine vegetated habitats in relation to adjacent less structurally complex estuarine or coastal habitats. In terms of latitudinal differences, estuarine environments seem to have provided sub-optimal quality habitats in the more southern area, where the lack of established and abundant populations might result from biotic resistance by other predator crabs absent from the northern area. To be effective, integrative management plans for mobile marine species should consider a broad spectrum of essential habitats.

KEYWORDS: Crabs, Density, Habitat-quality, Nucleic acids, Physiological condition, Population structure

INTRODUCTION

Habitat use by animals is determined by trade-offs between maximizing growth and minimizing mortality risk. Habitat quality and resource availability can thus regulate animal distribution and growth. Unlike habitat quantity, quality cannot be measured directly. High quality habitats are assumed to be those where growth and survival are enhanced and, ultimately, where reproductive potential is optimized (Gibson 1994, Beck et al. 2001).

The description of habitat quality poses numerous problems, not least the choice of appropriate indicators. Several indicators should be used, as no single measure provides an overall description of habitat quality (Beck et al. 2001, Gilliers et al. 2006). Hence, estimations of individual and population growth, condition and density have been used as relevant indicators of habitat quality, which should always be used on a comparative basis for a particular species (Gibson 1994, Beck et al. 2001, Gilliers et al. 2006). Biochemical indicators, and in particular RNA/DNA ratio, have been particularly valuable estimators of organism condition in studies of marine invertebrates and fishes (e.g. Wright & Hetzel 1985, Mayrand et al. 1994, Dahlhoff 2004, Amaral et al. in review). The principle is that RNA content reflects active metabolic rates canalized to growth and reproduction. As DNA content remains relatively constant in an individual due to be a function of chromosome number, higher RNA/DNA ratios are expected where, or when, conditions are favourable (Dahlhoff 2004). Nevertheless, few studies have attempted to use different bioindicators on marine species simultaneously at individual and population levels to assess the quality of respective essential habitats.

Estuarine and nearshore marine habitats are among the most important areas for fishes and marine benthic invertebrates, serving many vital ecosystem functions including spawning, recruitment, nursery and feeding (e.g. Orth & van Montfrans 1990, Beck et al. 2001). The quality of such habitats, and thus their contribution to ecosystem functioning, may vary both geographically, due to climate regime and species pool, and locally (Orth & van Montfrans 1990, Beck et al. 2001, Bas et al. 2005). Despite the recognized importance of these habitats, their degradation and loss continues at an alarming rate, resulting from a range of anthropogenic impacts. Loss of habitat has been highlighted as among the main causes of species decline or extinction (Gibson 1994, Gilliers et al. 2006).

Nearshore vegetated habitats, namely seagrass meadows and wetlands (especially marshes and mangrove forests) are important nursery areas for several marine species, as their high structural complexity enhances refuge and food availability (Orth & van Montfrans 1990, Fonseca et al. 2006). About half the studies on the effects of nearshore habitats on growth of marine species, have reported higher rates in vegetated habitats (Beck et al. 2001). Such areas also generally support higher densities of organisms than adjacent unvegetated substrates, which lack macrophytes, such as mud and sand flats (Polte et al. 2005, Fonseca et al. 2006, Paula et al. 2006). However, non-vegetated habitats such as oyster reefs, have also been recognized as high quality habitats (Glancy et al. 2003, Moksnes & Heck 2006). Furthermore, there is growing acknowledgement that structurally complex habitats are not always of higher quality than adjacent less complex ones (e.g. Diehl & Ekloev 1995, Parslow-Williams et al. 2001, Amaral et al. in review).

Crabs are among the most conspicuous and ecologically important organisms inhabiting intertidal areas of estuarine and nearshore marine ecosystems. They constitute, therefore, potentially valuable indicators of habitat quality. The European shore crab *Carcinus maenas* (L.) inhabits a variety of habitats throughout Europe, from rocky intertidal and subtidal areas to estuaries, saltmarshes and lagoons. It is a highly invasive brachyuran, which is native of the northeast Atlantic (Grosholz & Ruiz 1996, Carlton & Cohen 2003). Its diverse habitat occupations and wide tolerance to environmental conditions and ecological plasticity have allowed the establishment of abundant populations along both east and west coasts of North America, South America, South Africa, Australia and Tasmania (Grosholz & Ruiz 1996, Carlton & Cohen 2003, Hidalgo et al. 2005). Despite its vast range of habitat use, not all potential habitats support abundant shore crab populations. This is the case of rocky shore habitats: in southwest European waters (e.g. Portugal), abundant shore crab populations are only present in protected nearshore environments, such as estuaries and bays, while in north Europe (e.g. UK) they occupy continuously both estuarine and marine nearshore environments (Hayward & Ryland 1995). This geographical difference poses the question of quality of the diversity of habitats used by this species.

Our study investigated the quality of habitats used by *C. maenas*. We hypothesized that estuarine vegetated habitats should be of higher quality than estuarine and rocky shore habitats of lower structural complexity. In order to test this hypothesis, rocky shore and different vegetated and non-vegetated estuarine habitats were investigated in a southwestern (SW Portugal) and a northern (SW UK) European area. Shore crab population density, structure and physiological condition (expressed by RNA/DNA ratio) were assessed in five different habitats (*Zostera noltii* Hornemann, 1832, *Fucus* spp., clay mud, coarse mud and rocky shore) and compared to evaluate habitat quality.

MATERIALS AND METHODS

Habitats and sites

Five different intertidal habitats located in the Portuguese and English respective southwest mainland coasts were surveyed (Fig 4.1). Two estuarine habitats were studied in the Mira Estuary, Portugal: an habitat of high structural complexity characterized by clay sediments and a dense cover of *Zostera noltii* (*Z. noltii* habitat); and a non-vegetated habitat of low structural complexity, characterized by clay muddy sediments and cobbles (clay mud habitat). A replicate site of each habitat was sampled in the lower 2 km of the estuary, and the other at ~5 km upstream. Two estuarine and one rocky shore habitats were studied in the English coast around Plymouth: one estuarine habitat was characterized by silt sediments and medium-high covers of rockweeds *Fucus* spp., conferring a high structural complexity (*Fucus* spp. habitat); the other habitat was characterized by non-vegetated coarse muddy sediments and sparse pebbles, with lower structural complexity (coarse mud habitat); and the rocky shore habitat was characterized by a rock platform exposed to wave action, with gaps and ridges where sand to boulders substrates accumulate, conferring a medium structural complexity (rocky shore habitat). One site of the *Fucus* spp. and coarse mud habitats was located in Yealm Estuary, and the other in Salcombe-Kingsbridge Estuary. For the rocky shore habitat, the two sites were Downderry and Wembury Bay. As all three estuaries are sheltered mesotidal systems with low freshwater input where tidal flows dominate, all five sampled habitats are mainly marine in character (Amaral & Paula 2007, Marine Biological Association 1957).

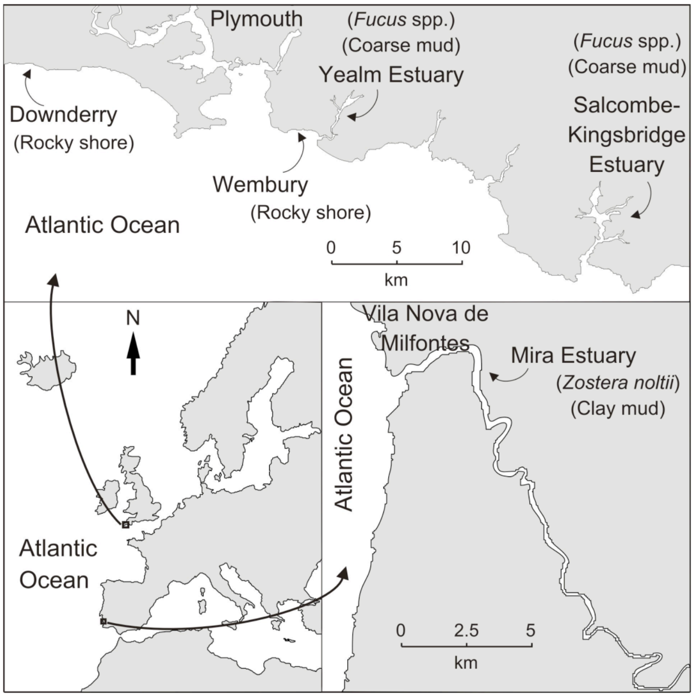

Fig. 4.1. Location of sampling areas. Habitats sampling on each area are between brackets.

The Mira Estuary is a small mesotidal system extending for 40 km inland, characterized by a single river channel with 400 m maximum width in the lower part and average depth of 5 to 10 m. Maximum tidal amplitude is ~3.5 m, and tidal penetration ranges from 2.5 to 7.5 km during neap and spring tides, respectively (Paula 1989). Water column structure varies from vertically homogeneous to slightly stratified during spring and neap tides, respectively (Blanton & Andrade 2001). Extensive meadows of *Z. noltii*, salt marshes and areas of sandy and muddy substrates with cobbles and pebbles characterize the intertidal zone (Amaral & Paula 2007).

The Yealm Estuary is a narrow, steep sided inlet with ~6.5 km long. The mouth is almost closed by a sand bar, sheltering the lower estuary from wave action, and a deep channel is left only on the southern side with an average depth of ~6 m during low tide. Water column stratification may occur in periods of high run off. Boulders and cobbles occur near the mouth and thick deposits of soft mud and cobbles further upstream and in secondary creeks characterize intertidal areas in the lower section. Small salt marsh patches characterize further upstream areas (Marine Biological Association 1957).

The Salcombe-Kingsbridge Estuary is a drowned river valley, covering an area of 674 ha with very little freshwater input. The mouth presents a sand bar offering protection from wave action. Maximal tidal amplitude is ~6 m, and tidal penetration reaches 8 km. Water column structure is only heterogeneous upstream and during neap tides. Sand and mudflats, mixed with pebbles and boulders, and colonized by fucoids, characterize intertidal lower and mid sections. Few small and sparse areas with *Z. noltii*, green algae and salt marsh are also present (Marine Biological Association 1957).

Wembury and Downderry are characterized by shores of laminated rock platforms with uneven series of gaps creating a series of ridges and pools where beach material, from boulders to sand, has accumulated. Both shores have a rich algal community, where the dominant fucoid species are luxuriant and a variety of other brown and red weeds colonize polls and sheltered edges. Southeastern winds dislodge laminarian and fucoid algae that accumulate on the upper shore (Marine Biological Association 1957).

Sampling

In each habitat, two separate replicate sites were considered, and two replicate stations, no less than 20 m apart, were sampled at each site. Each station was sampled on two different spring tide periods, between July and October 2005. At each date, and at each station, population density, size-structure and physiological condition of *C. maenas* were assessed during morning low tides. In all habitats, only areas where crabs seek refuge during low tides were sampled (i.e. areas with pebbles and cobbles or with vegetation).

Population density was estimated from 10 quadrats (0.5 x 0.5 m), randomly deployed throughout the whole intertidal range, lying at least 4 m away from each other, and from which individuals were collected by hand. Population size-structure was estimated by measuring (0.01 mm precision) the maximum carapace width (CW) of all individuals found within the deployed quadrats. When less than 200 individuals were found in the quadrats, more were collected from within the general area of sampling up to 200, assuring he collection of all individuals within the surveyed areas. Physiological condition was evaluated by quantifying RNA/DNA ratios. A minimum of 20 individuals (14 to 18 mm CW) in intermoult stage, as evaluated *in situ* by the hardness of the carapace, were collected within the general area of sampling. After collection, individuals were immediately frozen at -20 °C and transported to the laboratory, where they were stored at -80 °C until nucleic acid quantification. Seven individuals from those frozen were randomly selected for biochemical analyses.

Biochemical analysis

Nucleic acids were quantified following the Schmidt-Thannhauser method, according to the procedure of Amaral et al. (in review). Absorbance was measured with a NanoDrop® ND-1000 full spectrum spectrophotometer (NanoDrop, USA), which enables highly accurate analyses of 1 µl samples with high reproducibility (detection limits 2 - 3000, 3700 ng.µl^{-1} for RNA and double stranded DNA, respectively). A full spectrum feature allowed inspection of nucleic acid contamination concurrently with samples analysis by calculation of 260/280 and 260/230 nm ratios. All animals were weighted to the nearest 0.1 mg, and analyses were performed on 10 to 15 mg (freeze-dried weight) of white abdominal muscle. Three readings of each nucleic acid were performed per sample. All metal, plastic and glassware was autoclaved prior to use, and sterilized when used between individuals.

Statistical analysis

Preliminary analyses, revealed lack of significant differences at the date sampling level in all cases and for all evaluated indices ($p > 0.5$ in all cases). Data were, therefore, pooled together at each date level. To allow direct comparisons among all habitats, effects of habitat, site and station were evaluated using different three-way analyses of variance (ANOVA) for population density and RNA/DNA ratio data. Cochran's tests revealed homoscedasticity of variances, and Shapiro-Wilk's tests revealed normality of residuals in both analyses. *A posteriori* comparisons were performed using HSD Tukey's tests. To study population size-structure, CW data were arranged in frequency distributions of classes of increasing size (3 mm from 0 to 12 mm of CW, 4 mm from 12 to 20 mm of CW, 5 mm from 20 to 40 mm of CW, and 10 mm from then on), in order to avoid underestimation of smaller classes and overestimation of larger ones. Chi-square comparison tests were performed at the station, site and habitat sampling levels.

RESULTS

Significant effects of habitat and site on population density were revealed by ANOVA analysis (Table 4.1). Overall, vegetated habitats had significantly higher population densities than other habitats ($p < 0.001$ in all cases), which showed similar values between each other ($p > 0.15$ in all cases) (Fig. 4.2). Furthermore, population density in the *Z. noltii* habitat was significantly higher than in all other habitats (151 mean ind.m^{-2}). However, both *Z. noltii* and coarse mud habitats, revealed a significant effect of site on population density ($p < 0.001$ in both cases). Nevertheless, both sites of the *Z. noltii* habitat showed more than twice (165.38 and 136.12 ind.m^{-2}) the average density of *C. maenas* on the clay mud habitat (65.63 ind.m^{-2}).

All habitats were characterized by a specific population size-structure ($\chi^2 > 100$ df = 10, $p < 0.05$) similar at respective station and site sampling levels ($\chi^2 < 15$ df = 10, $p > 0.75$). A common aspect to all habitats was that the relative frequency of the size classes decreased consistently at least after 6 mm CW (Fig. 4.3). Juveniles of 3 to 6 mm CW dominated size-structures in both *Z. noltii* and clay mud habitats (59 and 50 %, respectively) (Fig 4.3).

Table 4.1. Results of ANOVA analyses performed to evaluate the effects of habitat, site and station on density and RNA/DNA ratio of *Carcinus maenas* populations. Significant p-values in bold.

	Density				RNA/DNA			
Source of variation	df	MS	*F*	p	df	MS	*F*	p
Habitat	4	10^5	191.22	**< 0.001**	4	78.45	117	**< 0.001**
Site (Habitat)	5	8355	15.94	**< 0.001**	5	0.59	0.89	0.50
Station (Habitat x Site)	10	897	1.71	0.07	10	0.55	0.83	0.60
Error	380	524			260	0.67		

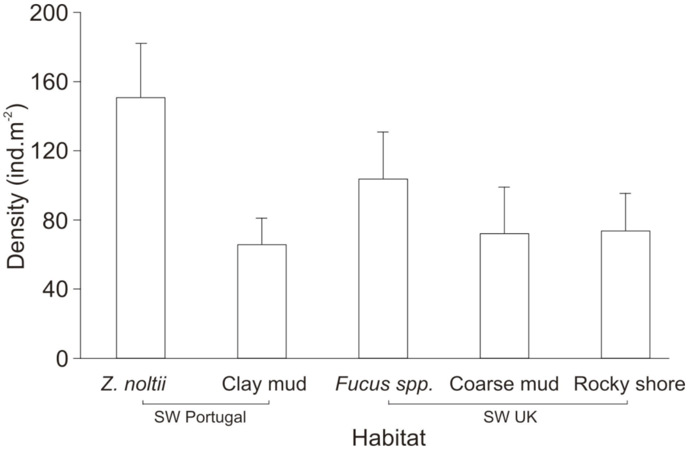

Fig. 4.2. *Carcinus maenas*. Mean population density in five different habitats. Error bars: SE.

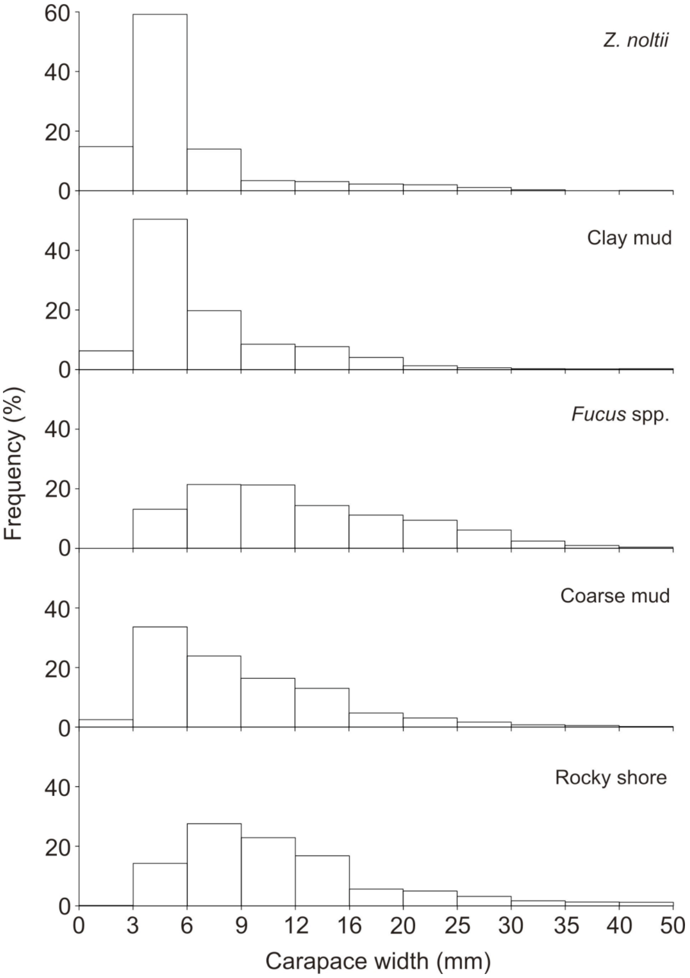

Fig. 4.3. *Carcinus maenas*. Frequency distribution of CW in the five different habitats.

That dominance was, however, more pronounced in the *Z. noltii* habitat, where new recruits (< 3 mm) were also relatively more abundant than in the clay mud habitat (Fig. 4.3). In the coarse mud habitat, new recruits occurred in lower frequencies and juveniles of 3 to 6 mm CW were also most abundant, with larger size classes gradually decreasing in abundance afterwards. In the *Fucus* spp. and rocky shore habitats, frequencies of juveniles with 3 to 6 and 6 to 9 mm CW were relatively similar and recently metamorphosed juveniles were virtually absent. In the *Fucus* spp. habitat, size classes of increasing CW declined gradually in abundance (Fig. 4.3).

ANOVA analysis revealed a significant effect of habitat on RNA/DNA ratio, but not of lower sampling levels (Table 4.1). RNA/DNA ratio was significantly different between habitats ($p < 0.001$ in all cases) with exception of both estuarine UK habitats (*Fucus* spp. and coarse mud), which showed the lowest ratios (3.6 and 3.3, respectively) (Fig. 4.4). Mean RNA/DNA ratio was significantly higher in the rocky shore habitat (6.0) than in all other habitats, followed by that of the *Z. noltii* and by that of the clay mud habitats.

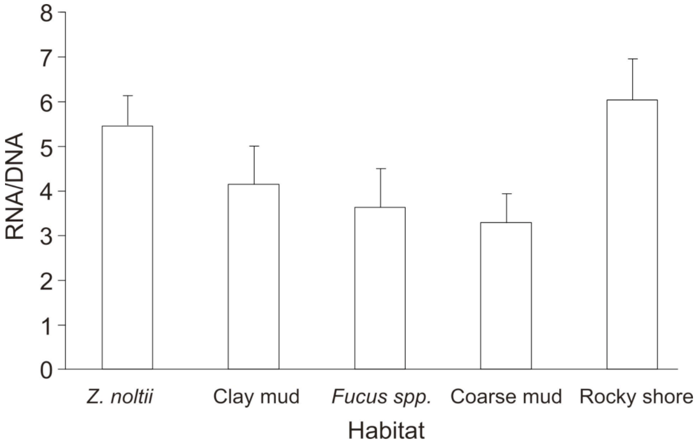

Fig. 4.4. *Carcinus maenas*. Mean RNA/DNA ratio in five different habitats (14 to 18 mm CW). Error bars: SE.

DISCUSSION

Habitats that enhance growth and survival of individuals are considered high quality habitats in relation to others, and estimation of growth, physiological condition and survival indices is assumed to reflect such quality. However, movements between habitats, a common behaviour in mobile marine species, might compromise this assumption. We dealt with such issues by using a short-term condition index (RNA/DNA), lowering the probability of the influence of other habitats on individuals studied.

Our results indicate that the quality of habitats used by *C. maenas* vary among habitats, and that different indices may result in different estimations of habitat quality. Contrary to population density and RNA/DNA ratio, which showed similarities between some habitats, the size-structure of shore crab populations was different among all habitats. In the southern area, population density in the *Z. noltii* habitat was more than twice that of the less structurally complex and unvegetated clay mud habitat; population size-structure revealed relatively higher frequency of small juveniles in both *Z. noltii* and clay mud habitats. There was lower relative frequency of larger individuals in the clay mud than in the *Z. noltii* habitats; and condition was significantly higher in the *Z. noltii* habitat. These results indicate *Z. noltii* habitat as that of higher quality in this southern area. Estuarine vegetated habitats (especially seagrass meadows) have been broadly recognized as higher quality recruitment and nursery areas, than adjacent unvegetated habitats, for juveniles of *C. maenas* (Moksnes 2004, Paula et al. 2006) and other marine benthic invertebrate and fish species (Orth & van Montfrans 1990, Polte et al. 2005, Fonseca et al. 2006). These habitats often present enhanced food and refuge availability, usually translated in higher growth and survival rates of individuals (Orth & van Montfrans 1990, Shervette & Gelwick 2007).

There is, however, growing recognition that unvegetated habitats might also be high quality habitats for several marine invertebrate and fish species, at least during part of the life-cycle (Diehl & Ekloev 1995, Lipcius et al. 2005). Furthermore, high population densities *per se* do not necessarily reflect a high habitat quality. The higher density on the *Z. noltii* habitat might have resulted from high abundances of young juveniles in a poor physiological condition (Amaral et al. in review). Habitats with higher densities of individuals of low physiological condition than adjacent ones, have been reported for several marine (Diehl & Ekloev 1995, Parslow-Williams et al. 2001, Moksnes 2004) and terrestrial mammal species (e.g. Wheatley et al. 2002).

In the northern area, the *Fucus* spp. habitat showed also significantly higher population density than the unvegetated coarse mud habitat; population structure revealed relatively lower frequency of early juveniles in the *Fucus* spp. habitat and of larger juveniles in the coarse mud habitat, with similar RNA/DNA ratio in crabs of both estuarine habitats. A population density similar to that of both non-vegetated estuarine habitats characterized the rocky shore habitat: juveniles dominated population structure, with a relatively higher frequency of larger animals in relation to other habitats. It also presented the overall highest physiological condition. A major unexpected finding was the appreciably highest RNA/DNA ratio in the rocky shore habitat, suggesting enhanced growth conditions in this habitat. Despite a relatively lower population density, the relatively higher quality of this habitat seems unequivocal. Several factors might account for this higher quality (Gibson 1994). Food and refuge quantity, quality and availability may be higher in the wave-exposed habitat. Higher quality of wave-exposed coastal habitats for several fish and marine invertebrate species has previously been reported, at least during some part of the life-cycle (Dahlhoff et al. 2001). On the other hand, estuarine environments are characterized by relatively higher temperature and salinity variations than adjacent coastal areas, causing higher osmotic and metabolic stress. Our results furthermore suggest that, in the northern area, both estuarine habitats have similar quality for shore crabs, notwithstanding the possible shift in their relative importance throughout ontogenesis. The notion that structurally complex vegetated habitats do not always have higher quality than less complex adjacent habitats has been supported in several studies (see Introduction).

No clear latitudinal trends in the quality of different estuarine habitats for the shore crab are apparent from our results. A similar pattern has been reported for the estuarine crab *Chasmagnatus granulatus* in South America (Bas et al. 2005). Nevertheless, considering the shore crab distribution in rocky shore areas and respective habitat quality, estuarine habitats at the southern area (SW Portugal) seem suboptimal to *C. maenas* compared to northern areas (SW UK), particularly rocky shores. The lack of abundant *C. maenas* populations in rocky shore habitats in the southern area cannot accordingly be attributable to low habitat quality in terms of environmental conditions, as these are similar to those further north. Furthermore, no significant genetic differences between populations of these regions have been found (Roman & Palumbi 2004). Such difference may, on the other hand, result from biological interactions. The existence of highly aggressive top-predator crab species in rocky shore habitats at the southern area, e.g. *Pachygrapsus marmoratus* and *Eriphia verrucosa* (Orth & van Montfrans 1990, Flores & Paula 2001, Cannicci et al. 2007), but not at the northern area, may limit the establishment of shore crab populations. Similar biotic resistance by native predator crabs, limiting the population abundance and geographic range of invasive shore crab, have been reported on the east coast of the United States by *Callinectes sapidus* (deRivera et al. 2005) and in the

Northeastern Pacific by *Cancer* spp. (Jensen et al. 2007).

In summary, our results suggest that vegetated estuarine habitats of high structural complexity, such as seagrass meadows, do not always provide higher quality than adjacent less structurally complex estuarine and rocky shore habitats. In fact, at the northern area, the rocky shore habitat showed highest quality in relation to vegetated and non-vegetated estuarine habitats. In terms of latitudinal differences, estuarine environments seem to provide sub-optimal quality habitats in the southern area, where the lack of established abundant populations might result from biotic resistance by other predator crabs, absent from the northern area. Habitat quality might change with ontogenetic development of the shore crab i.e., some habitats could function as quality nursery and recruitment areas, while others may act as quality habitats for later juvenile growth and reproduction. This shift in habitat quality is not surprising, as shore crabs, as other marine invertebrates and fishes, are characterized by habitat shifts throughout ontogenesis (Bystrom et al. 2003, Moksnes 2004). Management and conservation programmes should consider other habitats, in addition to the classic nearshore vegetated ones, as potentially high quality areas for mobile marine species.

ACKNOWLEDGEMENTS

We are in debt to A. Silva, A. Smith, I. Silva, G. Budd, L. Noel, M. Lilley, P. Masterson, P. Ribeiro, P. Rendle and, specially, J. Carvalho for their help with fieldwork. We acknowledge a PhD grant to V.A. (SFRH/BD/10471/2002) funded by Fundação para a Ciência e a Tecnologia (FCT). All work complied with Portuguese and UK government laws.

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CHAPTER 5

Cannibalistic interactions in two sympatric decapod species: effects of density, food, alternative prey and habitat

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J Exp Mar Biol Ecol (in review)

Cannibalistic interactions in two co-occurring decapod species: effects of density, food, alternative prey and habitat

ABSTRACT

Cannibalism is an intraspecific intra and inter-age class process that can structure and regulate populations in concert with other abiotic and biotic factors. *Cancer pagurus* and *Porcellana platycheles* are two co-occurring decapods on Atlantic rocky shores. In controlled laboratory experiments, we investigated their intra and intersize class cannibalistic behaviour and interspecific predatory interactions, evaluating the effects of prey and predator densities, food, starvation, alternative prey and habitat type. Only residual levels of aggressive competition were detected in *P. platycheles*, suggesting a non-aggressive intraspecific behaviour. *Cancer pagurus* heavily preyed on *P. platycheles*. Among *C. pagurus* juveniles of similar size, cannibalism was low and not affected by density, food or alternative prey. The costs of preying on a sibling juvenile may be too high, unless prey is vulnerable (i.e. at moulting). Intersize class cannibalism was high and influenced by prey and predator densities. With one predator, prey mortality increased with prey density at a decelerating rate, while with two it was inversely density-dependent. Strong predator interference was detected, which diminished prey consumption per capita. Food supply, alternative prey (*P. platycheles*) and habitat type significantly affected cannibalism rates. Small pebble and *Fucus* spp. habitats significantly reduced prey mortality in relation to sand and large pebble habitats. The combination of food and *Fucus* spp. habitat yielded 100 % prey survival.

KEYWORDS: *Cancer pagurus*, Density-dependent, Intraspecific predation, Marine, Mutual interference, Population dynamics, *Porcellana platycheles*

INTRODUCTION

The process of both killing and eating an individual of the same species, known as cannibalism or intraspecific predation, has in the past been considered a rare event in ecology, limited to confined, very dense or food limited populations. Only in recent decades has it been recognized as a normal and widespread response in the animal kingdom to several abiotic and biotic factors (e.g. Fox 1975, Polis 1981). Cannibalism has gained such recognition due to its potential importance in population regulation, generation of fluctuations in population structure and role in stock-recruitment relationships (Fox 1975, Polis 1981, Sainte-Marie & Lafrance 2002, Moksnes 2004, Wise

2006).

Cannibalism is generally recognized as a density-dependent event, capable of population regulation through three different mechanisms: intra and intersize class cannibalism and predator numerical effect on prey (Fox 1975, Polis 1981, Smith & Reay 1991, Moksnes 2004). Furthermore, cannibalism intensity and thus, population dynamics, can be significantly affected by predator-prey size ratio, food availability, predator starvation, alternative prey and habitat type (e.g. Fox 1975, Polis 1981, Smith & Reay 1991, Rickers & Scheu 2005, Wise 2006). Our understanding of the significance of cannibalism mainly results from the combined information of numerous different studies, each focusing separately on a few of these factors. This study evaluates the effects of prey and predator densities, food availability, predator starvation, alternative prey and habitat type on cannibalistic interactions in two coexisting species.

The edible crab *Cancer pagurus* (L.) (Custacea: Decapoda) is a large and active omnivorous predator inhabiting both hard and soft substrates from the low intertidal fringe to about 100 m offshore (Stevcic 1988). It occurs in the Northeastern Atlantic and Mediterranean Sea (Stevcic 1988, Lawton 1989). It constitutes an important fishery and attempts have been made to mass culture this species (e.g. Woll et al. 2006). Nevertheless, little data are available on the ecology of the edible crab, in particular regarding its cannibalistic behaviour.

The broad-clawed porcelain crab *Porcellana platycheles* (Pennant, 1777) (Custacea: Decapoda) is a conspicuous, small, and mainly microphagous filter feeder (Stevcic 1988). It co-occurs with *C. pagurus*, usually being found under boulders and cobbles in the low intertidal and shallow subtidal zones. It occurs from the Shetlands to the Canary Islands and the Mediterranean Sea (Smaldon 1972). Despite its feeding habits, the large cheliped size suggests that aggressive competition may occur among conspecifics. The large chelipeds are also important to resist predation, namely from *C. pagurus*, for which it is a preferred prey (Lawton 1989).

A series of laboratory experiments were conducted in flow-through mesocosms, to study both cannibalistic and interspecific interactions, leading to proportional appendage loss, prey mortality and prey consumption, in two co-occurring species, *C. pagurus* and *P. platycheles*, whose feeding and behaviour patterns largely differ. In particular, we addressed the following: (1) Is intrasize class cannibalism in *C. pagurus* determined by density, food supply or lack of alternative prey? (2) Is intrasize class cannibalism and predation in *P. platycheles* dependent on density and food supply? (3) Can intersize class cannibalism on both species be controlled by prey and predator density? And (4) which are the effects of food, alternative prey and habitat type on such cannibalistic behaviour?

MATERIALS AND METHODS

The experiments addressed three main aspects: (1) the effects of density, food and alternative live prey on intrasize class cannibalism; (2) the effects of food on juvenile interspecific predation; and (3) the effects of cannibal and prey densities, food, predator starvation, alternative prey, and habitat on intersize class cannibalism rates.

General procedures

Laboratory experiments were conducted during May and June 2007, at the Marine Biological Association, located in Plymouth (50°18-22' N, 4°5-13' W), on the southwestern UK coast. Field studies were made on adjacent shores (see Marine Biological Association 1957, for details).

Animals and habitats used were collected during low tides in intertidal areas of four different coastal sites. Only areas occupied by both species were considered (i.e. areas with boulders and pebbles). The natural densities of both species were estimated from 15 quadrats (0.5 x 0.5 m) per collection site, randomly deployed in the low intertidal, from where individuals were enumerated.

Low, medium and high densities of *C. pagurus* and *P. platycheles* (2, 4 and 6 and 2, 6 and 18 juveniles per tank, respectively) were used in the experiments representing natural juvenile densities, on areas with boulders and pebbles, during the spring season. The same rationale was used with low and high adult densities for both species (1 and 2 adults, respectively). Juvenile and adult *C. pagurus* (20-27 and 85-95 mm carapace width (CW), respectively) and *P. platycheles* (7-9.5 and 12-13.5 mm CW, respectively) were collected.

All experimental treatments were run in triplicate (n = 3) and conducted in small flow-through mesocosms: rectangular shaped tanks with round corners (0.27 x 0.27 x 0.18 m; 13 L), with a 2 cm deep layer of sieved (<1.0 mm) sun dried beach sand. This sand layer allowed complete burrowing of juvenile crabs of both species, and was also supplied with mussel shell debris of various sizes to provide a more natural environment. Running (~1.5 $L.min^{-1}$) natural and filtered (<750 µm) seawater from the Plymouth Sound entered at the bottom and left each tank at the surface. Water temperature varied between 15 and 17 °C and salinity between 32 and 36. Photoperiod was provided by an artificial light source giving 16 h light: 8 h dark, approximating the natural conditions in southwest UK.

Animals were kept in flow-through 120 L containers and fed fresh blue mussel, fish and shrimp for at least 5 d before being used. Adults further maintained without food for 3 d were considered "starved" predators. Unless stated otherwise, excess food (fresh blue mussel, fish and shrimp) was provided every day, and uneaten remains were siphoned out daily. Adult predators were allowed to adapt to the mesocosms for 8 h before prey were added. Habitats used in respective experiments (pebble and *Fucus* spp., but not sand) were rinsed in saltwater and cleaned of epiphytes and fauna prior to use. Upon termination of each trial, habitats were carefully removed from the tanks and surveyed for animals. Animals were used only once, and fed for 2 d before release in their original natural habitats.

ANOVA models were used to study intrasize class cannibalism and interspecific predation, with proportional mortality (number of crabs missing / total number) and proportional appendage loss (number of appendage missing / total number) as dependent variables, and also to assess intersize class cannibalism using proportional prey mortality (number of prey missing / total number) as the depend variable. Cochran's tests revealed homoscedasticity of variances, and Shapiro-Wilk's tests revealed normality of residuals in all analyses. *A posteriori* comparisons among factors were performed by Tukey's tests of honest-significant-differences (HSD) and Dunnett's tests.

Intrasize class cannibalism in *C. pagurus*: effects of density, food and alternative prey

To test whether density could regulate intrasize class cannibalism, a 15 d experiment was conducted using low (2 ind.tank^{-1}), medium (4 ind.tank^{-1}) and high (6 ind.tank^{-1}) *C. pagurus* juvenile densities (Experiment 1). Density was used as the independent variable in one-way ANOVA.

In Experiment 2 we studied the effects of food supply and the presence of *P. platycheles*, as alternative live prey, on cannibalistic responses of *C. pagurus* juveniles. A 5 d experiment was conducted using only medium juvenile densities in four different treatments: (1) juvenile *C. pagurus* with food; (2) juvenile *C. pagurus* without food; (3) juvenile *C. pagurus* and *P. platycheles* with food; and (4) juvenile *C. pagurus* and *P.*

platycheles without food. Food supply and alternative live prey were used as factors in two-way ANOVA analysis. The addition of alternative prey resulted in a crab density increase, potentially causing a confounding factor. However, Experiment 1 revealed that this was likely not the case, as intrasize class cannibalism in *C. pagurus* was independent of density (see Results).

Intrasize class cannibalism and predation in *P. platycheles*: effects of density and food

To test whether density could regulate intrasize class cannibalism, a 15 d experiment was run comparing low (2 ind.tank^{-1}), medium (6 ind.tank^{-1}) and high (18 ind.tank^{-1}) *P. platycheles* juvenile densities (Experiment 3). Density was used as the independent variable in one-way ANOVA.

The effects of food supply and juvenile *C. pagurus*, as potential predators, on *P. platycheles* survival were assessed in a 5 d experiment (Experiment 4) using medium juvenile densities in four different treatments: (1) juvenile *P. platycheles* with food; (2) juvenile *P. platycheles* without food; (3) juvenile *P. platycheles* and *C. pagurus* with food; and (4) juvenile *P. platycheles* and *C. pagurus* without food. Food supply and presence of juvenile predators were used as factors in two-way ANOVA. A potential confounding factor in this experiment is the increase in crab density with the addition of juvenile *C. pagurus* predators. However, Experiment 3 revealed that this was likely not the case, as cannibalism in *P. platycheles* was not detected irrespective of density (see Results).

Intersize class cannibalism: effects of prey and predator density

To study the effects of predator and prey density on incidence of intersize class cannibalism, 80 h experiments were ran independently for each species (Experiments 5 and 6, for *C. pagurus* and *P. platycheles*, respectively), using all combinations of low (2 ind.tank^{-1}), medium (4 or 6 ind.tank^{-1}, respectively) and high (6 or 18 ind.tank^{-1}, respectively) juvenile prey densities and one or two conspecific adult predators (6 treatments). Control trials were ran with the same prey densities, but without predators. Prey and predator densities (including control) were used as factors in two-way ANOVA, in relation to prey proportional mortality. Prey consumption rate (number of prey missing / number of predators) was used as dependent variable in two-way ANOVA analyses, using prey and predator densities as factors, but excluding control treatments. The addition of predators increased stock density, possibly causing a confounding factor, which was not addressed as these experiments were about densities, rather than stock density. Analyses were conducted independently for each species. No intersize class cannibalism was detected in *P. platycheles* (see Results), and no further experiments were conducted on this species.

Intersize class cannibalism in *C. pagurus*: effects of food, alternative prey and habitat type

To test whether intersize class cannibalism in *C. pagurus* could be an artefact caused by predator starvation, an 80 h experiment (Experiment 7) was conducted using medium density of juvenile *C. pagurus* and one predator in four different treatments: (1) fed predator without food during the trial; (2) starved predator with food during the trial; (3) starved predator without food during the trial; and (4) control, fed predator with food during the trial. Predator starvation state and food supply were used as factors in two-way ANOVA.

To evaluate the effects of alternative live prey and food supply, another 80 h experiment (Experiment 8) was conducted with medium densities of juvenile *C. pagurus* and *P. platycheles* (alternative prey) and one predator per tank, in four different treatments: (1) juvenile *C. pagurus* with food; (2) juvenile *C. pagurus* without food; (3) *C. pagurus* and *P. platycheles* juveniles with food; and (4) *C. pagurus* and *P. platycheles* juveniles without food. Food supply and alternative live prey were used as factors in two-way ANOVA. As in Experiment 2, the potential confounding factor caused by the addition of alternative prey was likely not relevant, as intrasize class cannibalism in *C. pagurus* was independent of density (Experiment 1, see Results).

A final experiment was performed to study the effects of habitat type and food on intersize class cannibalism in *C. pagurus* (Experiment 9). An 80 h experiment was conducted using medium density of juvenile *C. pagurus* and one predator per tank in four different habitats: sand; small pebbles (8-12 mm long); large pebbles (16-22 mm long); and *Fucus* spp.. Each habitat was tested with and without food supply (8 treatments). Habitats were laid on 2 cm of sieved sand. Habitat type and food supply were used as factors in two-way ANOVA.

RESULTS

Intrasize class cannibalism in *C. pagurus*: effects of density, food and alternative prey

Cannibalism was independent of density (Experiment 1), with an average of ~12 % of both proportional mortality and appendage loss (ANOVA: *F* = 1.26, df = 2, p = 0.35, in both cases). Only crabs that moulted were cannibalized. Experiment 2 revealed no effects of food supply, alternative live prey (*P. platycheles*) and interaction (Table 5.1), and both juvenile proportional mortality and appendage loss remained below 19 % in all treatments.

Intrasize class cannibalism and predation in *P. platycheles*: effects of density and food

Cannibalism was not detected (Experiment 3). Proportional mortality and appendage loss were identical, low (~2 %) and independent of juvenile density (ANOVA: *F* = 1.00, df = 2, p = 0.42, in both cases). *Cancer pagurus*, food supply and interaction (Experiment 4) significantly affected survival and appendage loss of *P. platycheles* juveniles (Table 5.1). Mortality was significantly higher when predators were present and no food was supplied (67 %, HSD p < 0.01, in all cases), being similar among the other treatments (HSD p > 0.2, in all cases). Both with and without food supply, appendage loss was significantly higher when predators were present (36 and 68 %, respectively, HSD p < 0.05, in all cases), being similar among treatments with no predators (HSD p > 0.07, in all cases).

Table 5.1. Results of two-way ANOVA analyses on the effects of food supply and presence of other crab species on intrasize class cannibalism, as proportional mortality and appendage loss on *C. pagurus* (Experiment 2) and *P. platycheles* (Experiment 4). Significant p-values in bold.

	Proportional mortality				Proportional appendage loss			
C. pagurus	df	MS	*F*	p	df	MS	*F*	p
Food (A)	1	0.01	0.50	0.50	1	0.001	0.08	0.78
Alternative prey (B)	1	0.05	4.50	0.07	1	0.48	4.75	0.06
A x B	1	0.01	0.50	0.50	1	0.01	1.06	0.33
Error	8	0.01			8	0.01		
P. platycheles	df	MS	*F*	p	df	MS	*F*	p
Food (A)	1	0.17	16.76	**< 0.01**	1	0.11	10.96	**< 0.05**
Predator (B)	1	0.42	41.71	**< 0.001**	1	0.55	56.12	**< 0.001**
A x B	1	0.10	9.70	**< 0.05**	1	0.05	5.40	**< 0.05**
Error	8	0.01			8	0.01		

Intersize class cannibalism: effects of prey and predator density

No mortality and appendage loss were recorded in *P. platycheles* (Experiment 5). In contrast, intersize class cannibalism was common among *C. pagurus* and, despite not affected by prey density, prey mortality was significantly affected by predator density and interaction (Table 5.2) (Experiment 6). Overall, prey mortality was only higher than control at high predator density (35 %, HSD $p < 0.01$). At high predator density, prey mortality was inversely density-dependent, decreasing significantly from low to medium prey density (from 67 to 25 %, HSD $p < 0.05$), but not further on (13 %, HSD $p = 0.97$) (Fig. 5.1A). At low predator density, prey mortality increased significantly from low to medium prey density (from 0 to 50 %, HSD $p < 0.01$), decreasing afterwards, but not significantly (29 %, HSD $p = 0.579$), possibly indicating satiation.

Prey consumption was significantly affected by prey (ANOVA: $F = 2.04$, df = 2, $p < 0.001$) and predator densities (ANOVA: $F = 3.56$, df = 1, $p < 0.001$) and interaction (ANOVA: $F = 2.76$, df = 2, $p < 0.001$) (Experiment 6). Overall, consumption rate was higher with one than with two predators (1.44 and, respectively, HSD $p < 0.001$). With one predator, prey consumption increased significantly from low to medium prey densities (0 to 2, HSD $p < 0.001$), but not further on (2.3, HSD $p = 0.643$) (Fig. 5.1B). With two predators, prey consumption was similar among prey densities (0.50 to 0.67, HSD $p > 0.967$, in all cases) (Fig. 1B).

Table 5.2 Results of two-way ANOVA analyses on the effects of prey and predator densities, predator starvation and food supply, alternative prey and food supply, and habitat type and food supply on intersize class cannibalism in *C. pagurus*, as proportional mortality (Experiments 6 to 9). Significant p-values in bold.

	Experiment 6				Experiment 7			
	Prey (A) & predator (B) densities				Starvation (A) & food (B)			
Factor	df	MS	*F*	p	df	MS	*F*	p
A	2	0.01	0.51	0.61	1	0.05	4.50	**< 0.07**
B	2	0.11	5.82	**< 0.05**	1	0.42	40.50	**< 0.001**
A x B	4	0.23	11.54	**< 0.001**	1	0.13	12.50	**< 0.01**
Error	18	0.02			8	0.01		
	Experiment 8				**Experiment 9**			
	Alternative prey (A) & food (B)				Habitat type (A) & food (B)			
Factor	df	MS	*F*	p	df	MS	*F*	p
A	1	0.63	40.33	**< 0.001**	3	0.35	22.44	**< 0.001**
B	1	0.05	3.00	0.12	1	0.42	2.67	0.12
A x B	1	0.01	0.33	0.58	3	0.09	5.78	< 0.01
Error	8	0.02			16	0.02		

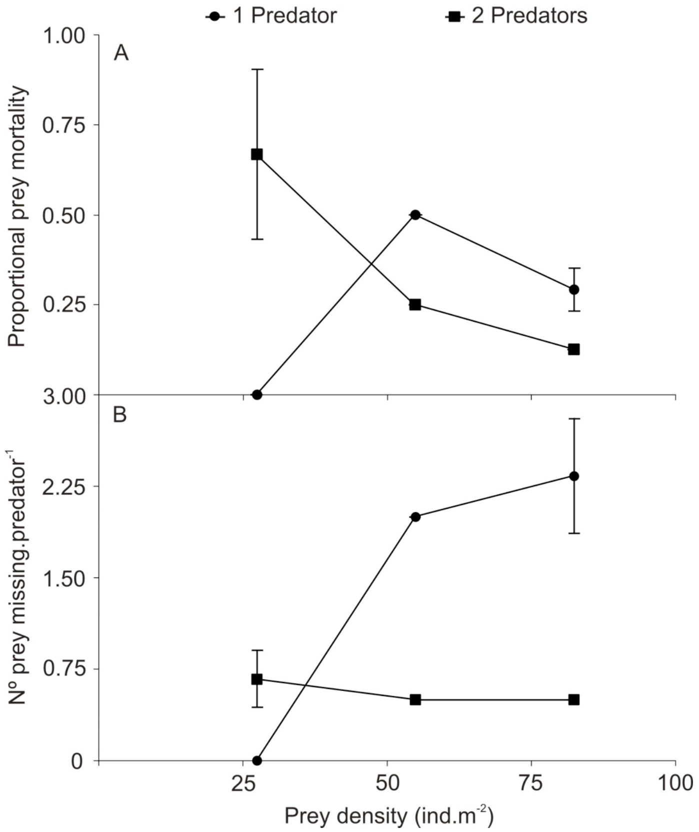

Fig. 5.1. Intersize class cannibalism of *C. pagurus*. Effects of predator and prey densities (A) as proportional prey mortality and (B) prey consumption rates. Error bars: ±SE.

Cannibalism in *C. pagurus*: effects of food, alternative prey and habitat type

Predator starvation did not have significant effects on proportional prey mortality, which, however, was significantly affected by food supply and interaction (Table 5.2) (Experiment 7). Prey mortality was significantly higher in treatments with starved predators not fed during the trials than in control (100 %, Dunnett $p < 0.001$), and was similar among the other treatments (42-67 %, Dunnett $p > 0.2$, in both cases).

The presence of *P. platycheles* significantly decreased mortality of juvenile *C. pagurus* (from 58 to 13 %, HSD $p < 0.001$), without any effect of food supply (Table 5.2) (Experiment 8). Experiment 9 revealed that habitat type and interaction with food supply significantly effected proportional prey mortality (Table 5.2). Sand and large pebble habitats showed similar proportional prey mortality (58 and 50 %, respectively, HSD $p = 0.663$), which were significantly higher than that on the *Fucus* spp. and small pebble habitats (HSD p < 0.05, in all cases) (Fig. 5.2). The overall lowest proportional prey mortality was attained in the *Fucus* spp. habitat (4 % HSD p < 0.05, in all cases), which yielded 100 % prey survival when food was provided.

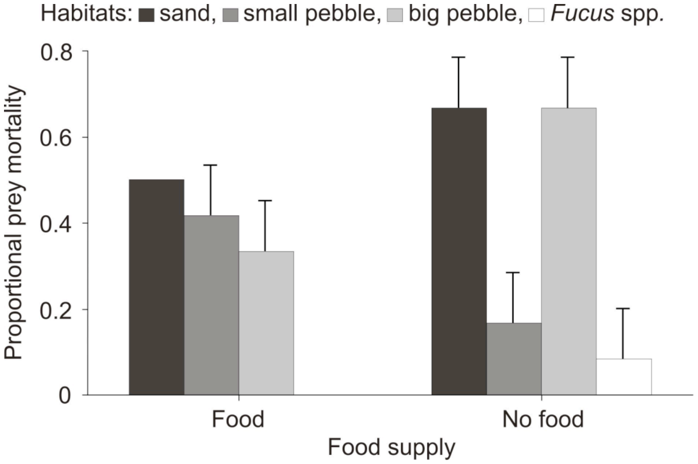

Fig. 5.2. Results of the effects of habitat type and food on intersize class cannibalism of *C. pagurus* as proportional prey mortality. Error bars: ±SE.

DISCUSSION

The complex behaviours underlying different cannibalistic functional response types include activity of predators, search time, handling time and digestion of the prey, as well as possible agonistic interactions between predators interacting with these behavioural components. The assessment of such responses can be accurately achieved in laboratory experiments, in which the experimental setup allow all those behaviours room to act in an environment that, as much as possible, represents the natural habitat. In the present study, a variety of factors were addressed always attempting to reproduce a natural environment, while manipulating specific factors. Nevertheless, mesocosm experiments are always constrained by space availability and caution must be taken in extrapolating results and drawing conclusions on the effects of cannibalism in natural populations. On the other hand, such extrapolation and conclusions may be extremely useful and more directly applied for aquaculture populations.

Cannibalism has been pointed as the main cause of juvenile mortality in size-structured populations of crabs, and affects several other aspects of population dynamics (Moksnes 2004). Only low levels of aggressive interaction were detected among *P. platycheles* during our experiments. *Cancer pagurus* showed clear cannibalistic behaviours, more intense among individuals of different sizes. Prey and predator density, food, alternative prey as well as habitat type influenced such behaviours. *Cancer pagurus* predation on *P. platycheles* was relatively intense, and also affected by food availability.

Intrasize class cannibalism

In addition to the somehow expected absence of cannibalism among *P. platycheles*, due to its filter-feeding habits, no clear evidence of aggressive competition has been detected, independently of juvenile density. In the wild, *P. platycheles* were usually found at high densities hiding under rocks, which together with our results, suggests a non-aggressive behavioural pattern.

Cannibalism among juvenile *C. pagurus* was low and not significantly affected by density, food or the presence of *P. platycheles*. Cannibalism is generally considered a size-dependent process, and thus more intense among individuals of different size (Fox 1975, Polis 1981, Fernandez 1999, Wise 2006). Rejection of conspecific prey also has been suggested in *C. pagurus* (Lawton 1989). Furthermore, only crabs in moult stage were cannibalized, suggesting that sibling juvenile *C. pagurus* do compete for resources, but the costs of cannibalism may be too high, unless the prey presents some vulnerability (Fox 1975, Polis 1981, Moksnes et al. 1997, Wise 2006). The 5 d duration of our experiment (Experiment 2) may have contributed to the lack of strong effects of food supply and alternative live prey on intrasize class cannibalism, but we feel it was an appropriate amount of time in relation to natural conditions.

Interspecific juvenile predation

In comparison with the literature on crustacean predation on molluscs, predatory interactions among decapod crustaceans are scarce. Juvenile *P. platycheles* were heavily preyed upon by *C. pagurus*. Despite proportional appendage loss of porcelain crabs have been significantly higher in the presence of *C. pagurus*, and regardless of food supply, their mortality was only significantly higher when no food was provided. In accordance with Lawton (1989), these results suggest that juvenile edible crabs preferentially prey upon broad-clawed porcelain crabs than upon conspecifics.

Intersize class cannibalism

Despite the size asymmetry and predator density, no aggressive interaction was detected among *P. platycheles*. Together with the results from the experiment on intrasize class cannibalism, this suggests that the large and broad chelipeds might serve as protection exclusively for interspecific predation (Stevcic 1988, Mansour & Lipcius 1991).

On the contrary, *C. pagurus* showed intense intersize class cannibalism, with significant effects of both prey and predator densities. With one predator, prey mortality increased with prey density, approaching saturation at high prey density, which resembles a type III predator functional response (Holling 1959, Lipcius & Hines 1986). With two predators, prey mortality was inversely density-dependent, resembling a type II functional response, with a risk of local prey extinction at low prey densities (Hassel 1978, Lipcius & Hines 1986). The form of the predator functional response to prey density is known to vary with environmental conditions (Murdoch & Oaten 1975, Hassel 1978), and a shift from types III to II has been reported for blue crabs as a result of a decrease in habitat heterogeneity (Lipcius & Hines 1986, Moksnes et al. 1997). However, the effects of variable predator densities are somehow unknown (but see Moksnes et al. 1997).

The shift in predator response when its density doubled, might have resulted from enhanced encounter rates (Hassel 1978). In fact, a significantly lower *per capita* consumption rate (~60 %) was detected at high than at low predator density, suggesting mutual interference. Interference competition among predators is a common process (Fox 1975, Polis 1981), being usually associated with agonistic behaviours in brachyuran crabs (e.g. Moksnes et al. 1997, Fernandez 1999, Moksnes 2004). Therefore, and in addition to environmental conditions, predator density can affect predator response to prey density, because of predator interactions.

Feeding history of predators has been mostly disregarded in cannibalism studies, even though it is generally recognized that starved individuals present increased propensity to cannibalize (Fox 1975, Polis 1981, Smith & Reay 1991, Wise 2006). Predator starvation *per se* had no significant effect on prey mortality, which has been reported also for shore crabs (Moksnes et al. 1998). However, when associated with no food supply during the trials, it resulted in 100 % prey mortality. On the other hand, food supply alone significantly decreased prey mortality, which is generally observed in cannibalistic interactions (Fox 1975, Polis 1981, Smith & Reay 1991, Wise 2006).

The presence of *P. platycheles*, as alternative prey, significantly reduced intersize class cannibalism among *C. pagurus*, especially when no other food source was available (~78 %). In fact, cannibals of several species, including crabs, prey preferentially on alternative prey then on conspecifics (Fox 1975, Polis 1981, Smith & Reay 1991, Rickers & Scheu 2005). Altogether, this suggests cannibalism avoidance behaviours in *C. pagurus*, as previously observed by Lawton (1989). Nevertheless, because the size of porcelain crabs was much smaller than of *C. pagurus* juveniles, the different predation rates might have also resulted from the fact that smaller prey was easier to catch and handle. Furthermore, *C. pagurus* predation upon *P. platycheles* was lower in presence of alternative food, indicating that *C. pagurus* might choose the most cost effective prey. These explanations are not mutually exclusive.

Habitats of higher structural complexity are usually recognized as providing more valuable refuges to prey (Fox 1975, Polis 1981, Smith & Reay 1991, Wise 2006, Amaral et al. in press). Prey mortality was significantly reduced in the more structurally complex small pebble and *Fucus* spp. habitats. In fact, no mortality was observed when food was supplied in the *Fucus* spp. habitat. *Cancer pagurus* predators sought refuge under pebbles in the respective habitat, dislodging and cannibalizing smaller conspecifics. In the *Fucus* spp. habitat, predators had movement restrictions, as their appendages usually got stuck between vegetation branches. Similar restraints on cannibal movement have been recorded for shore crabs in algae habitats (Moksnes et al. 1998). These results suggest that the small pebble and *Fucus* spp. habitats provided significant valuable refuges for prey, and that habitat type is more important than food supply for juvenile *C. pagurus* to escape intersize class cannibalism, at least for short periods.

In summary, our results indicate that *P. platycheles* is a non-cannibalistic species, which is heavily preyed upon by juvenile and adult *C. pagurus*. Intrasize class cannibalism among *C. pagurus* does not seem to be an important source of juvenile mortality or population regulation. On the contrary, intersize class cannibalism is affected by both prey and predator density, and might be an important population structuring and regulating mechanism. Doubling the number of *C. pagurus* cannibals can lead to local extinction of juvenile prey. Food supply, alternative prey and habitat structural complexity significantly reduced intersize class cannibalism. These seem promising results for *C. pagurus* aquaculture potential. Field validation of these results will help us clarify the role of cannibalism in edible crab population dynamics.

ACKNOWLEDGEMENTS

We are indebted to A. Silva for her help with crab collection. We acknowledge a PhD grant to V.A. (SFRH/BD/10471/2002) funded by Fundação para a Ciência e a Tecnologia.

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CHAPTER 6

RNA/DNA ratio of crabs as indicator of mangrove habitat quality

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Aquat Conserv (in review)

RNA/DNA ratio of crabs as indicator of mangrove habitat quality

ABSTRACT

Mangrove ecosystems suffer from severe cutting and deterioration, putting at risk their future persistence and that of local communities that depend on them. Pollution, namely wastewater discharge, is a major problem in these ecosystems, especially in peri-urban mangroves. Biochemical indicators of organism physiological condition have been widely used to evaluate habitat quality and early detect impact of stressors, improving the success of management and conservation plans. Grapsid and ocypodid crabs are potential good bioindicators of the quality of mangrove habitats, as they are important and characteristic species of such environments. The potential of RNA/DNA ratio of *Perisesarma guttatum* and *Uca annulipes*, as indicators of habitat quality, was studied in a peri-urban and two relatively pristine Mozambican mangroves, in the rainy and dry seasons. RNA/DNA ratio was significantly lower in *P. guttatum* than in *U. annulipes*. Such differences were due to higher DNA, rather than lower RNA cellular contents in tissue claw muscle of *P. guttatum*. Our results indicate that RNA/DNA ratio of *U. annulipes* may be a useful and good indicator of the quality of mangrove habitats in terms of pollution and seasonal fluctuation. Both species showed seasonal effects on RNA/DNA ratio, but only *U. annulipes* was significantly affected by pollution. These results suggest that the physiological condition of mangrove crabs was more affected by seasonal fluctuations than by pollution, especially on *P. guttatum.* We propose the RNA/DNA ratio of *U. annulipes* as a valid and sensitive bioindicator of quality of mangrove habitats.

KEYWORDS: Biochemical indicators, Environmental conditions, Mozambique, Nucleic acid, *Perisesarma guttatum*, *Uca annulipes*

INTRODUCTION

Mangroves are wetland ecosystems formed by the association of trees and shrubs and various animal taxa that thrive naturally in intertidal zones of brackish and marine coastal habitats (Kathiresan & Qaim 2005, Hogarth 2007). They are limited to zones of low wave energy at tropical and sub-tropical latitudes. Mangroves serve several important ecological functions e.g., they provide recruitment, nursery and feeding areas for many fish and invertebrate species, are a source of nutrients and organic matter to primary and secondary producers, prevent siltation of coral reefs, offer protection and stabilization of shorelines and also act as a sink for pollutants (Abuodha & Kairo 2001, Kathiresan & Qaim 2005, Hogarth 2007). Thus, they constitute coastal resources of economical relevance, especially for local communities, as they provide a variety of fishing products and areas, wood supply and other forestry products (Abuodha & Kairo 2001, Kathiresan & Qaim 2005, Hogarth 2007).

This potential for many uses, together with global increase in human populations, climate change and associated sea-level rise have led to severe clearance of mangrove areas for aquaculture, urbanization and costal landfill purposes, and to its deterioration due to direct and indirect effects of pollution and upstream land use (Kathiresan & Qaim 2005, Duke et al. 2007, Hogarth 2007). As mangrove areas become smaller, scattered and degraded, their medium and long-term persistence is at serious risk, jeopardizing essential ecological functions and associated resources, as well as the future of local communities (Alongi 2002, Kathiresan & Qaim 2005, Duke et al. 2007). Consequently, it is now unanimously agreed that there is an urgent need for effective policies, strategies and actions leading to sustainable maintain, protect, and restore these valuable wetland ecosystems (Duke et al. 2007).

Biochemical indicators of organism health and environmental quality of its habitats have become central in such an integrative approach (Dahlhoff 2004, Amaral et al. in press, Amaral et al. in review-a). They are key components of synthetic or metabolic pathways, reflecting metabolic activities directly or indirectly linked to growth, reproduction, survival, and thus physiological condition (Dahlhoff 2004). Biochemical changes usually occur before those in organism growth or reproduction become discernable, which may help early identification of effects of stressors, such as pollution, and thus contribute for preventing the loss of ecosystem functions. RNA/DNA ratio has been especially valuable as a biochemical estimator of organism condition in studies of marine invertebrates and fishes (e.g. Wright & Hetzel 1985, Gilliers et al. 2004, Amaral et al. in press, Amaral et al. in review-a). The principle is that RNA content correlates with new protein synthesis, usually interpreted as beneficial to the organism, reflecting active metabolic rates canalized to growth and reproduction. As DNA content remains relatively constant in an individual, as a function of chromosome number, higher RNA/DNA ratio is expected to arise when environmental conditions are favourable (Dahlhoff 2004).

Because the benthos integrates conditions over time, and the associated fauna is the main link between primary producers and secondary consumers, macrobenthic organisms are considered good indicators of environmental quality of its habitats (Smith et al. 1991, Lohrer et al. 2004, Pagliosa & Barbosa 2006). Crabs constitute an important macrofauna taxon in terms of abundance, species richness and biomass in mangrove ecosystems (Kathiresan & Qaim 2005, Hogarth 2007). Brachyuran crabs, particularly Grapsidae and Ocypodidae, further enhance ecosystem functioning, as their burrowing and feeding activities substantially improve biotic properties of the sediment, tree colonization, primary and secondary productivity, nutrient cycling and energy flow (e.g. Smith et al. 1991, Skov et al. 2002).

Despite the relevance of such crabs in mangrove ecosystems, and their potential as an evaluation tool of habitat quality, knowledge is lacking on their physiological responses to stressors, namely pollution and seasonal fluctuations. This study was therefore set up to investigate the bioindicator potential of the grapsid *Perisesarma guttatum* (A. Milne Edwards, 1869) and the ocypodid *Uca annulipes* (H. Milne Edwards, 1837) to evaluate habitat quality of polluted and non-polluted mangrove areas, in different climatic seasons, as measured through organism physiological condition. Another objective was to provide information on the physiological condition of those species, creating a baseline for future studies. RNA/DNA ratio in crabs of both species was assessed in peri-urban and relatively pristine mangrove systems at Mozambique, in the rainy and dry seasons.

MATERIALS AND METHODS

Sampling sites

This study was conducted in three different mangrove systems located in southern Mozambique, East Africa: a peri-urban mangrove (Costa do Sol) at Maputo Bay, on the mainland continent; and two relatively pristine mangroves (Saco and Ponta Rasa) at Inhaca Island (Fig. 6.1). All mangrove systems are located at similar latitudes within Maputo Bay, in a transitional region of tropical to warm subtropical climate, characterized by hot and rainy (September to March) and warm and dry (April to August) seasons, and where the tidal regime is typical semidiurnal (Kalk 1995).

Costa do Sol mangrove is characterized by a small and shallow seawater swamp located ~7 km north of Maputo city (25º55’ S, 32º35’ E). Maximum tidal amplitude is ~3.5 m, and water temperature and salinity vary from 18 to 35 ºC and 20 to 35, respectively (Litulo 2005, PUMPSEA 2007). The small seasonal river Quinhenganine discharges in to the swamp after passing, artificially canalized, through the city. *Avicennia marina* and small patches of *Rhizophora mucronata* dominate the vegetation (PUMPSEA 2007). The mangrove is bordered by a residential area, and has received domestic sewage, aquaculture residuals and solid dumps of various sources throughout the last decades (PUMPSEA 2007).

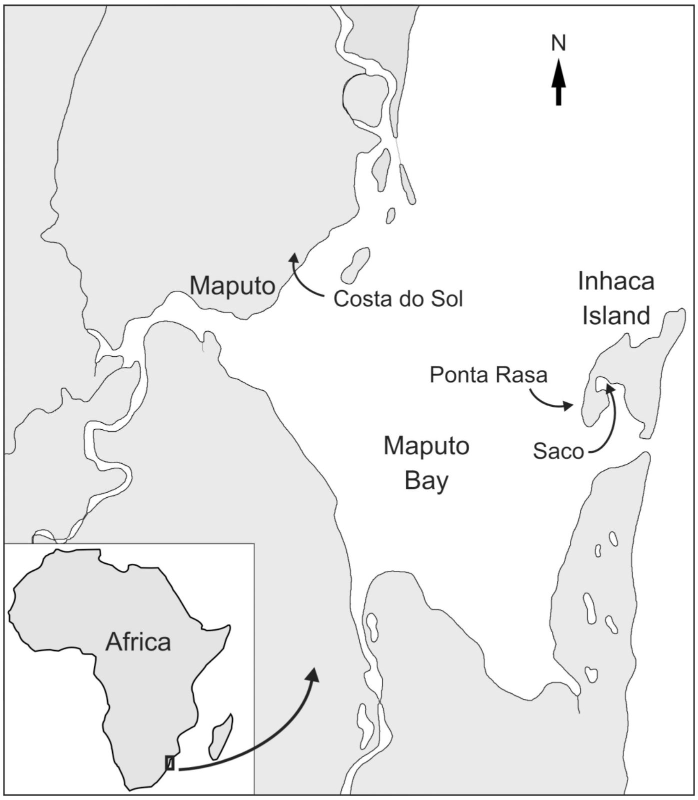

Fig. 6.1. Location of Costa do Sol, Ponta Rasa and Saco mangrove systems

Inhaca is a small island at ~32 km east of Maputo (26° S, 32º55' E), constituting a barrier between Maputo Bay and the Indian Ocean: the west and south coast are sheltered, while the east coasts are exposed to the Indian Ocean (Kalk 1995, Paula et al. 2003) (Fig. 6.1). Maximum tidal amplitude is ~3.7 m, and average water temperature and salinity vary between 20 and 33 °C, and 22 and 39, respectively (PUMPSEA 2007). No rivers are present, and freshwater supply results from diffused ground water flow and rainfall. There are few human communities in the south part of the island, and the absence of industries contribute to the persistence of relative pristine areas in relation to those of the mainland (Kalk 1995). Saco mangrove covers an area of ~2.1 km^2 and is located in a small, enclosed and shallow bay in the south of the island (Fig. 6.1). *Avicennia marina*, bordering the entire bay, and *R. mucronata*, *Ceriops tagal* and *Bruguiera gymnorhiza*, lining channel banks and creeks, dominate the vegetation (PUMPSEA 2007). Ponta Rasa mangrove is the smallest of the island, covering ~0.2 km^2, and is located in the southwest coast, facing Maputo Bay (Fig. 6.1). The creek is densely bordered by *R. mucronata*, sparser patches of *C. tagal* and *B. gymnorhiza* dominate higher areas of the mangrove, and the uppermost more sandy zone is characterized by *A. marina* (Paula et al. 2003).

Several pollution indicators have been recently studied in the three mangroves (PUMPSEA 2007). Higher concentrations of nutrients, especially nitrates and nitrites, characterize Costa do Sol in relation to Saco and Ponta Rasa mangroves. As a direct result, the benthic microalgae community is significantly more abundant in Costa do Sol. This mangrove system also presents much higher abundances of both total and faecal coliform bacteria, such as *Escherichia coli*, *Vibrio cholerae* and *Salmonella* spp., with a frequent occurrence of levels above those recommended (PUMPSEA 2007). In terms of granulometry, both Saco and Ponta Rasa mangroves are mainly formed by sandy sediments, while silt sediments dominate at Costa do Sol (PUMPSEA 2007). Altogether, these conditions allow us to discriminate between Costa do Sol, a peri-urban and more polluted ecosystem, and Saco and Ponta Rasa mangroves, relatively pristine ecosystems.

Field sampling

The physiological condition of the mangrove crabs *P. guttatum* and *U. annulipes* was evaluated by RNA/DNA ratio in both rainy (February) and dry (August) seasons, in the three mangrove systems. Crabs of both species are mainly active during low tides, usually foraging for food around the burrow that serves as refuge, and to which they quickly return when threatened by human presence (Skov et al. 2002). Only active individuals of *P. guttatum* and *U. annulipes* with dimensions corresponding to adult stages, were considered in this study: from 15 to 22 and 13 to 16 mm of carapace width (CW), respectively. At each season, the same habitat was sampled at the three mangrove systems: more landward zones characterized by the presence of *A. marina*, and where the different crab species form fairly monospecific crab assemblages that succeed each other throughout an intertidal fringe. Observing these conditions, sampling consisted in the haphazard collection of 40 to 50 adult male crabs of each species on the intertidal zone during morning low tides, in days of one spring tides period.

A general feature in crabs is a largest claw size in males than females, with a particular case occurring in species of the genus *Uca*, where males possess extreme claw asymmetry. We have chosen the muscle tissue of claws of male individuals of both species to determine the RNA/DNA ratio. Furthermore, as RNA/DNA ratio exhibit higher amplitude changes around moult events (Chang 1995), only individuals in intermoult, as evaluated *in situ* by the hardness of the carapace, were considered.

After collection, the largest claw of each individual was removed and transported to the laboratory in cool boxes. In the laboratory, claws were promptly freeze-dried and stored at -80 ºC until nucleic acid quantification. Claws of seven individuals of each species, season and mangrove system combination were then randomly selected for biochemical analysis.

Biochemical analysis

Analyses were performed on 10 - 15 mg (freeze-dried weight) of white claw muscle. RNA/DNA quantification was conducted following the Schmidt-Thannhauser method, according with the procedures of Amaral et al. (in review-a). Absorbance of nucleic acids was determined in a NanoDrop® ND-1000 full spectrum spectrophotometer (NanoDrop, USA), which ensures highly accurate analyses with high reproducibility (detection limits 2 - 3000, 3700 ng.µl^{-1} for RNA and double stranded DNA, respectively) of samples as small as 1 µl. Concurrently to samples analyses, the full spectrum feature of the NanoDrop® allows inspection of nucleic acid contaminations by estimation of 260/280 and 260/230 nm ratios. Three readings were performed per sample. All metal, plastic and glassware was autoclaved before used.

Statistical analysis

Effects of species, season and mangrove system on the physiological condition of mangrove crabs, as evaluated by RNA/DNA ratio, were estimated using three-way analysis of variance (ANOVA). Cochran's tests revealed homoscedasticity of variances, and Shapiro-Wilk's tests revealed normality of residuals. *A posteriori* comparisons were performed using HSD Tukey's tests.

RESULTS

ANOVA analysis (Cochran's $C = 0.245$, $p = 0.49$) revealed significant effects of species, season and mangrove system, and all interactions on the RNA/DNA ratios (Table 6.1). In both species, the overall ratio was significantly higher in the dry than in the rainy season (HSD $p < 0.001$, in all cases). In Ponta Rasa and Costa do Sol mangroves RNA/DNA ratios were also higher in the dry season (HSD $p < 0.001$, in both cases), while in Saco mangrove there were no differences among seasons (HSD $p = 0.76$) (Fig. 6.2).

Table 6.1. Results of three-way ANOVA analyses on the effects of species, season and mangrove system on RNA/DNA ratio of *Perisesarma guttatum* and *Uca annulipes*. Significant p-values in bold.

Source of variation	df	MS	*F*	p
Species (A)	1	42.34	841.66	**< 0.001**
Season (B)	1	7.56	150.27	**< 0.001**
Mangrove (C)	2	4.62	91.85	**< 0.001**
A x B	1	0.57	11.33	**< 0.01**
A x C	2	2.88	57.20	**< 0.001**
B x C	2	1.27	25.22	**< 0.001**
A x B x C	2	0.10	2.04	**< 0.001**
Error	72	0.05		

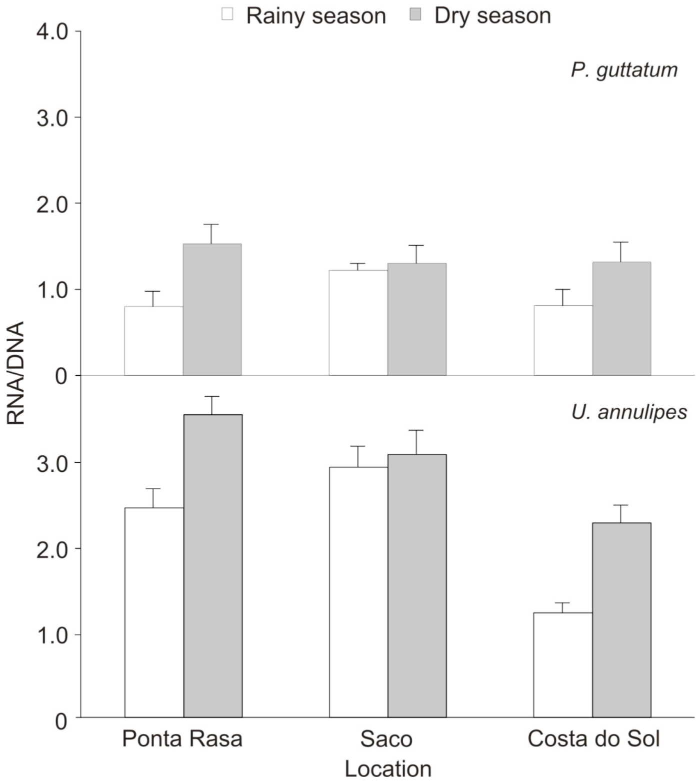

Fig. 6.2. Mean RNA/DNA ratio of *Perisesarma guttatum* and *Uca annulipes* in three different Mozambican mangrove systems, in the rainy and dry seasons. N = 42. Error bars: SE.

Overall, RNA/DNA ratio was significantly lower in *P. guttatum* than in *U. annulipes* (1.2 and 2.6, respectively, HSD $p < 0.001$) (Fig. 6.2). In fact, this was so at all times, irrespective of mangrove system (HSD $p < 0.05$, in all cases). The overall average of RNA content was similar in both species (Student's *t* = 1.57, df = 82, p = 0.12), with values of 2.3 and 2.0 µg.mg^{-1} of freeze-dried muscle in *P. guttatum* and *U. annulipes*, respectively. DNA overall average content was however significantly higher in *P. guttatum* than in *U. annulipes* (2.2 and 0.8 µg.mg^{-1} of freeze-dried muscle, respectively; Student's *t* = 7.72, df = 82, p < 0.001). Therefore, the overall lower RNA/DNA ratio in *P. guttatum* did not result from lower RNA, but from relatively higher DNA content of the muscle tissue, in relation to that of *U. annulipes*.

Regarding the effects of mangrove system, there were no significant differences in the RNA/DNA ratio of *P. guttatum* among mangrove systems (HSD p > 0.20). Contrastingly, the RNA/DNA ratio in *U. annulipes* was significantly lower in Costa do Sol than in both pristine mangrove systems (HSD p < 0.001, in both cases), which showed no differences among them (HSD p = 1.00) (Fig. 6.2).

DISCUSSION

Biochemical indicators are important tools in the assessment of impacts caused in organisms by environmental stressors, namely seasonal oscillations and those of anthropogenic origin, such as pollution. The theory behind their use is that concentrations of biochemical components of metabolism change as a direct result of varying exposure conditions, leading to adjustments in metabolic rates that impact physiological condition (Dahlhoff 2004, and references therein). As for other bioindicators, they present several advantages over physical and chemical measures of pollution: they can give earlier indication of stress or impact; they assess bioavailable pollutants and cumulative and synergetic impacts; and they can also measure effects even after the cessation of impacts (Linton & Warner 2003).

The RNA/DNA ratio has been recognized as a useful index to assess the effects of environmental constraints on protein synthetic capacity, growth rate and overall physiological health of fishes (e.g. Malloy & Targett 1994, Buckley et al. 1999, Gilliers et al. 2004) and marine invertebrates (e.g. Wright & Hetzel 1985, Dahlhoff & Menge 1996, Parslow-Williams et al. 2001, Buckley & Szmant 2004), including crabs (e.g. Wang & Stickle 1986, Mayrand et al. 2000, Amaral et al. in press). While high RNA levels are usually interpreted as being beneficial, reflecting active metabolic states and elevated protein production for growth and reproduction, increased protein synthesis might in some cases reflect a stress response. Organisms exposed to stressful conditions may up-regulate the expression of stress-inducible heat shock proteins and protein phosphatases, that can also promote elevated RNA levels (Buckley & Szmant 2004, Dahlhoff 2004). Laboratory calibration is therefore of highly importance for the use of biochemical indicators of stress and metabolism, such as the RNA/DNA ratio, to investigate the effects of environmental impacts on the physiological health of organisms in a natural context (Buckley & Szmant 2004, Dahlhoff 2004).

The values and magnitude of the RNA/DNA ratios obtained in this study are consistent with the findings of Amaral et al. (in review-b), who measured the effects of sewage load and vegetation type in the RNA/DNA ratio of mangrove crabs in controlled mesocosm conditions. The ratios of crabs of the genus *Uca* from pristine mangrove habitats were similar to those obtained in the present study for the relatively pristine Ponta Rasa and Saco mangroves (Amaral et al. in review-b). These results seem to support the use of RNA/DNA ratio of mangrove crabs to evaluate their physiological health and assess the environmental quality of the habitats where they live.

The RNA/DNA ratios of *P. guttatum* and *U. annulipes* were sensitive to all studied factors, which suggests a high sensitivity of such ratios to the studied environmental constraints. In relation to climatic season, both species showed analogous patterns: lower ratio in the rainy season, with exception of Saco mangrove, where no seasonal differences were observed. These results are in agreement with those of Amaral et al. (in review-b), that also found more responsiveness on those ratios to the effects of climatic season, than to those possibly caused by naturally treated sewage discharges. In Mozambique, higher mean temperature and humidity characterize the rainy season in relation to the dry season. Temperature is known to be a major factor affecting several ecological aspects, and an optimum temperature interval generally favours metabolic activities, translated in enhanced growth and reproductive output (e.g. Ota & Landry 1984, Wagner et al. 2001, Clemmesen et al. 2003, Melzner et al. 2005). The lack of seasonal differences at Saco mangrove was due to relatively higher RNA/DNA ratio in the rainy season, rather than lower in the dry season. Peculiarities of such mangrove system, such as its enclosed character and strong influence from the Indian Ocean (Kalk 1995, Paula et al. 2003), may attenuate the impact of seasonal fluctuations on the system, and thus on the physiological condition of mangrove crabs.

Despite the good resolution of RNA/DNA ratios of both species to assess effects of climatic seasonal fluctuations, such ratios were consistent and significantly lower in *P.*

guttatum than in *U. annulipes*, irrespective of mangrove system. In addition, no differences between the peri-urban and the relatively pristine mangroves were observed for *P. guttatum*. These results suggest that *P. guttatum* may possess a lower metabolic activity than that of *U. annulipes*. Furthermore, the low RNA/DNA ratio observed for *P. guttatum* may only allow the detection of changes in metabolic rates of higher magnitude. Altogether, it seems that RNA/DNA ratio of *P. guttatum* might not constitute a sensitive and valid indicator of the environmental quality of mangrove habitats.

Interestingly, the relatively overall low RNA/DNA ratio of *P. guttatum* did not result from lower RNA content of the muscle tissue, but from higher DNA content in relation to that of *U. annulipes*. A possible explanation is a naturally higher cellular DNA content in *P. guttatum*, which is a well documented feature amongst closely related species of several animal taxa, including crustaceans (Hartley 1990, Gregory et al. 2000, Boulesteix et al. 2006). The relatively different claw size between both species may have also accounted for the observed pattern. The larger claw in male crabs of the genus *Uca* is supported by larger muscle fibres (Govind et al. 1986, Rhodes 1986), which may likely be originated by larger muscle cells (Penney et al. 1983, Nader 2007). Consequently, the muscle tissue of the claws of *U. annulipes* males would have fewer cells than the same amount of that of *P. guttatum*, and thus lower DNA content. These two explanations are not mutually exclusive, and only histological and genetic studies may appropriately clarify this issue.

RNA/DNA ratio of *U. annulipes* showed clear and consistent differences among the peri-urban and both pristine mangrove systems, contrasting with the lack of pollution sensitivity of *P. guttatum*. Irrespective of season, RNA/DNA ratio was significantly lower at the peri-urban than at the relatively pristine mangroves, which did not differ among each other. Several studies conducted in different organisms have previously reported, and supported, the usefulness of RNA/DNA ratio as indicator of pollution (e.g. Malloy & Targett 1994, Wo et al. 1999, Dahl et al. 2006). Contrastingly, lack of significant effects of pollution on RNA/DNA ratios of animals has also been reported, especially under good feeding conditions (Houlihan et al. 1994).

Fiddler crabs, such as *U. annulipes*, are deposit-feeders and shredders, feeding mainly on microphytobenthos and organic matter associated with sediment surface, thus benefiting under enriched situations, nutrient recycling and energy (Skov et al. 2002). The higher nutrient levels characterizing the peri-urban mangrove have been reported to led to an increase in the fiddler crab community (PUMPSEA 2007). Furthermore, stable isotope signatures have revealed a clear change in their diet between relatively pristine sites, where a broad range of food sources was identified, and polluted sites, where microphytobenthos was almost exclusively the only food source (PUMPSEA 2007). If such increase in food availability would theoretically favour energy availability, and thus physiological condition, such food may not be of proper quality for fiddler crabs. The higher levels of both total and faecal coliform bacteria in the sediment and water of the peri-urban mangrove indicate the presence of much sewage, which may have deleterious impacts on fiddler crabs, negatively affecting synthetic or metabolic pathways and rates, and resulting in a general lower physiological condition (Meziane & Tsuchiya 2002).

Elevated temperatures, both due to seasonal fluctuations and long-term trends of global climate change, may pose additional threats to condition, growth and, ultimately, survival of marine ectotherms, by synergistic effect with other stressors, such as pollution (Walther et al. 2002). Although this combination is a relevant and unfortunately widespread scenario, few studies have so far addressed its effects (Lannig et al. 2006). The RNA/DNA ratio of *U. annulipes* was sensitive to a synergistic effect of season and pollution. It showed an average decrease of ~64 % in the polluted mangrove in the less favourable season, while showing reductions of only ~17 and ~43 % when considering separately season and pollution, respectively. This suggests that pollution associated with the relatively higher temperatures and humidity of the rainy season may lead to serious deleterious effects on fiddler crabs.

In summary, our results indicate that RNA/DNA ratio of *U. annulipes* may constitute a useful and good indicator of environmental quality of mangrove habitats, at least in terms of pollution and seasonal fluctuations. Only the physiological condition of *U. annulipes* was significant and negatively affected by pollution, but both crab species showed seasonal effects on RNA/DNA ratio, suggesting that these may have more impact than those possibly caused by pollution. Resolution of issues related to cellular DNA content of claw muscle tissue of both species will only be assessed through histological and genetic approaches, but *U. annulipes* seems to have a higher rate of new protein synthesis than *P. guttatum*. We thus propose RNA/DNA ratio of *U. annulipes* as a valid and sensitive biochemical indicator of environmental quality of mangrove habitats. Future research should focus on mangrove crab responses to other environmental constraints, in order to such organisms be considered sentinel species, and be used in integrative manage and conservation programmes.

ACKOWLEDGEMENTS

We are in debt to M.J. Alves for providing laboratory facilities for biochemical analyses. We acknowledge a PhD grant to V.A. (SFRH/BD/10471/2002) and G.P.-L. (SFRH/BD/25277/2005) funded by Fundação para a Ciência e a Tecnologia. This study forms part of the PUMPSEA project (funded by the European Commission: FP6, INCO-CT2004-510863).

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CHAPTER 7

Effects of vegetation and sewage load on mangrove crab condition using experimental mesocosms

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Estuar Coast Shelf Sci (in review)

Effects of vegetation and sewage load on mangrove crab condition using experimental mesocosms

ABSTRACT

Constructed wetlands, especially mangroves, have been studied as successful alternative sewage treatment areas. Nevertheless, few studies have focused on the effects of vegetation and sewage load on mangrove macrofauna. Ocypodid crabs are important components of mangrove forests and constitute good bioindicators of ecosystem functioning as a whole. In a constructed mangrove mesocosm system, three vegetation treatments (bare substrate, and *Avicennia marina* and *Rhizophora mucronata* seedlings) were subject to 0, 20, 60 and 100 % sewage loads, supplied by a nearby hotel facility. The physiological condition of introduced *Uca annulipes* and *Uca inversa* was evaluated by RNA/DNA ratio, after one, five and twelve months, and used as an indicator of habitat quality. Crab condition in 0 % sewage load was similar to that of wild crabs at all times, suggesting no significant effects of the mesocosms on the response variable RNA/DNA ratio. Overall, both species coped well with the sewage loads administered, suggesting resistance to stress. Both species showed similar patterns of RNA/DNA ratio, being more affected by seasonal fluctuations than by sewage load and vegetation. Higher RNA/DNA ratio was recorded in the long than in the short-rainy season. Sewage enhanced crab condition on bare substrate and *R. mucronata* treatments, probably as a result of higher food availability. These trends were especially apparent after one year. *Uca inversa* may be more sensitive to sewage pollution than *U. annulipes*. In *A. marina*, no difference in crab condition was observed among sewage loads, yielding the best minimization of sewage impacts. Our results support the potential of constructed mangrove areas used for sewage treatment, especially if planted with *A. marina*, in supporting physiologically healthy ocypodid crabs, which in turn may enhance system performance through burrow and feeding activities.

KEYWORDS: Habitat quality, Mangroves, Mesocosm, Ocypodidae, RNA/DNA ratio, Tanzania, Wastewater treatment

INTRODUCTION

Local communities and researchers have long realized the support that mangrove forests provide to important ecological and economical functions. They protect and stabilize the coast, provide breeding, recruitment, nursery and feeding areas for many fish and invertebrate species, and also attract many other kinds of wildlife (Alongi 2002, Kathiresan & Qaim 2005, Hogarth 2007). They also provide wood and several other forestry and pharmaceutical resources, as well as, and perhaps most importantly, a variety of fishing areas and products (Costanza et al. 1997, Kathiresan & Qaim 2005, Hogarth 2007).

Mangrove forests further supply organic matter to primary producers, and are, as other wetland ecosystems, naturally deficient in nutrients and a sink for both nutrients and heavy metals (Kathiresan & Qaim 2005, Hogarth 2007). This makes them particularly important areas buffering nearshore waters from land-based pollution (Boonsong et al. 2003, Hogarth 2007). Accordingly, mangrove forests have been extensively studied as successful low cost and low maintenance alternative sewage treatment areas, especially in developing countries, where resources and appropriate technology are scarce (Wong et al. 1997, Ye & Tam 2002, Boonsong et al. 2003). The present study forms part of PUMPSEA project (funded by the European Commission: FP6, INCO-CT2004-510863), which aims to demonstrate the ecosystem services performed by mangroves in filtering discharged wastewater, and thereby limiting coastal sewage pollution, and to examine its ecological and socio-economical effect, in East Africa (PUMPSEA 2007).

Overexploitation of resources, together with global trends of human activities, climate change and sea level rise, have led to severe clearance and deterioration of mangrove forests, with increased rates in developing countries (Kathiresan & Qaim 2005, Duke et al. 2007, Hogarth 2007). As a result, many natural mangrove forests are now protected or in the process of being so, and it would be counterproductive to impact them with sewage discharges (Kathiresan & Qaim 2005, Hogarth 2007). Instead, the use of constructed mangrove areas for sewage treatment may be a valid alternative (Boonsong et al. 2003). By definition, such areas will allow a more effective control of sewage and water circulation, and confine sewage discharges to specific and appropriate areas.

Several studies have reported effective sewage treatment by constructed mangrove areas, associated with enhanced growth of mangrove flora (e.g. Ye & Tam 2002, Boonsong et al. 2003). However, it is not yet clear if such areas fulfil, at medium and long term, the valuable ecological functioning and services of natural mangrove forests, especially since only one or two tree species are commonly used (Alongi 2002, Bosire et al. 2004, Kathiresan & Qaim 2005, Walton et al. 2006). The introduction of macrofauna in such areas is of particular importance for a healthy ecological performance of the system. Macrofauna is an essential component of mangrove systems, being responsible for several vital ecological processes, such as bioturbation and detritus processing

(Kathiresan & Qaim 2005, Hogarth 2007). Ecological functioning may be properly evaluated by biological indicators of the system status or health, such as the physiological condition of ecological relevant organisms in a natural context (Ashton et al. 2003, Bosire et al. 2004, Amaral et al. in press, Amaral et al. in review-b). The principle is that adjustments in rates of physiological processes, as a consequence of organism exposure to different environmental conditions, result in alteration of protein synthesis or metabolism, which impact performance, growth, reproductive output and, ultimately, survival (Bosire et al. 2004, Dahlhoff 2004, Amaral et al. in review-b).

Brachyuran crabs are amongst the most abundant and conspicuous macrofauna of mangrove systems (Kathiresan & Qaim 2005, Hogarth 2007). They also play important ecological roles, as their burrowing and feeding activities substantially improve biotic properties of the sediment, tree colonization, primary and secondary productivity, nutrient cycling and energy flow (Smith et al. 1991, Kathiresan & Qaim 2005). They are thus considered good bioindicators of environmental quality reflecting, furthermore, conditions integrated in the benthos over time and being a main link between primary producers and secondary consumers (Smith et al. 1991, Ashton et al. 2003, Pagliosa & Barbosa 2006). In a recent study, RNA/DNA ratio of *Uca annulipes*, a characteristic mangrove ocypodid crab, has been pointed as a good biochemical indicator of habitat quality, regarding both pollution and environmental stressors, and thus a good estimator of system health and functioning (Amaral et al. in review-b).

The present study was designed to evaluate the effects of vegetation and sewage load on crab physiological condition, in constructed mangrove mesocosms used for sewage treatment. Specifically, we addressed whether vegetation presence and type, and sewage load may yield favourable conditions for mangrove crabs, and thus for a healthy ecological system functioning. *Uca annulipes* (H. Milne Edwards, 1837) and *U. inversa* (Hoffman, 1874) were exposed to different sewage loads in mesocosms provided with three vegetation treatments: bare substrate, and *Avicennia marina* (Forsk.) and *Rhizophora mucronata* Lamk. 1804 seedlings. The physiological condition was estimated by RNA/DNA ratio after exposure times from one to twelve months, using wild crabs as control.

MATERIAL AND METHODS

Field experiments

The effects of vegetation and sewage load on the physiological condition of *U. annulipes* and *U. inversa*, as estimated by RNA/DNA ratio, were evaluated in mesocosms constructed at Kunduchi mangrove, near Dar es Salaam, Tanzania. Fauna, substrate and vegetation used were collected locally. In the scope of PUMPSEA project, a system of 36 (3.0 x 3.0 x 0.8 m) surface-flow cement mesocosms (plots) was constructed at Jangwani Beach, and protected from tidal influence by a soil embankment. Each plot was provided with independent in- and outflow pipes. Sewage from a nearby hotel facility was drained into an 86 m^3 storage pond, where it was subject to primary treatment before entering the mesocosm system.

Three vegetation treatments were manipulated: bare substrate, and *A. marina* and *R. mucronata* seedlings. In early February 2006, substrate was laid and seedlings planted on haphazardly selected plots, and allowed to grow without disturbance. For each vegetation treatment, three replicate plots per sewage load (see below) were haphazardly assigned. In mid August, 5 ind.m^{-2} of both sexes of *U. annulipes* and of *U. inversa* males, and 3 females.m^{-2} of *U. inversa*, were introduced reflecting minimum natural densities (PUMPSEA 2007). Average carapace width of males and females of *U. annulipes* and *U. inversa* was 14 and 11, and 16 and 12 mm, respectively.

From early September, the system was inundated exclusively with natural seawater during one month. Initial RNA/DNA ratio of crabs was evaluated using 3 male crabs of each species, collected from each of two haphazardly chosen bare substrate plots. From early October, sewage loads were applied. Sewage loads of 0, 20, 40 and 60 % to natural seawater were studied, as they cover a broad range of concentrations, and have shown to support growth of both *A. marina* and *R. mucronata* in the laboratory (PUMPSEA 2007). A daily 12 h period of sewage inundation of 0.1 m, starting at 11:00 a.m., was applied based on a pilot study conducted on bare substrate plots: higher water quality was reached after a total of 12 h, irrespective of inundation period and sewage load (PUMPSEA 2007). Average height (m), and number of nodes and leaves of *A. marina* and *R. mucronata* were at the time 0.50, 14, 26 and 0.55, 7 and 10, respectively. Crabs were sampled for biochemical analyses after one, five and twelve months (November 2006 and March and October 2007, respectively). At each date, three male crabs of each species were collected from each of two plots, haphazardly selected in each vegetation treatment and sewage load combination. Due to sampling constraints, no crabs were collected from plots of 40 % sewage load.

The system capacity to support higher sewage load and an extended inundation periods were also studied from March 2007. One haphazardly chosen plot from each vegetation treatment and sewage load combination, started receiving 100 % sewage load in one of two inundation schemes: the 12 h period continued to be applied to plots of previous 0 and 60 % sewage load; and a 3 d period was applied to those of previous 20 and 40 %. On October 2007, three male crabs of each species were also collected from each of these plots for biochemical analyses.

To account for the effects of the mesocosms on crab condition, 6 male crabs of each species were collected from wild bare mangrove substrates concurrently with all crab samplings in the plots. In all cases, only active individuals and in intermoult stage, as evaluated *in situ* by the hardness of the carapace, were considered. After collection, the largest claw of each individual was removed and immediately transported to the laboratory in cool boxes, where it was promptly freeze-dried, and stored at -80 ºC until nucleic acid quantification.

Biochemical analysis

Nucleic acid quantification was conducted based on the Schmidt-Thannhauser method, according with the procedures of Amaral et al. (in review-a). Analyses were performed on ~15 mg (freeze-dried weight) of white claw muscle. Absorbance of RNA and DNA was measured in a NanoDrop® ND-1000 full spectrum spectrophotometer (NanoDrop, USA). The full spectrum feature allows inspection of nucleic acid contamination by estimation of 260/280 and 260/230 nm ratios, concurrently to sample analysis.

Statistical analysis

To evaluate the effects of the mesocosms on RNA/DNA ratio, Student's *t*-tests were performed independently for each crab species, at all sampling dates, between wild and 0 % sewage-exposed crabs of bare substrate treatments. Effects of vegetation, sewage load and exposure time on RNA/DNA ratio, were estimated for each species using three-way analysis of variance (ANOVA).

When the system capacity to support higher sewage load and extended inundation periods started to be tested, on March 2007, no significant differences on RNA/DNA ratios were observed among any sewage load in any vegetation treatment (see Results). Therefore, data from plots subjected to 100 % sewage load were pooled by inundation period (12 h and 3 d) for comparison proposes among sewage loads. The effects of vegetation, sewage load and inundation period were estimated for each species using two-way ANOVA analyses. Cochran's tests revealed homoscedasticity of variances, and Shapiro-Wilk's tests revealed normality of residuals in all ANOVA analyses. *A posteriori* comparisons were performed using HSD Tukey's tests.

RESULTS

Student's *t*-tests revealed similar RNA/DNA ratio on wild crabs and on those of bare substrate treatments under 0 % sewage load, at all times and in both species ($p > 0.05$, in all cases), suggesting that the mesocosms had no significant effect on crab physiological condition. Under such conditions, RNA/DNA ratio was significantly higher in *U. annulipes* than *U. inversa* (2.8 and 2.5, respectively, Student's $t = 2.11$, df = 94, $p < 0.05$).

Sewage load accentuated differences among species, yielding a RNA/DNA ratio of 3.0 and 2.6 in *U. annulipes* and *U. inversa*, respectively (Student's $t = 3.74$, df = 322, $p < 0.001$). Nevertheless, patterns of RNA/DNA ratio were generally similar in both species, and both ANOVA analyses revealed significant effects of vegetation, sewage load, exposure time and interactions (Fig. 7.1, Table 7.1). Under 0 % sewage load, both crab species showed similar RNA/DNA ratio among the three vegetation treatments, at each sampling date (HSD $p > 0.05$, in all cases). In both species, overall RNA/DNA ratio was significantly lower in *A. marina* (HSD $p < 0.001$, in all cases, except for bare substrate in *U. inversa* with $p < 0.05$) and similar among bare substrate and *R. mucronata*. In the wild, and in all vegetation and sewage load (0, 20 and 60 %) treatments, RNA/DNA ratio of both species decreased significantly from November 2006 to March 2007 and increased significantly afterwards to October 2007, revealing a strong influence of climatic season (HSD $p < 0.001$, in all cases) (Fig. 7.1).

For both *U. annulipes* and *U. inversa*, and at each sampling date, RNA/DNA ratio in each vegetation treatment was similar among sewage loads, except in two and four occasions, respectively. In *U. annulipes* on October 2007, in both bare substrate and *R. mucronata*, RNA/DNA ratios were similar among 20 and 60 % sewage loads, and significantly lower in crabs under 0 % sewage load (HSD $p < 0.01$, in all cases) (Fig. 7.1).

In *U. inversa*, in November 2006 and October 2007, RNA/DNA ratio was significantly higher in crabs of treatments of 20 % sewage load than in those of 0 and 60 %, in both bare substrate and *R. mucronata* (HSD $p < 0.05$, in all cases). In both species, no differences were observed in *A. marina* at any time (Fig. 7.1).

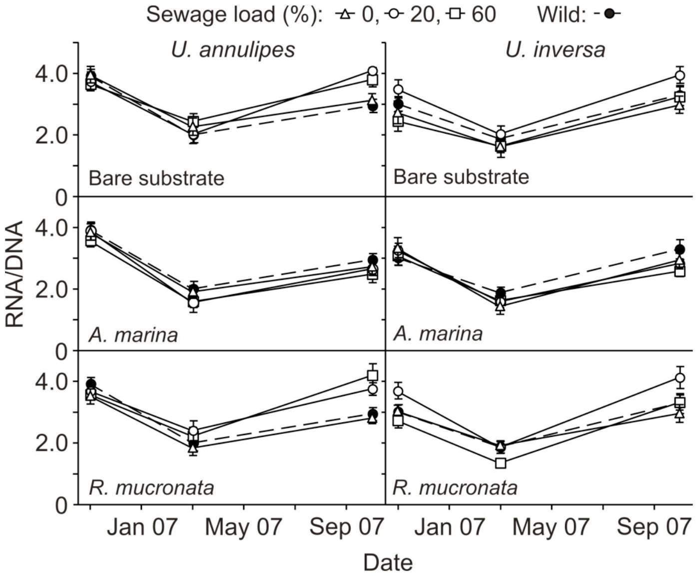

Fig. 7.1. Mean RNA/DNA ratio of *U. annulipes* and *U. inversa* exposed to different sewage loads, in three different habitats, throughout twelve months. Error bars: ±SE.

Table 7.1. Results of three-way ANOVA analyses on the effects of mangrove habitat, sewage load and exposure time on RNA/DNA ratio of *U. annulipes* and *U. inversa*. Significant p-values in bold.

	U. annulipes				*U. inversa*			
Source of variation	df	MS	*F*	p	df	MS	*F*	p
Habitat (A)	2	4.32	57.56	**< 0.001**	2	0.91	10.80	**< 0.001**
Sewage load (B)	2	0.64	8.55	**< 0.001**	2	4.34	51.53	**< 0.001**
Exposure time (C)	2	41.49	552.88	**< 0.001**	2	39.31	466.59	**< 0.001**
A x B	4	0.97	12.96	**< 0.001**	4	0.86	10.22	**< 0.001**
A x C	4	1.80	23.93	**< 0.001**	4	1.18	14.04	**< 0.001**
B x C	4	0.91	12.15	**< 0.001**	4	0.45	5.28	**< 0.001**
A x B x C	8	0.38	5.11	**< 0.001**	8	0.30	3.57	**< 0.001**
Error	135	0.08			135	0.08		

Considering in addition the treatments of 100 % sewage load, ANOVA analysis revealed significant effects of vegetation, sewage load and interaction on RNA/DNA ratio in both species (Table 7.2). However, no differences in inundation period were observed among 100 % treatments in any vegetation treatment ($p = 1.00$, in all cases) (Fig. 7.2). Overall RNA/DNA ratio of both species remained significantly lower in *A. marina* (HSD $p <$

0.001, in all cases) and similar among bare substrate and *R. mucronata* treatments (Fig. 7.2). The general patterns of RNA/DNA ratio in October 2007 also remained similar: in *U. annulipes*, overall RNA/DNA ratio was significantly lower under 0 % sewage load (HSD p < 0.001, in all cases), and similar among other loads; while in *U. inversa*, was significantly lower under 0 and 60 % sewage loads (HSD p < 0.001, in all cases), and also similar among other loads.

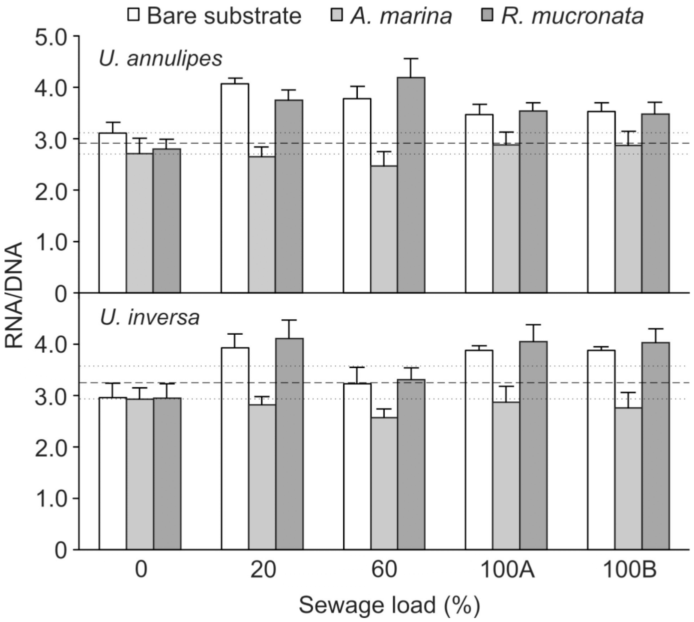

Fig. 7.2. Mean RNA/DNA ratio of *U. annulipes* and *U. inversa* exposed to different sewage loads (100A - 12 h exposure; 100B - 3 d exposure) in three different habitats, on October 2007. Dashed and doted lines indicate mean RNA/DNA ratio and ±SE of wild crabs, respectively. Error bars: SE.

Table 7.2. Results of two-way ANOVA analyses on the effects of mangrove habitat and sewage load (100 % included) on RNA/DNA ratio of *U. annulipes* and *U. inversa*. Significant p-values in bold.

	U. annulipes				*U. inversa*			
Source of variation	df	MS	*F*	p	df	MS	*F*	p
Habitat (A)	2	7.34	112.93	**< 0.001**	2	7.20	90.81	**< 0.001**
Sewage load (B)	4	1.12	17.24	**< 0.001**	4	1.99	25.10	**< 0.001**
A x B	8	0.68	10.43	**< 0.001**	8	0.52	6.51	**< 0.001**
Error	75	0.07			75	0.08		

DISCUSSION

Evaluation of ecological health and functioning of constructed wetlands, such as mangroves, are of crucial importance since these areas mimic natural ecosystems, and their performance at medium and long term depends on the system auto-sustainability, independently of the purposes for which they were built e.g., sewage treatment, wood supply or reforestation (Kairo et al. 2001, Bosire et al. 2004, Walton et al. 2007). The results of RNA/DNA ratio of both *U. annulipes* and *U. inversa* suggest that the constructed mesocosms can support an system functioning equivalent to that of natural areas. Crab condition was overall more affected by natural environmental conditions, namely seasonal climatic fluctuations, than by sewage load and vegetation, and presented similar patterns in both crab species.

The values and magnitude of RNA/DNA ratios obtained for both crab species agreed with those reported for *U. annulipes* from pristine mangrove habitats (Amaral et al. in review-b), suggesting an overall healthy physiological condition in the mesocosms. In addition, RNA/DNA ratio response to seasonal fluctuations, vegetation, sewage load and exposure time was generally similar for both species. Furthermore, increased sewage load and exposure time did not influenced significantly crab physiological condition. Altogether, our results indicate that both crab species coped well with the sewage loads administered, irrespective of vegetation treatment, and have similar metabolic responses to the stressors studied. The mesocosm system thus seems to provide quality habitats for ocypodid crabs. In fact, such crabs are characteristic pioneers in young mangrove plantations, including those of *A. marina* and *R. mucronata*, where high crab densities have been reported (Ashton et al. 2003, Bosire et al. 2004, Walton et al. 2007).

In both species, RNA/DNA ratio was significantly lower in the long (March to May) than in the short-rainy season (November to December). However, due to lack of short-rainy season replication, caution is needed in interpreting these results. Seasonal climatic fluctuations affect several ecological aspects, and optimum conditions generally favour metabolic activities, resulting in enhanced growth and reproductive output (Walther et al. 2002, Dahlhoff 2004). Lower RNA/DNA ratio in the rainy season has been recently recorded for *U. annulipes* and *Perisesarma guttatum* in mangroves of Mozambique (Amaral et al. in review-b). That study has also reported higher sensitivity of both crab species to seasonal climatic fluctuations than to pollution, as well as a respective synergistic negative effect on crab condition. Natural elevated temperatures characterize mangrove systems, and further temperature increase, both due to seasonal fluctuations and long-term trends of global change, may additional stress organism condition, growth and survival, by synergistic effects with other stressors, such as pollution (Eshky et al. 1996, Walther et al. 2002). Such synergism was not detected in this study, probably due to the efficient sewage treatment performed by the mesocosm system (PUMPSEA 2007), reducing the overall impacts of pollution on crab condition.

In *U. annulipes*, enhanced RNA/DNA ratio was obtained in bare substrate and *R. mucronata* treatments under both 20 and 60 % sewage loads, in October 2007. In *U. inversa* this occurred both in November 2006 and October 2007, but only under 20 % sewage load. The overall low RNA/DNA ratio in March 2007 may have invalidated the detection of humble metabolic changes. Ocypodid crabs are deposit-feeders and shredders, relying on organic matter and microalgae for food, and can thus benefit from situations of enriched nutrient and organic matter contents (Ashton et al. 2003, Lim & Heng 2007). Organic matter and nutrient contents of higher sewage loads yielded higher microalgal productivity, resulting in less time spent by both species foraging for food (PUMPSEA 2007). Furthermore, both species significantly decreased the microalgae and organic matter contents of the sediment (PUMPSEA 2007). Enhanced RNA/DNA ratio in both species may have thus resulted from higher food consumption rates. Reduced pollution effects on organisms exposed to good feeding conditions have been reported in other animal taxa, such as fish (e.g. Houlihan et al. 1994). This would only be possible due to the effective sewage treatment performed by the mesocosms, since in otherwise similar conditions, RNA/DNA ratio of *U. annulipes* is known to decrease (Amaral et al. in review-b).

The differences among species suggest that *U. inversa* responds quicker to microalgal and organic matter increase, and that it may be more sensitive to sewage pollution than *U. annulipes*. Environmental stressors, such as pollution, may decrease the food conversion efficiency by lowering the rate of protein synthesis, and thus the RNA/DNA ratio (Houlihan et al. 1994). Above 60 % sewage load, nitrification was limited and there was accumulation of organic matter, which could have affected the metabolism of *U. inversa* (PUMPSEA 2007). However, under 100 % sewage loads, RNA/DNA ratio was similar to that obtained under 20 %. Only through specific toxicological studies will it be possible to attest on the higher sensitivity of *U. inversa* to sewage pollution.

By contrast, no significant differences in crab condition were detected among sewage loads in *A. marina*. Higher growth rate and nutrient uptake, resulting in a more efficient sewage treatment were also recorded in plots with *A. marina* (PUMPSEA 2007). Overall, this suggests that such vegetation type provided the best combination of crab condition and sewage treatment efficiency. We therefore propose that constructed mangrove areas provided with ocypodid crabs, especially if planted with *A. marina*, may support a sustainable and healthy ecological system functioning, inferred through crab condition, and simultaneously, a good sewage filtering capacity (PUMPSEA 2007). Ideally, several genera of mangrove trees should be used in constructed mangrove areas and reforestation programmes. Mono-specific plantations of economical interest are, however, usually undertaken (Kairo et al. 2001, Alongi 2002, Walton et al. 2006). As suggested by our study, such mono-specific plantations can successfully rehabilitate mangrove areas and recover an ecological functioning similar to that of natural mangrove areas (Bosire et al. 2004, Walton et al. 2007).

In summary, our results indicate that both *U. annulipes* and *U. inversa* coped well, in terms of physiological condition, with sewage pollution irrespective of vegetation treatment, being more sensitive to impacts of climatic seasonal fluctuations. However, *U. inversa* may be more sensitive to such pollution than *U. annulipes*. Furthermore, this study suggests that constructed mangrove areas used for sewage treatment may be able to support physiologically healthy ocypodid crabs, especially if planted with *A. marina*, and thus a healthy system functioning. The present study therefore supports the potential of such areas to preserve mangrove forests, recover valuable ecological and economical resources, and address land-based pollution issues in developing countries, where there are many competing demands but only limited funds for development.

ACKNOWLEDGEMENTS

We are in debt to M.J. Alves for providing laboratory facilities for biochemical analyses. We acknowledge a PhD grant to V.A. (SFRH/BD/10471/2002) and G.P.-L. (SFRH/BD/25277/2005) funded by Fundação para a Ciência e a Tecnologia.

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SECTION III

GENERAL DISCUSSION AND CONCLUSIONS

This study addressed the effects of important environmental constraints on population structure and condition of coastal and estuarine crabs inhabiting heterogeneous environments, and the relation of such response variables with habitat quality. The relative importance of recruitment versus post-recruitment processes in determining distribution and abundance of organisms and populations is still debated among researchers. Nevertheless, the structuring and limiting potential of different abiotic and biotic processes acting during both planktonic and benthic life stages of open marine populations have been recognized (Roughgarden et al. 1988, Ólafsson et al. 1994, Caley et al. 1996, Hixon et al. 2002, Jenkins 2005). Initial patterns established by recruitment and settlement mechanisms can be modified, or even overridden, by strongly selective post-settlement processes such as competition, predation, food limitation and physical and chemical disturbances (Ólafsson et al. 1994, Caley et al. 1996).

The results from this study corroborate that different environmental factors, acting in both planktonic and benthic life stages of coastal and estuarine crabs, have the potential to determine distribution and abundance patterns of organisms and populations. More relevant than determining which life stage or set of constraints is more important for population structure and physiological condition, is to understand the mechanisms by which those contribute to the patterns observed in natural environments. This study, furthermore suggests that more than any of those environmental constraints *per se*, all of them jointly must be taken into account for a full and correct understanding of the population ecology of estuarine and costal organisms in heterogeneous environments.

The patchiness and type of settlement habitats may constitute recruitment constraints of special relevance in species that possess megalopae with strong swimming capacity, and that actively search for appropriate settlement conditions, such as *Carcinus maenas* (L.) (Hedvall et al. 1998, Moksnes 2002). The investigation presented in Chapter 1 revealed that artificial collectors of two different materials, both with high structural complexity, yield very similar settlement rates and patterns, at both high and low settlement intensities. These are promising results for the potential replacement of hog's hair collectors, specifically by those of plastic grass, which are easier to process in the laboratory, more durable and easier and cheaper to obtain than the previous.

In relation to habitat patchiness, settlement of *C. maenas* megalopae per surface area of artificial collector generally decreased with increasing surface area, associated with decreasing perimeter:area ratio. This trend was more pronounced when settlement was more intense (Chapter 1). Patchy settlement habitats are usually associated with high perimeter:area ratio (i.e. more patch edge is exposed), which results in higher encounter rates between settling organisms and those habitats, and thus higher concentration of settled individuals (Eggleston et al. 1998, Bologna & Heck 2002, Hovel & Lipcius 2002).

Availability of high quality settlement habitats is, however, of no use if organisms are not available or are not capable of settling. In the lower Mira Estuary, Portugal, a decoupling between maxima supply of megalopae during spring tides and maxima settlement during neap tides, characterize *C. maenas* recruitment patterns (Queiroga et al. 2006). Despite the presence of suitable settlement habitats, such as seagrass meadows, and of competent megalopae for settlement during both spring and neap tides, settlement only takes place during neap tides, when co-occurrence of darkness and flood tides is minimal (Paula et al. 2006, Queiroga et al. 2006). The investigation of this subject (Chapter 2) suggests that this recruitment pattern deviates from that of the conceptual model for brachyuran crabs in mesotidal systems (Little & Epifanio 1991, Queiroga 1998, Tankersley et al. 2002, Forward et al. 2004) not due to abnormal larval behaviours, but due to physical constraints, namely high turbulence levels preventing megalopae settlement. High turbulence levels may also override megalopae swimming inhibition by light during spring tides, resulting in upstream movement of megalopae also during daylight flood tides (Pineda 2000, Welch & Forward 2001, Amaral et al. 2007).

Fragmentation of preferred settlement and nursery habitats, such as seagrass meadows (Moksnes & Heck 2006, Paula et al. 2006), together with settlement restriction during spring tides, may act synergistically on *C. maenas* recruitment patterns in the lower Mira Estuary. Megalopae would settle during neap tides at even higher densities in such patchy habitats, both due to lower habitat availability and higher encounter rates with such habitats (Chapters 1 and 2), at least until their quality is maintained. Furthermore, such density increase of new recruits may affect the quality of habitats where they recruit (Chapter 3). Due to crowding, density-dependent competition for food, space and aggressive interactions may result in reduced growth and physiological condition in such habitats, and thus, in a lower survival potential within the population (Ólafsson et al. 1994, Diehl & Ekloev 1995, Moksnes 2004a). In this context, the results from the investigation presented in Chapter 4, regarding the implications of habitat type on growth, size-structure and physiological condition of early juvenile *C. maenas* populations in the lower Mira Estuary, corroborate such effects of increased recruit density. Reduced growth and lower physiological condition characterized early juvenile cohorts in the seagrass habitat, where young recruits were more abundant and constituted the most significant part of the juvenile population, in comparison with the sandy habitat.

Such differences between sandy and seagrass habitats could be even greater resulting from higher juvenile density in seagrass habitats, and caused for example by habitat fragmentation. On the other hand, competition among recruits in such habitats may result in secondary dispersal to habitats providing better survival conditions (Moksnes 2004a). Such habitats e.g., sandy beaches with boulders, may provide higher growth rates and better physiological condition to young recruits, and later juvenile and adult stages (Chapter 3). In fact, many aquatic organisms, including benthic invertebrates, shift their habitats, as well as their feeding habits, at some point during juvenile life (Werner & Gilliam 1984, Gosselin & Qian 1997, Bystrom et al. 2003). Thus, the relative importance of different habitats is likely to change throughout ontogenesis.

Estuarine vegetated habitats may also be of relatively lower quality than adjacent non-vegetated areas for local *C. maenas* popultions (Chapter 4). These habitats are usually considered of higher quality for crabs than adjacent non-vegetated and rocky-shore areas, due to their capacity to support larger populations (Polte et al. 2005, Fonseca et al. 2006, Paula et al. 2006). However, the high quality of non-vegetated habitats, with substrates such as shell debris, has been recognized for different fish and invertebrate species, but it has not yet received due recognition (Diehl & Ekloev 1995, Glancy et al. 2003, Moksnes & Heck 2006).

In the case of *C. maenas*, the quality of both vegetated and non-vegetated estuarine native habitats, evaluated by measures of population density, size-structure and physiological condition, seem to be similar for each local population, despite differences in their relative importance throughout ontogenesis (Chapters 3 and 4). Further north within its native distribution range, in Southwest Britain, rocky shore habitats revealed the highest quality for *C. maenas* populations. However, this trend was not observed further south, namely in SW Portugal, where biological interactions with other species, particularly top-predator crabs, may severely restrict the establishment of abundant *C. maenas* populations along the coast (Chapter 4). Nevertheless, in both northern and southern locations, estuarine vegetated habitats supported significantly larger crab populations than other habitats.

As suggested for the early juvenile stages, density-dependent intraspecific competition in densily populated estuarine vegetated habitats may also account for the relatively low quality of these habitats for *C. maenas* populations. In fact, this species is characterized by intense aggressive intraspecific competition, capable of population regulation by intra and intersize class cannibalism and mutual interference among cannibals (Moksnes 2004b). Even in less aggressive species, lower cannibalism intensity and interspecific predation, can constitute important environmental processes dictating distribution and abundance patterns (Chapter 5). Cannibalism is a density-dependent mechanism of recognized importance in population regulation, causing fluctuations in population structure and being also implicated in stock-recruitment relationships (Fox 1975, Polis 1981, Smith & Reay 1991, Moksnes 2004b, Wise 2006).

No cannibalistic behaviour was detected in *Porcellana platycheles* (Pennant, 1777), which was heavily predated by *Cancer pagurus* (L.), especially in the absence of food, suggesting avoidance behaviour of *C. pagurus* for conspecific prey (Chapter 5). Contrastingly, juvenile-adult and adult-adult cannibalistic interactions might regulate intertidal populations of *C. pagurus* (Chapter 5). In fact, irrespective of juvenile density, only vulnerable individuals (in moulting stage) were cannibalized by juvenile siblings. This suggests that in otherwise similar circuntances cannibalism may not constitute a gainful behaviour. Among different size classes, cannibalism was more intense and varied with both prey and cannibal densities. Predator response to prey density may vary with environmental conditions, namely changes in habitat heterogeneity (Murdoch & Oaten 1975, Hassel 1978, Lipcius & Hines 1986, Moksnes et al. 1997), but also with cannibal density (Fox 1975, Polis 1981, Moksnes et al. 1997, Moksnes 2004b, Chapter 5).

In addition to organism density, cannibalistic behaviour in *C. pagurus* has revealed to be also significantly affected by food, alternative live prey and habitat type, being less intense when the first two were supplied, and in structurally complex habitats (Chapter 5). This investigation also revealed that the relative importance of a specific factor might change as a result of other components of the environment. Food supply was crucial to minimize intersize class cannibalism of *C pagurus* in sandy habitats, being of less importance if proper refuges were provided (Chapter 5). Furthermore, when food was supplied to the crabs in the *Fucus* spp. habitat, cannibalism ceased to occur.

The natural processes and resulting patterns hitherto discussed may be severely modified by the increasing global rates of habitat and ecosystem loss and deterioration. Consequently the identification and comprehension of the mechanisms by which critical environmental processes structure populations and affect their physiological condition assume increasing relevance, as the consequences of global climate change and associated sea-level rise, and impacts of anthropogenic activities cascade through the biosphere (Costanza et al. 1997, Walther et al. 2002, Dahlhoff 2004, Hogarth 2007). This knowledge is crucial to evaluate the health of organisms and populations and the quality of their habitats (Hixon et al. 2002, Dahlhoff 2004). In this context, biological indicators, including brachyuran crab populations and assemblages, assessed at different levels of organization, can constitute valuable tools (Smith et al. 1991, Linton & Warner 2003, Lohrer et al. 2004, Pagliosa & Barbosa 2006).

The ratio of nucleic acids (RNA/DNA) of *Uca annulipes* (H. Milne Edwards, 1837) (an ocypodid crab) revealed to be a good and promising biochemical indicator of mangrove habitat quality in terms of pollution, seasonal fluctuations and synergistic effects (Chapter 6). This was not the case, however, for the grapsid *Perisesarma guttatum* (A. Milne Edwards, 1869). As ocypodid crabs are deposit-feeders and shredders, they can be greatly affected by the quality of the microphytobenthos and organic matter associated with the sediment, serving as food (Skov et al. 2002, Ashton et al. 2003, Lim & Heng 2007). The higher levels of coliform bacteria present in the water and sediments of polluted areas may have been incorporated in such food sources, and thus have deleterious effects on ocypodid crabs (Meziane & Tsuchiya 2002).

The physiological condition of both species, as estimated from that ratio, was more affected by seasonal climatic variations than by pollution, which may reflect the fact that mangrove systems, and associated fauna, are naturally exposed to elevated temperatures (Chapter 6). The further increase in those temperatures may pose additional threats to condition, growth and survival of mangrove organisms (Walther et al. 2002). This gains special relevance, since pollution seems to act synergistically with seasonal fluctuations, yielding lower physiological condition of crabs in the more wet and warm, rainy season (Chapter 6).

Wastewater pollution caused by discharges of untreated sewage is particularly problematic in mangrove systems located in developing countries, where resources and appropriate technology for sewage treatment are scarce (Wong et al. 1997, Ye & Tam

2002, Boonsong et al. 2003). Mangrove areas constructed for natural sewage treatment may benefit from the presence of mangrove crabs, namely *U. annulipes and Uca inversa* (Hoffman, 1874), due to their vital importance in several ecological functions (Smith et al. 1991, Kathiresan & Qaim 2005, Chapter 7). Furthermore, the physiological condition of such crabs can also serve as bioindicators of habitat quality and ecosystem health (Chapters 6 and 7).

The mesocosm investigation presented in Chapter 7 showed that both crab species were able to cope with significant sewage loads, irrespective of vegetation treatment, under effective filtering of sewage discharges by the system. As in natural ecosystems, crab condition was more affected by seasonal variations than by pollution, with better physiological condition in the long than in the short-rainy season. Furthermore, no synergistic effect of pollution and climatic season was detected. Mesocosms planted with *Avicennia marina* (Forsk.) yielded physiological condition in both crab species similar to that in natural environments. In mesocosms planted with *Rhizophora mucronata* Lamk. 1804 and with bare substrate, crabs showed a relatively enhanced condition, especially after one year, probably as a result from higher food availability (Ashton et al. 2003, Lim & Heng 2007). These results seem promising for the potential of such constructed wetland areas to support healthy mangrove crab assemblages, and to preserve mangrove areas and their valuable ecological and economic resources.

Final remarks

Considering the above discussion, one may compromise that a full understanding of the patterns of population structure and physiological condition of coastal and estuarine crabs, depends on the comprehension of the mechanisms by which environmental processes operate and of the factors capable of modifying them, such as habitat fragmentation and pollution. Different environmental constraints, whether of natural or anthropogenic origin, can affect population patterns at particular phases during the life cycle of coastal and estuarine crabs, and the relative importance of a specific constraint may also change according to such phases. Furthermore, the mechanism by which a specific process affects a particular assemblage or population characteristic interacts with other environmental variables and processes, which may result in a synergistic impact.

By addressing several environmental constraints in different stages of the life cycle of coastal and estuarine crabs, and doing it in distinct crab species and ecosystems, this study benefited from a broad approach to the ecological processes determining distribution and abundance patterns of crab populations. This study also supports the potential of crab individuals and populations to be used as bioindicators of habitat quality and ecosystem status. In addition, brachyuran crabs are good model organisms to study the processes affecting and structuring open marine populations, namely those of fish and benthic invertebrate species, which in turn, support vital ecological functions, economically important fisheries and aquatic farming industries.

Most, if not all, of the world natural estuarine and coastal ecosystems are becoming extensively degraded, due to climate change and consequent sea-level rise, global trends in human activities, including habitat destruction, overexploitation of resources and increasing pollution. The information compiled in this study can thus be useful for the integration of conservation, protection, restoration and sustainable managment programmes of several of those valuable ecosystems and associated resources.

This study represents a contribution to the knowledge of the environmental agents and mechanisms contributing to the patterns of distribution and abundance of coastal and estuarine crabs, and for the use of crab populations and assemblages as indicators of ecological status of habitats and ecosystems. Notwithstanding, further research is necessary to address other important environmental constraints affecting such patterns, particularly as global trends continue to alter the natural regulation of ecosystems. Given the complexity of life cycle, and the characteristic habitat shift throughout ontogenesis of marine fish and invertebrate species, future studies should focus on the continuous evaluation of environmental impacts throughout ontogenesis, with especial emphasis on cumulative and synergistic effects.

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