Universidade de Aveiro Departamento de Biologia

Ano 2016

Carolina Nunes Ladeira

ECOLOGY, DISTRIBUTION, HABITAT SEGREGATION AND TIDAL MIGRATION OF GREEN CRAB CARCINUS MAENAS IN RIA DE AVEIRO, PORTUGAL

ECOLOGIA, DISTRIBUIÇÃO, SEGREGAÇÃO POR HABITATS E MIGRAÇÃO DE MARE DO CARANGUEJO VERDE CARCINUS MAENAS NA RIA DE AVEIRO, PORTUGAL

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Marinha, realizada sob a orientação científica do Professor Doutor Henrique José de Barros Brito Queiroga, Professor Associado com Agregação, e a co-orientação do Doutor Juan Bueno Pardo, ambos do Departamento de Biologia da Universidade de Aveiro.

DECLARAÇÃO

Declaro que este relatório é integralmente da minha autoria, estando devidamente referenciadas as fontes e obras consultadas, bem como identificadas de modo claro as citações dessas obras. Não contém, por isso, qualquer tipo de plágio quer de textos publicados, qualquer que seja o meio dessa publicação, incluindo meios eletrónicos, quer de trabalhos académicos.

À minha família, “como se fosse água.”

o júri

presidente

Doutora Ana Isabel Lillebo Batista
Investigadora Principal, Universidade de Aveiro

Doutor Ricardo Jorge Guerra Calado
Equiparado a Investigador Principal, Universidade de Aveiro

Professor Doutor Henrique José de Barros Brito Queiroga
Professor Associado com Agregação, Universidade de Aveiro

acknowledgements

This study was supported by Centro de Estudos do Ambiente e do Mar da Universidade de Aveiro (CESAM), an Associate Laboratory of the University of Aveiro.

The author wishes to thank Prof. Dr. Henrique Queiroga and Dr. Juan Bueno Pardo for the advice and guidance during the work and for the critical revisions of the paper. Prof. Dr. Sizenando Abreu, Mr. Fernando Mouro and Pedro Soares for the advice and help with technical issues during experimental trials. Prof. Dr. Sizenando Abreu, Mr. Aldiro Pereira, Pedro Amaro and Mr. João Silva for the precious help during field work. Ricardo Dionísio and Diogo Oliveira for the crucial help during lab work. Also, Carolina Ventura, Abel Mirassol, Fernando Pilão and Daniela Marques for volunteering to help with field and laboratory work when needed. A very special thanks to my friend and co-worker Frederico Selores for the always present help during field and laboratory work and for the helpful discussions during the writing of this document. This paper could not have been so successfully executed without his presence.

agradecimentos

Este estudo contou com o apoio do Centro de Estudos do Ambiente e do Mar da Universidade de Aveiro (CESAM), laboratório associado da Universidade de Aveiro.

A autora deseja agradecer ao Prof. Dr. Henrique Queiroga e ao Dr. Juan Bueno Pardo pelos conselhos e orientação durante a realização do trabalho e revisão e criticas do artigo. Ao Prof. Dr. Sizenando Abreu, Sr. Fernando Mouro e Pedro Soares pelo auxílio e conselhos técnicos na criação e montagem do equipamento de filmagem. Ao Prof. Dr. Sizenando Abreu, Sr. Aldiro Pereira, Pedro Amaro e Sr. João Silva pela ajuda preciosa durante o trabalho de campo. Ao Ricardo Dionísio e ao Diogo Oliveira pela ajuda crucial durante o trabalho de laboratório. À Carolina Ventura, ao Abel Mirassol, ao Fernando Pilão e à Daniela Marques por terem prestado serviços de voluntariado no campo e no laboratório sempre que fora necessário. Por fim, um muito especial agradecimento ao meu amigo e colega de trabalho Frederico Selores pela presença assídua e indispensável durante os trabalhos de campo e laboratório e pelas úteis conversas durante a escrita deste documento. Sem a sua presença este trabalho jamais teria sido conseguido com o mesmo sucesso.

keywords

Carcinus maenas; Habitat segragation; Geografic distribution; Gregarism; Tidal migration; Baited hoop nets; Underwater video; Estuaries; Ria de Aveiro; Portugal.

abstract

Coastal transition systems, such as estuaries and coastal lagoons, are among the most productive and valuable aquatic ecosystems. They have a great variety of habitats which perform vital functions for the species that inhabit them and that have, consequently, an overall ecologic importance in those systems. Ria de Aveiro is the largest coastal lagoon in Portugal with 4 main channels radiating from a common inlet. The aim of this paper was the study of one of the most conspicuous species present in Ria de Aveiro - the green crab *Carcinus maenas.* Intertidal crabs have extensive linkage to estuarine ecosystems processes being considered keystone species. An overall understanding of this species ecologic patterns (habitat use, spatial distribution, monthly condition, gregarious behavior and tidal migration mechanisms) may contribute to a better understanding of its interaction with the ecosystem, and, also, increase the comprehension of the ecological status of this lagoon. In order to do so, we used baited hoop nets' in a monthly sampling programme that encompassed the first 6-month period of a year. The sampling was conducted in 5 different types of habitat (Subtidal, Sand, Mixed, Zostera and Marsh) in 5 different sites along the lagoon. To study the tidal migration of this species, we used an underwater video camera to film the flood migration during the day and night time in a sandy habitat located in Mira's sub-estuary in Ria de Aveiro.

From our study, we found that the female and male population of crabs in Ria de Aveiro search more actively for food during different periods of the year for every site. We found that site and its proximity to the lagoon's mouth appear to be important factors when comparing sex segregation data. The green crab was found to presents spatial and temporal differences in the use of habitat in Ria de Aveiro. While studying the allometry of the population, we found that both sexes presented similar trends of condition through the months, with lower levels during March and higher levels in June, the last month sampled. Our findings also suggested that adult crabs may form aggregations with others with similar sizes (SD between 7 mm for females and 8 to 12 mm for males) We verified the presence of groups of 35 to 45 adult individuals with similar characteristics of size for each sex, especially evident in females. We found that tidal migrations were performed later in time during the day, around 90 to 120 minutes from low tide, than during night. Most crabs migrating were of 20-50 mm carapace width; none smaller than 10 mm was observed. We also found that the migratory behavior in this species seemed not to be segregated by size.

palavras-chave

Carcinus maenas; Segregação de habitats; Distribuição geográfica; Gregarismo; Migração de maré; Nassas; Filmagem subaquática; Estuários; Ria de Aveiro; Portugal.

resumo

Os sistemas de transição costeira, como estuários e lagunas costeiras, estão entre os ecossistemas aquáticos mais produtivos e valiosos. Com uma grande variedade de habitats que desempenham funções vitais para as espécies que os habitam, tendo, consequentemente, uma enorme importância no funcionamento ecológico desses sistemas. A Ria de Aveiro é a maior laguna costeira de Portugal com 4 canais principais radiando da embocadura. O objetivo deste trabalho foi o estudo de uma das espécies mais características da Ria de Aveiro - o caranguejo verde Carcinus maenas. Os caranguejos inter-litorais têm uma intensa ligação com os processos dos ecossistemas estuarinos, sendo considerados uma key-stone specie na Ria de Aveiro. Um estudo global dos padrões ecológicos desta espécie (uso do habitat, distribuição espacial, estado da sua condição, comportamento gregário e mecanismos de migração de maré) pode contribuir para uma melhor compreensão do seu relacionamento e interação com o ecossistema de que faz parte, fornecendo também informação sobre o estado ecológico da região. Para tal, utilizaram-se nassas como método de amostragem e a colheita de amostras foi realizada mensalmente durante os primeiros 6 meses de um ano. A amostragem foi realizada em 5 tipos de habitat (Sublitoral, Areia, Misto, Zostera e Sapal) em 5 locais diferentes ao longo da laguna. Para estudar a migração de maré desta espécie utilizamos uma câmara de vídeo com a qual gravamos a migração de enchente durante os períodos do dia e da noite num habitat arenoso localizado no sub-estuário de Mira, na Ria de Aveiro.

Verificamos que a população de fémeas e machos de caranguejos da Ria de Aveiro procura mais ativamente por alimento em períodos diferentes do ano. Descobrimos que o local e sua proximidade com a entrada da laguna parecem ser fatores importantes para a compreensão das diferentes distribuições espaciais de cada sexo pela Ria. Verificamos que o caranguejo verde apresenta diferenças espaciais e temporais no uso do habitat na Ria de Aveiro. Ao estudar a alometria da população, verificamos que ambos os sexos apresentavam tendências semelhantes de condição ao longo dos meses, com níveis mais baixos durante Março e níveis maiores em Junho, último mês amostrado. Os nossos resultados também sugiram que caranguejos adultos podem formar agregações com outros com tamanhos semelhantes (SD entre 7 mm para fémeas e 8 a 12 mm para machos). Verificamos a presença de grupos de 35 a 45 indivíduos adultos com características semelhantes de tamanho para cada sexo, especialmente evidente nas fémeas. Descobrimos que as migrações de maré durante o dia, cerca de 90 a 120 minutos após a maré baixa, em comparação com a noite, parecem ser realizadas num período mais tardio. A maioria dos caranguejos possuíam uma largura de carapaça de 20-50 mm; nenhum menor que 10 mm foi observado a executar a migração. Constatamos também que o comportamento migratório nessa espécie não parece ser segregado temporalmente por tamanho.

INDEX

1. INTRODUCTION

1.1. *CARCINUS MAENAS* – A KEY STONE SPECIES AS A STUDY MODEL

Intertidal crabs that are active during tidal exposure on the sediment surface are known to be important players in the biological dynamics of estuaries (Kristensen et al. 2008; Amaral et al. 2009; Vermeiren & Sheaves 2014). These organisms are intimately involved in many estuarine ecological processes via trophic interactions and active ecosystem engineering (Kristensen et al. 2008; Amaral et al. 2009; Vermeiren & Sheaves 2014). Shifts in crabs' populations and their distribution may have great influence in estuarine communities, which could have major effects in ecosystem functioning (Vermeiren & Sheaves 2014). Intertidal crabs have extensive linkage to estuarine ecosystems processes, with constant migration to and from intertidal zones during tide periods (Sheaves 2005) and tight relation with different subtidal and intertidal habitats and animal communities. This makes them great ecological model species to study in estuarine environments. Changes in intertidal crabs' behavior, survival, biomass, species richness and burrow morphology in response to pollution have been demonstrated in several estuaries (Bartolini et al. 2009; Cannicci et al. 2009; Penha-Lopes et al. 2009), showing that intertidal crabs are also good bio-indicators of ecosystems condition. Furthermore, studies have shown the successful use of the composition of intertidal crabs' communities to indicate condition in degraded areas and rehabilitated areas, measuring this way the ecological success of estuarine rehabilitation programs (Ashton et al. 2003; Macintosh et al. 2002; Nordhaus et al. 2009).The Ria de Aveiro hosts one of the most important and large population of green crab, *Carcinus maenas*, Linnaeus (1758), in Portuguese coastal areas (Queiroga 1996). The green crab is a littoral crab (Ingle 1983) and a major benthic inhabitant of coastal and estuarine habitats along the North and Central Europe (Queiroga 1996; Almeida et al. 2011), from where he is a native (Leignel et al. 2014). The green crab is one of the most conspicuous, ecologically important (Amaral et al. 2009) and most studied (Moksnes et al. 2003) organisms in intertidal marine and estuarine ecosystems. With high tolerance to fluctuations of environmental features, including oxygen, salinity and temperature (Leignel et al. 2014), this specie is capable to acclimate in conditions ranging from fully marine environments to low estuarine mesohaline regions (Broekhuysen 1936).

Besides being a great ecologic model, *C. maenas* is also famous for being one of the most widely distributed intertidal crabs and marine invaders in the world (Leignel et al. 2014). This crab is one the most characteristic decapod that inhabits European estuaries and rocky shores. Beside it's distribution through Northwest Europe (Baltic Sea, Iceland, and Central Norway), this species is also distributed in North African coasts - Morocco and Mauritania (Leignel et al. 2014), Mediterranean sea (Leignel et al. 2014), east and west coast of North America (Cohen et al. 1995; Grosholz & Ruiz 1995) and Australia (Vermeiren & Sheaves 2014; Thresher et al. 2003). *C. maenas'* eurytopic nature, high breeding potential, aggressive competition for resources and voracious predatory activity suggest major extensive ecosystem alteration specially in places where he is a non-native species (Conhen et al. 1995), making them strong interactors in natural local communities (Leignel et al. 2014). In such regions, this species is recognized as one of the most ecologically potent and economically damaging predators in coastal communities (Lowe et al. 2000). Due to ecological and evolutionary consequences of invasions, the ecological study of invasiveness model species in places where they are native may be an important key to help guide the control and management programs at non-native regions.

When breeding, female *C. maenas* move to the lower parts of the estuaries and hatching occurs during night-time ebb tides (Queiroga et al. 1994). During ebb, newly hatched larvae migrate vertically to the superficial layers of the water column, ensuring this way that shortly after spawning all larvae is exported to the sea (Queiroga 1995). With four pelagic zoeal stages and a megalopal stage, the larval development of the green shore crab lasts from 4–6 weeks, depending on the abiotic conditions of the development, in shelf waters (Almeida et al. 2008). With planktonic duration of up to about 50 days, *C.maenas* presents late stage larvae well distributed across the continental shelf (Lindley 1987; Domingues et al. 2011; Queiroga et al. 1994). In the Portuguese coast, higher levels of abundance of reproductive events occur during winter (Almaça 1982; Queiroga 1995), although larvae can be found in Europe’s coastal waters during most of the year (Lebour 1928; Paula 1989). The last larvae stage and the one that reinvades the estuary is the megalopa (Queiroga 1996). When analyzing results that involve megalopal supply and settlement rates, we should consider green crabs’ populations at Ria de Aveiro to be recruitment-limited (Almeida et al. 2011), so densities in this region may be smaller when comparing with studies other regions (Hedvall et al. 1998; Moksnes 2002).

In Portuguese estuaries, two peaks of recruitment can be identified: the first in late winter and the second in early summer (Queiroga 1995). In Ria de Aveiro, which is further at the north of the country, recruitment events are higher during spring (Queiroga 1993). After settlement early juveniles tend to accumulate at very high densities in biogenic substrates with complex physical structures (Almeida et al. 2011; Hedvall et al. 1998; Moksnes 2002). Green crabs are epibenthic omnivores (Mosknes 2004) with cannibalistic behavior (Almeida et al. 2011). It is important to mention that mortality by predation in green crabs is both size and habitat-specific and, from settlement to adulthood, drops exponentially, especially in highly complex habitats (Moksnes 2004; Almeida et al. 2011). Thus, the growth and survival of green crabs’ early stages is very dependent of the settlement in physically complex habitats (nursery habitats) which megalopa actively select over bare sediments (Hedvall et al. 1998). Those habitats provide food supply and, specially, shelter from predators. The status of biogenic complex substrates in intertidal and shallow-water estuarine areas are important features controlling green crab populations’ size and densities (Almeida et al. 2011).

Greens crabs’ spatial distribution patterns change ontogenetically (Almeida et al. 2011). While juvenile this species is mainly intertidal. However, as green crabs increase in size and attain maturity, they begin migrating from intertidal to subtidal habitats. Habitat-dependent predation (Moksnes et al. 1998) and crab movements (Moksnes 2004), which range increases with crab size (Almeida et al. 2008), are the main reasons behind the modification of initial occupation patterns. Also, as reported by Almeida et al. (2011), when population densities increase in intertidal complex habitats (e.g. recruitment season) and its carrying capacity exceeds, juveniles in advanced stages may be forced to disperse to less structured habitats. For this stage and beyond, this dispersal to other habitats may be a natural and necessary step promoted by growth. As carapace width ranges get bigger and individuals size increases, less adapted this crabs are for movement within highly physically structured habitats (Almeida et al. 2011). Their growth and survival is actually enhanced when they disperse to less structured habitats (Mosknes et al. 2002; Amaral et al. 2009). Although many current studies are trying understand them, the green crabs' ontogenetic migration density dependent mechanisms in intertidal habitats remain yet uncertain.

The mature and bigger sized crabs that spend most of their life in deeper areas of the estuaries still migrate with the flood to feed in intertidal habitats, returning to the subtidal habitats with the ebb - a process called tidal migration. This species has a great ability to express biological rhythms, predicting flood and ebb, which is a great adaptive value (Styrishave et al. 2004). Thus, their tidal migration activities to feed in intertidal (highly productive) habitats is in line with tidal invasion of intertidal banks (Hunter & Naylor 1989; Warman et al. 1993). Green crabs' migration is expected to be higher during nighttime as in happens with many other crustaceans (Styrishave et al. 2004). The execution of the tidal migration during nighttime periods may reduce the risk of exposure to desiccation and low salinities (Reid & Aldrich 1989), while providing effective protection against predation (Dumas & Witman 1993). Furthermore, it has also been suggested that intraspecific differences may occur in such migratory behavior dependent upon size (Naylor 1962) and moult stage (Warman et al. 1993).

1.2. MAIN CHARACTERISTICS OF COASTAL LAGOONS: THE CASE STUDY OF RIA DE AVEIRO

Estuaries are highly productive systems containing a high diversity and density of fish and invertebrates (Beck et al. 2001) which are directly dependent on estuarine particular habitats for a variety of ecological functions such as spawning, recruitment, nursery, feeding and refuge (Amaral et al. 2009).

The Ria de Aveiro is the most extensive shallow coastal lagoon in the Northwest coast of Portugal (Teixeira 1994), with 45 km long and 10 km wide, comprising an extension of 74 km^2 (Teixeira 1994). Located between 40 138ºN and 40 157ºN (Dias 2001), this complex system is formed by four main channels: Mira and Ílhavo at the south, and Ovar, and Espinheiro at the north. It is a very shallow lagoon, with an average depth of 1 m (Lopes et al. 2008), reaching the highest depths just near the inlet (Rodrigues et al. 2011). Ocean tides and river flows combined effect is one of the main drivers of the estuarine dynamics (Rodrigues et al. 2012). These variables create two axes of environmental forces shaping the habitats found at Ria de Aveiro. First, the lunar semi-diurnal component (Dias 2001) creates a diversity of zones related to the coverage of water during tides. Tides at the Ria de Aveiro range from 0.6 m in neap tides to 3.2 m in spring tides (Rodrigues et al. 2012), with a tidal range of 2.1 m at the inlet (Morgado et al. 2003). These zones are the subtidal (always covered by water), intertidal (only covered during flood tide), and supratidal (never covered by water). Second, a longitudinal gradient is created by the distance to the mouth of the estuary, being that salinity and water temperature values are similar to that of the sea close to the lagoon's mouth and similar to freshwater further upstream (Dias 2001). Due to these environmental and hydrological features, each one of the main channels can be understood as individual estuaries (Araújo et al. 2008).

Hence, the physical characteristics of a given location at the Ria de Aveiro can be described according to these combined forces: 1) the duration of the exposure to tide, indicating the proportion of time covered by water, and 2) the distance to the mouth of the estuary, which indicates the range of values of salinity and temperature. These main environmental gradients shape the kind of habitats present within this estuarine ecosystem, and are the main drivers of the types of sediments, vegetation and fauna present.

In this sense, subtidal habitats are more constant over time than intertidal habitats because they are constantly covered by water. The intertidal zone represents the 87% of the total estuarine area and hence represents the most important feature of the Ria de Aveiro (Vasconcelos 2009). Close to the mouth and from the subtidal to supratidal areas, the main intertidal habitats that can be found with increasing level of structural complexity are: sand banks, mixed mudflats, *Zostera* prairies, and salt marshes. Sand banks are mainly characterized as non-vegetated areas and represent the main linkage between subtidal and medium - upper intertidal habitats. Mixed habitats are a generally sparsely vegetated habitat and more structurally complex. Their composition varies considerably, but they are mainly characterized by high presence of shells and muddy sediments with a sparse cover of algae (Almeida et al. 2011). Mixed habitats are very important as transition zones between non-vegetated and vegetated intertidal habitats. The prairies of *Zostera* are the main habitats providing shelter for fish and invertebrates during ebb tides. Populations of *Zostera noltii* are common in the Ria de Aveiro, but *Zostera marina* (typical from the subtidal zone), seems to be extinct since 2008 (Azevedo et al. 2013). Finally, salt marshes are important vegetated habitats from the intertidal and supratidal zones. In fact, the Ria de Aveiro hosts some of the healthiest populations of *Halimione portulacoides*, *Salicornia ramosissima* and *Sarcocornia perennis* of Portugal and some of the largest of Europe (Lillebø et al. 2009).

As the distance from the mouth increases, the horizontal disposition of the habitats partially changes. Mixed mudflats and *Zostera* habitats disappear and the horizontal transition from sandy habitats to salt marshes or artificial rocky shores is more evident. Rocky shores are still quite uncommon in the Ria de Aveiro, being mostly found in upstream locations in the Mira channel. Similarly to the Mixed sediments, rocky habitats can be characterized as structurally complex habitats, and are mainly formed by rock blocks with sparse coverage of algae.

1.3. ECOSYSTEM SERVICES OF RIA DE AVEIRO

From the point of view of human activities and benefits, estuaries are known for supporting high levels of productivity and diversity of ecosystem goods and services essential to mankind (Sousa et al. 2013; Vermeiren & Sheaves 2014). A long-term continued provision of ecosystem services depends on a thorough understanding of the system and proper integrated large-scale management (Lillebø et al. 2009). In this sense, the Ria de Aveiro is a quite vulnerable system, hosting a great amount of industry activities and a growing urbanization pressure with high seasonal fluctuations. In terms of environmental quality, this lagoon has a moderately low degree of eutrophication (Lillebø et al. 2009) and has some problems in sediment quality regarding metal contamination (Vasconcelos 2009). An overall report comparing all main estuaries in Portugal and their anthropogenic pressures classified Ria de Aveiro as one of the most pressurized by resource exploitation (fishing, agriculture and aquaculture) and port activities (Vasconcelos 2009). In fact, the fishing descriptor in Ria de Aveiro had the highest score comparing to other Portuguese estuaries. In this lagoon, agriculture and aquaculture prime source of pressure is mostly related with bank reclamation and loss of habitat, whereas those activities take place mostly in former intertidal flats or saltmarshes (Vasconcelos 2009). Sousa et al (2013), whose research aimed to include the population knowledge in the identification of Ria de Aveiro ecosystem services, also mentioned fishing, shellfish harvesting and port activities (commercial ship traffic areas and intense recreational traffic) as being crucial activities for the local and regional economy. In this study, the interviewed stakeholders recognized that those activities had a strong in influence in the lagoon's ecosystem and the need to be properly managed (Sousa et al. 2013). It was mentioned as well the great diversity of fish and shellfish with commercial value that was harvested in this region (Sousa et al. 2013) and whose populations have been declining over the years (e.g. lamprey, eel, bass, bream, sole, cuttlefish, crucian carp, flounder, crab, cockles, oysters, clams and substrate worms). Ria de Aveiro's needs for conservation is not recognized only by the local population. In fact, this region is classified under the protection status of the Natura 2000 Network, within Birds and Habitats Directives, as a Zone of Special Protection (ZSP) and a Zone of Special Conservation (ZEC), respectively. This empathizes the importance of conservation of this lagoons' species and habitats (ICNF 2014). Some migratory fishes that were highly harvested in this region, and for whom this lagoon is essential to complete its breeding cycle, are under the protection provided by the directives and its fishery has been limited. This is the case of the lamprey (*Petromyzon marinus*), the allis shad (*Alosa alosa*), the twait shad (*Alosa fallaxe*) and the eel (*Anguilla anguilla*), whose conservation status is related with the conservation of spawning habitats in estuarine areas (ICNF 2014).

Increasing human pressure in estuaries is jeopardizing ecological functions and accordingly all goods and services provided from them (Vermeiren & Sheaves 2014). Degradation of an estuary can irreversibly impair the services provided with severe environmental, economic and social implications to the region (Lillebø et al. 2009). In addition to all local pressures, globalized matters as climate change may already have important consequences in estuarine habitats. Conservation of estuarine habitats seem to not have brighter future expectations if human and climatic pressures continues to increase. So it is necessary to understand how estuarine' habitats and their ecological functions stand in the current situation. The study of model organisms and the monitoring of environmental variables arise as crucial tools to understand these changes and extrapolate the main findings to other regions.

1.4. OBJECTIVES

A general overlook of this key stone species' ecology, distribution, habitat use and tidal migration could contribute to a better understanding of Ria de Aveiro's ecosystem. The study of this organism in a place where he is native can also improve the management options in those where its presence constitutes a problem. Other aspect is that this also allows future researchers to have an overall ecological status of this species in an estuarine system as past reference from which they could compare their results.

In order to do so, this study is divided in two parts. In the first part, we studied the spatial and temporal distribution of the number of crabs and its size (using carapace width as reference) in relation to habitat type. While studying local distributions and habitat use, we essentially tried to understand the following topics: 1) how did frequencies in the number of crabs changed through time and space; 2) how did crabs' size, sex and life cycle stage influence their distribution per site, habitat and month. This study was followed by a modal analysis of the size distributions aiming to understand if different cohorts could be detected per habitat sampled. Then, we executed an analysis of the allometry of size, which is the study of the relationship between body size and weight, with the aim of finding changes related to the physical condition of individuals during the period of study (Crothers 1968a). When a crab moults, it can increase in size, volume and linear dimensions, so we tried to understand how fitness condition in crabs with the same size and sex changed through time. Then, to finalize Part I, the gregarious behavior by sex of the crabs captured was studied. Size and sex data from each sample of crabs captured per baited hoop net were used to characterize the aggregations of the crabs from Ria de Aveiro.

In Part II, the migration behavior of crabs during flood tides was studied. For this, we analyzed the number of crabs moving to the intertidal flats, and the size distribution of migrants through time. This study is important to understand the dynamics of this species in estuaries with high tidal amplitude, and also to understand the importance of crab size in tidal migrations. Coupled with the information obtained in the study of gregarious behavior in Part I, this analysis could throw some light on other ecological aspects of crab size such as predator avoidance, search of food, among others.

2. METHODS

PART I – DISTRIBUTION AND HABITAT USE

2.1. SAMPLING SITES AND HABITATS

Samples were collected from January to June of 2016 at five sampling sites distributed along the Ria de Aveiro (Fig. 1). Sampling sites 1 to 3 are located along the Canal do Mira, site 4 in the Canal de Espinheiro and site 5 near the Largo do Laranjo. The Canal do Mira behaves as a single sub-estuary with a full estuarine gradient. The Canal do Espinheiro is the continuation of the Vouga River, the main freshwater course discharging into the Ria de Aveiro. Site 5, near the Largo do Laranjo, is located in the northeast arm of the lagoon, receives smaller freshwater contributions and salinity shows smaller tidal fluctuations during the summer. Tidal delay may be of 1.5 hours or more in the farthest study sites from the mouth, site 3 and site 5, an important issue to have in consideration during sampling.

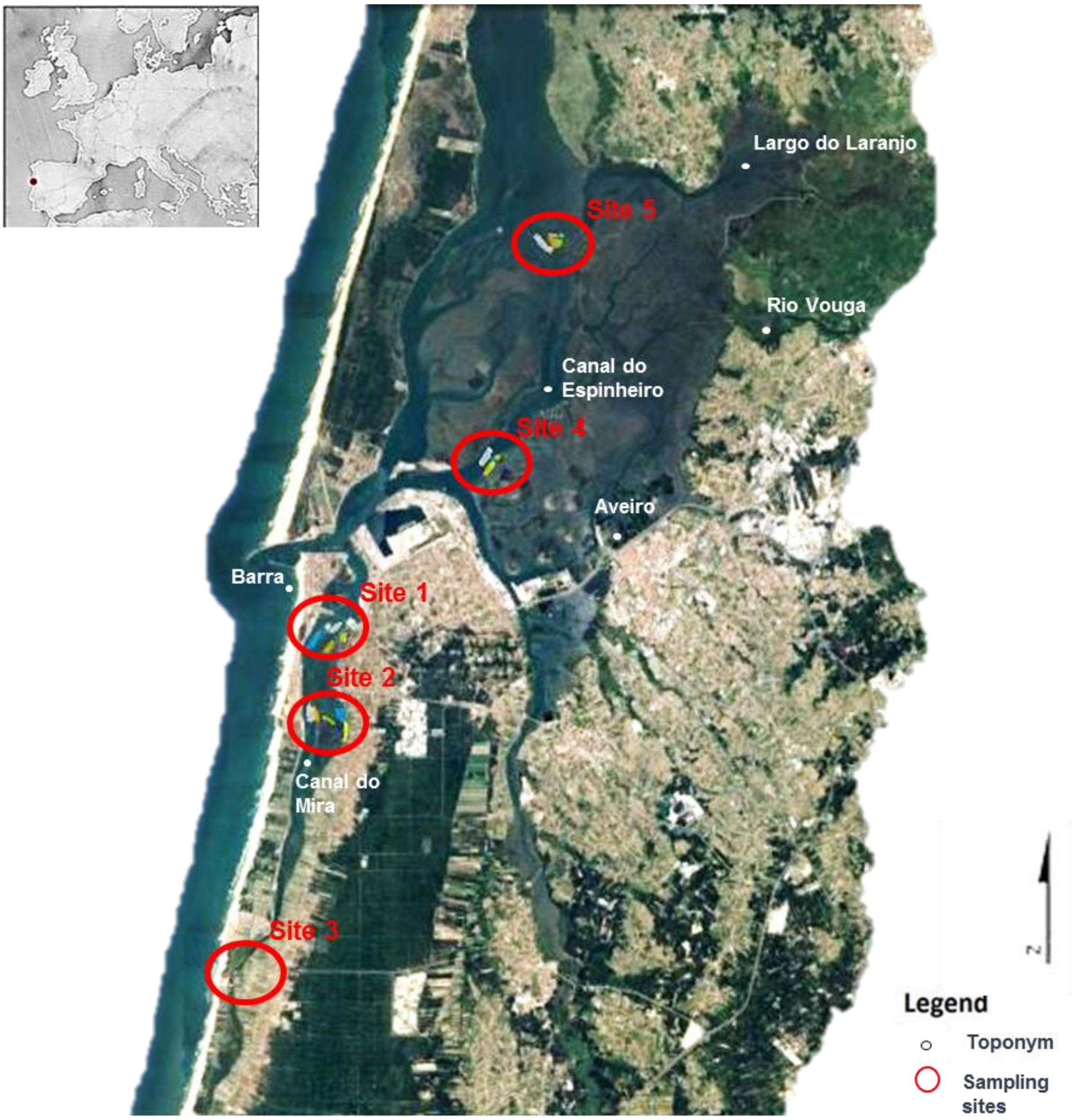


Figure 1 – Sampling sites in Ria de Aveiro, Portugal (adapted from Google Earth 2016).

The five habitat types in analysis were: i) Subtidal habitats, areas that are always underwater even in low tide and characterized by sandy sediments (Subtidal); ii) non-vegetated sandy habitats (Sand), iii) muddy sediments with sparse cover of algae and shells (Mixed); iv) seagrass beds, particularly dominated by *Zostera noltii* (Zostera); and v) salt marsh habitats (Marsh). Captures were executed under similar conditions and were always carried out during the highest tides of the month. This way every habitat was submerged and crabs, during flood, would have time to reach its habitat of choice.

Before the sampling campaign, in December of 2015, habitats' areas in each site were delimited during low tide using a GPS device (GPSmap 62st Garmin) (see Annex 1), so we could have georeferenced data to help navigation and locate the sampling sites during high tide Then, every month, three random points within each area were selected for sampling, leaving a reasonable distance (10 m approximately) between them. Each baited hoop net was submersed during 30 minutes. After that, nets were emptied and crab samples collected. Hence, for each site and month, a total amount of 3 replicates in each of the 5 habitats per site were collected.

In the Ria de Aveiro, salinities can range from 30 to 38 near the mouth and 1 to 10 at the head (Vaz et al. 2005; Quintino et al. 2009). According to this, site 3 is very different from the other sampling sites, presenting low salinity ranges, particularly during high rainfall seasons in spring. Additionally, site 3 does not include both mixed and *Zostera* habitats. For that reason, samples from site 3 were not considered in the study of the habitat.

2.2. SAMPLING METHODS

In each sampling site, baited hoop nets (area of 19.63 cm^2) were deployed in the previously defined areas corresponding to the different habitats. In order to standardized the crabs' abundance in the catches, the following rules were applied. First, mussels were caught on the day before the beginning of each month campaign. Approximately the same weight of smashed mussels were prepared as bait in each individual net. The odor of mussels in the beginning of its decomposition stage is a low cost bait to marine researchers and an attractive food supply to crabs. Although for carnivorous crabs, such as *C.maenas,* carrion forms only a small part of their diet (Warner, 1977), decaying food supply naturally releases a sufficient amount of compounds which serves as primary feeding stimulants (Hayden et al. 2007). Second, we made sure that the bait in each trap had proximally the same quality and attractiveness to crabs in all sampling sites. This was made by renewing, if necessary, the bait in each net before beginning sampling at a different site. At last, every month campaign was realized during highest tides days so that every habitat type in the analysis would be underwater and equally predisposed to crabs. Sampling was made in two consecutive days during the highest tides of each month. Additionally, in each habitat, temperature, salinity and depth were also collected using a Aanderaa Instruments hand held probe.

2.3. DATA COLLECTION AT THE LABORATORY

During triage and data collection, crabs were preserved alive at 4 ºC in the laboratory. Besides the number of individuals per replicate, sex, carapace width (CW) and weight were recorded for each individual. Males and females' morphologic description for *C. maenas* specie were established using Crothers' nomenclature standards (Crothers 1968). Carapace width was measured to the nearest 0.5 mm, using a caliper or, in the case of the smallest crabs, under a stereoscopic microscope (equipped with an eye piece graticule). Crabs' CW was measured between the 5th tooth of the antero-lateral borders (Queiroga 1993). Wet weight was measured in grams to the nearest 0.05 g with a laboratory balance, properly calibrated. When preserved in cold, crabs lose most of the water they normally have in the branchial chambers and adherent to the body. Even thought, the crabs were dried up with soft paper tissue to remove excess of water before weighting. Crabs were released alive after these procedures.

All features mentioned above were collected from all sampled individuals with the exception of weight, which was measured from a random subsample of 30 individuals from each habitat.

2.4. DATA ANALYSIS

Baited hoop net method presents some disadvantages as a sampling method, e.q. only active hungry crabs are caught and, as response to that, number of crabs is lower when naturally food supply is higher (Crothers 1968b). Another concern is that crabs do not usually feed during period of moult and so they are not likely to be captured at that time, which means that for each crab moult cycle they can only be caught using bait 80% of the time (Crothers 1968b). Furthermore, smaller crabs, paired crabs and parasitized crabs are unlikely to be captured in this nets, probably due to their reduced mobility (Crothers 1968b). Ovigerous females do not feed, as a result their presence in the catches using this method is as well unlikely. Finally, concerning, typical intraspecific behavior between crabs, it is considered a great possibility that smaller and injured individuals won't come with ease to baited nets where they represent an easy (and live) prey to larger carnivorous and cannibalistic crabs (Almeida et al. 2011) attracted to the nets by the smell of the bait.

In short, baited hoop nets sampling method is selective regarding the size of the crabs they attract. This should be considered while analyzing the results.

Habitat and Site segregation through time

Habitat and Site segregation study through time was done separately for females and males. The analysis was done in two steps, first looking at the number of crabs (Nº of crabs) and secondary at their size (carapace width - CW). In both analyses using statistical models, the objective was to understand the patterns of distribution according to time, site and habitat. Month and Site were considered as random factors with 6 (January, February, March, April, May and June) and 4 levels (Site 1, Site 2, Site 4 and Site 5), respectively. Habitat was considered as a fixed factor with 5 levels (Subtidal, Sand, Mixed, *Zostera* and Marsh) with decreasing degrees of structural complexity (Almeida et al. 2011) and depth.

For both variables the model design was the same and could be considered as slightly unbalanced. This was because in some situations during the field collection of the samples, particularly during the first 3 months (January, February and March), the number of replicates was 2 instead of 3 because of accidental reasons (e.g. loss of nets, nets wrongly placed). This situation was fairly rare during this 6-month experiment, can be considered as not affecting the analysis analysis of the results.

To choose which statistical model to apply to each variable, we needed to verify the normality of the distributions and its homocedasticity (or homogeneity of variances) (Marôco 2014). Initially, both variables were tested with Shapiro-Wilk normality test and was found to neither had a normal distribution for both females (Nº of crabs: W=0.73729, p-value $< 2.2 \times 10^{-16}$; CW: W=0.82155, p-value $< 2.2 \times 10^{-16}$) and males' samples (Nº of crabs: W=0.77681, p-value $< 2.2 \times 10^{-16}$; CW: W=0.93031, p-value $< 3.096 \times 10^{-10}$). When normality was rejected, raw data of the variables were transformed using the natural logarithm and the normality was re-tested using first, the Shapiro-Wilk normality test, and second, the Kolmogorov-Smirnov normality test (Marôco 2014). Again, for both variables and normality tests, the transformed variables presented p-values<<0.05 (See Annexes 2 and 3). Levene's test of equality of variance was also verified and samples were always heterogeneous. Homoscedasticity (for unbalanced designs) which compares the correlation between the number of observations and groups' variances (Marôco 2014) was tested as well for both variables. Despite the variable, the test didn't show neither positive nor negative correlation, which means that we didn't have to have any particular concerns while interpreting significant results. Another way of saying this is that neither conservative or liberal sceneries of the test became an issue during the interpretation of p-values (Marôco 2014). Descriptive information about normality, homogeneity and homoscedasticity test for both variables (N and CW) can be found in Annexes 2 and 3.

From this analyses, we found that the number of crabs (Nº of crabs) transformed distribution was extremely flat (Marôco 2014) and the maximum number of observations from de combination of factors was very small (<5), as result it wasn't possible to invoque the robustness of parametric methods such as ANOVA. Therefore, differences on crabs' numbers were examined through a non-parametric type III PERMANOVA, using the raw data of the variable and executed with PRIMER software.

In the case of CW, it was possible to invoque parametric methods' robustness (Marôco 2014), especially because the sampling size of each group combination was large (Underwood 1997) and the variables distribution seemed fairly normal (Marôco 2014) (See Annex 3). So for CW, differences between crabs throughout study factors were examined using a multivariable ANOVA model, type III, in our study computed using SPSS software.

Finally, all data figures presented in the results were created using R software. When boxplots were used they represent the minimum and maximum values (whiskers), the first and third quartile (boxes) and the median (horizontal bar).

Modal analysis

In order to identify the average size of the cohorts composing the population, the size distributions of crabs in the different habitats and months was analyzed using the Bhattacharya (1967) method. No statistical indices were used to separate crabs' instars. For instance, Almeida et al. (2011), also used the Bhattacharya method to identify moult cohorts in this crabs' juveniles and for that they attributed to crabs' instars only when separation indices were equal or higher than 2.0. Instead, in our study, the model components were discriminated using biological criteria such as the expected appearance of certain groups of crabs through time. This routine was accomplished using *MixedTools* package in R software. The number of model components was adapted to the CW frequency histograms individually for every combination of habitat and month. We used the ecological knowledge of this species life cycle events in Ria de Aveiro, e.g. recruitment (Domingues et al. 2011; Queiroga et al. 1994) and reproduction peaks (Queiroga et al. 1994) through the year, as guidance in the determination of the model components.

Allometry

To understand how fitness condition in crabs changed through time, we estimated the linear regressions between the natural logarithm (ln) of CW and weight for each month, separately for each sex, by pooling data from all sites and habitats. Comparisons between monthly regression lines were done by means of an Analysis of Covariance (ANCOVA) univariate type III model, with the CW as co-variable and month as a fixed factor. All descriptive and statistical analysis behind the use of the transformed variables and ANCOVA univariate model can be found in Annex 4. The weight of a 40 mm CW standard individual was then estimated from the monthly regression lines and the changes through time were interpreted using ANCOVA's model results.

Gregarious behavior

To study gregarious behavior, we used each crab size that we could possibly capture using baited hoop nets and we compare itself with: first**,** the mean size of its netmates; second, the standard deviation of its netmates and; third, the number of netmates. The netmates are the crabs which are captured in the same baited hoop net of the analyzed crab. To finalize we also included an analysis, for each sex, of the average number of captures in each net in relation with its average size. The aim of this study was to understand if groups of crabs with similar sizes exhibit gregarious behavior.

PART II – TIDAL MIGRATIONS

2.5. STUDY SITE LOCATION

Site 1 was the location chosen for the migrations experiments. This site was chosen because preliminary results showed densities of crabs in this region, specifically in the sandy habitat, which were assumed to be migrating from subtidal to intertidal habitats. In this habitat, the experiment was conducted near the low intertidal limit, represented in white in Fig. 2 (N 40º37'26.3''; W 008º 44'26.2'').

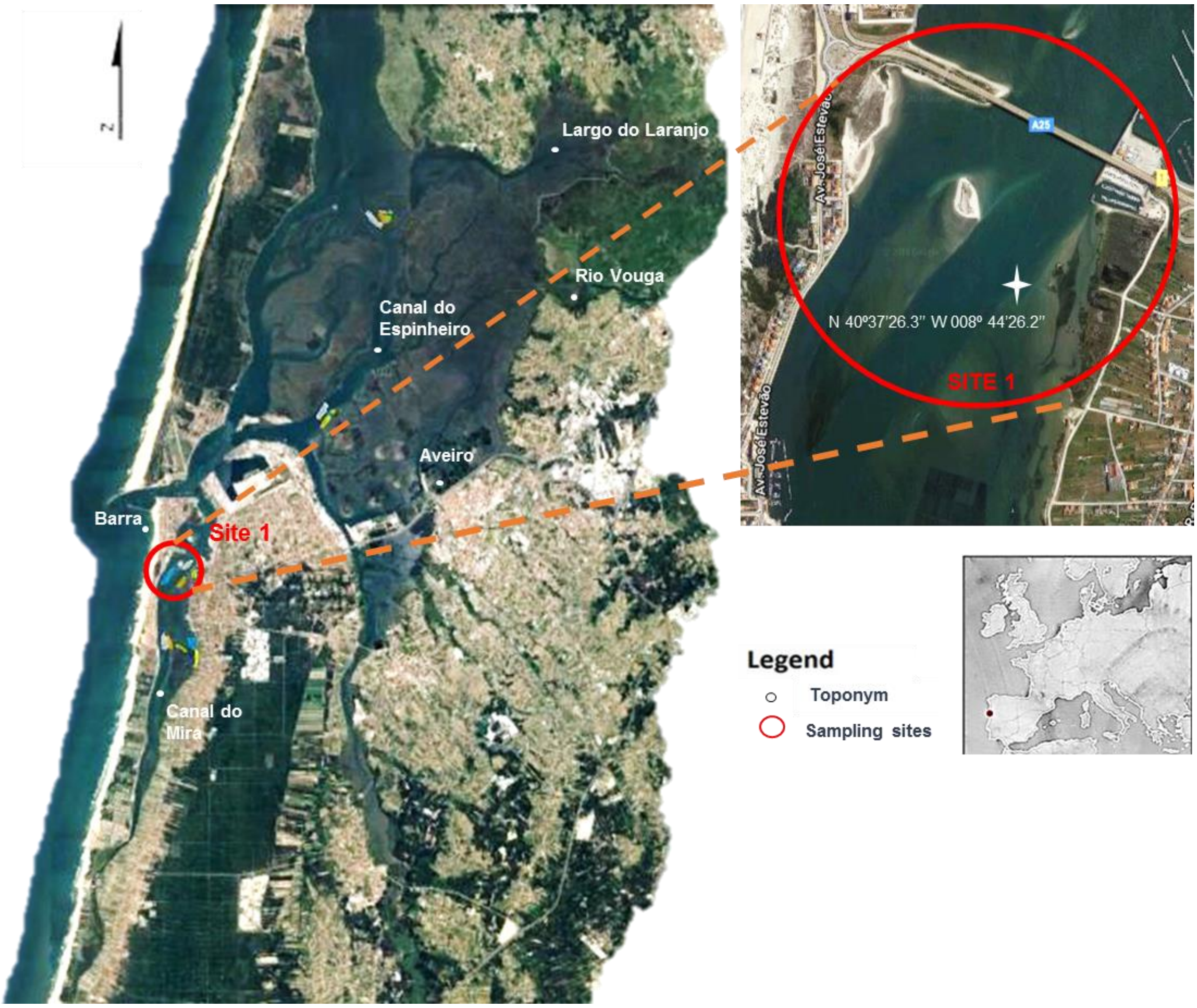


Figure 2 – Location of the tidal migration experiment in Ria de Aveiro. The figure on the right was adapted from Sousa et al. 2016 and the figure on the left was designed using the Google earth satellite image of the site sampled.

2.6. EXPERIMENTAL SETUP

Two recording experiments were conducted from low tide to high tide peak, in June 20th an 21th during the day and in July 6th and 7th during the night. Information about the tidal conditions and the starting time of each recording experiment can be found in Table 1.

Table 1 – Tidal migration experiment's dates, the hour of low tide, the range of the flood, the starting time of the experiment and its delay from low tide.

	Date	Low tide (h)	Range (m)	Starting time of the experiment	Experiment delay from low tide
Day 1	20/06/2016	10:42:00	3.18	11:32:25	00:50:25
Day 2	21/06/2016	11:17:00	3.22	11:32:30	00:15:30
Night 1	06/07/2016	23:46:00	3.28	22:34:31	00:12:31
Night 2	07/07/2016	00:29:00	3.20	00:38:00	00:09:00

The video camera used was a **IP Bullet 2MP Ambrella S2** (Fig. 3 A). It was concealed in a waterproof cylindrical inox case designed specifically for this experiment (Fig. 3 B).

Figure 3 – Real images of the IP Bullet Video camera S2 (A) (source: Ambrella) and its waterproof cylindrical inox case (B).

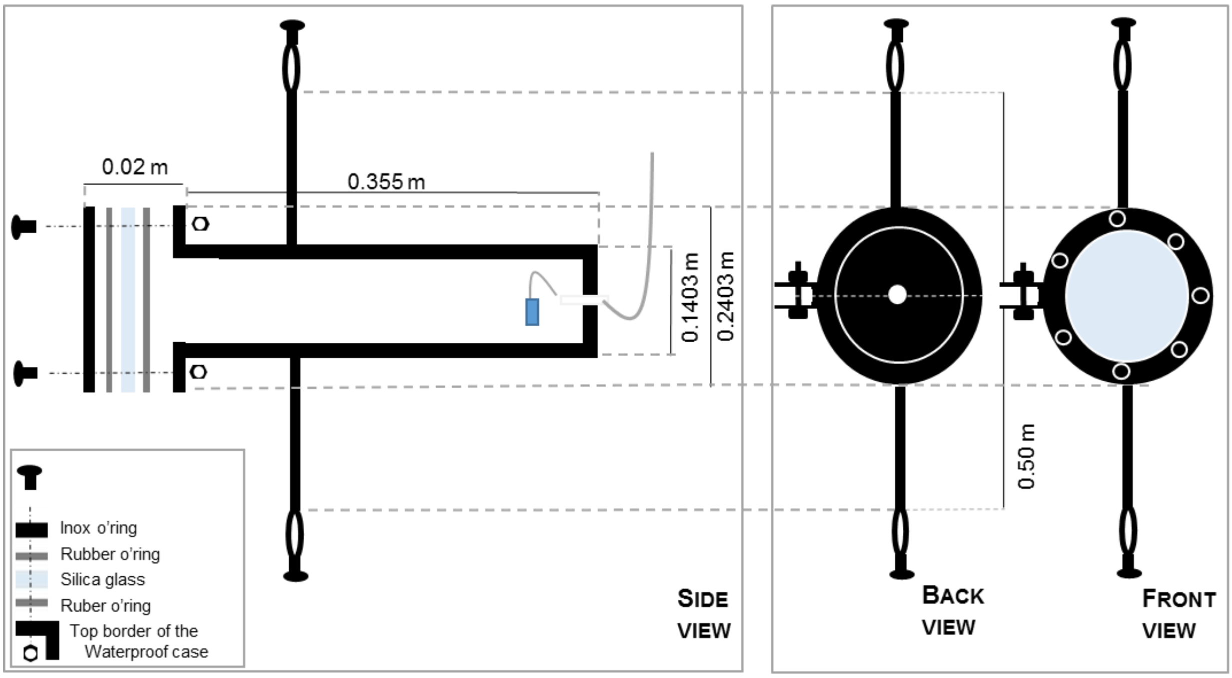


Figure 4 - Waterproof inox case technical scheme: Side view (on the left), Back and Front view (on the right).

The waterproof inox case technical scheme, which shows the indications for its assemblage, is presented in Fig. 4. Detailed information related to the practical setup of the scheme presented in Fig. 4 can be found in Annex 5. There are also listed the main materials used in the creation of this waterproof case, its market names and references. The experimental setup is presented in Fig. 5.

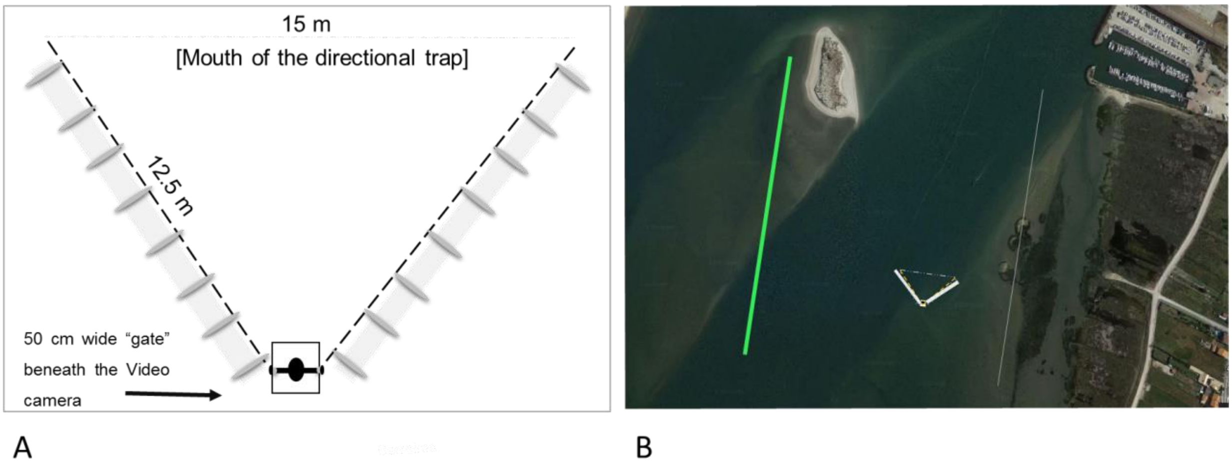


Figure 5 – Top view of the experiment set up (A) and representation of the position of the experiment in relation to the center of Canal do Mira in Ria de Aveiro (B), refereced with a green line. The figure on the left was designed using the Google earth satellite image of the experiment site.

Two 12.5 m long x 0.5 m high plastic fence was fixed to the bottom with metal stakes at approximately 90º angle, so that an open 15 m wide front faced the channel and a 0.5 m wide gate was located on the opposite side. The video camera was positioned at a height of 0.40 m of the bottom at the gate. This arrangement was meant to conduct the crabs during the flood migration, so that the density of the crabs at the gate would be 30 times higher than at the open front, therefore increasing the probability of recording migrating individuals. A similar setup was used by Dare & Edwards (1981) and Holsman et al. (2006). A 0.5m x 0.5 m gridded plate was placed below the camera providing a frame of reference to estimate crabs' CW. The color of the plate used was white for daytime experiments and black for nighttime experiments, in order to increase crabs' contrast against the bottom. . The open mouth of the setup was located perpendicular to, both, the center of Canal do Mira and the low water line, as presented in Figs. 5B and in Fig. 6.

Figure 6 – Experimental setup.

2.7. VIDEO ANALYSIS

The Network Video Monitor System software was used to enhance the quality of image during the recordings in the field. The video analysis was performed with VLC Media Player software. All crabs recorded during the two 5 – 6 h experiments were counted and its CW was estimated from the video image of each individual while crossing the gridded plate (Fig. 7). CW was estimated to the nearest 5 mm.

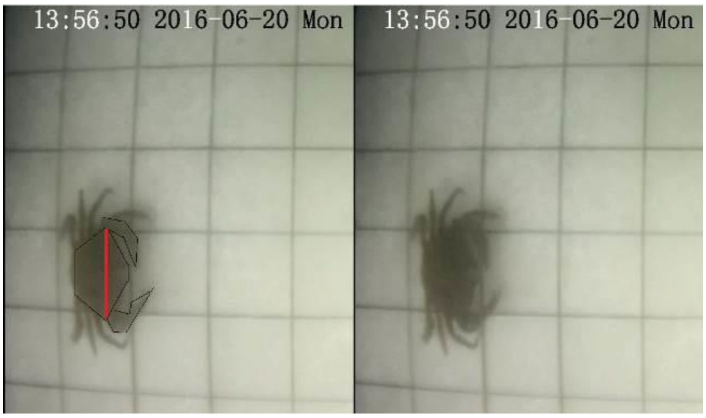


Figure 7 – Example of the approach used in order to estimate each single individual's CW: an individual categorized as size group 5 - crabs with CW ≥ 50 mm.

3. RESULTS

PART I – DISTRIBUTION AND HABITAT USE

3.1. NUMBER OF CRABS AND ITS RELATION WITH SITE, HABITAT AND MONTH

The total number of crabs captured per month, from January to June of 2016, is presented by sex in Fig. 8. An average of 1 916 crabs were collected per month, with a maximum of 2 191 in February and a minimum of 1 508 in April. As illustrated in Fig. 8, female crabs represent around two thirds of total amount of crabs collected (7 293 females in 11 457 captures).

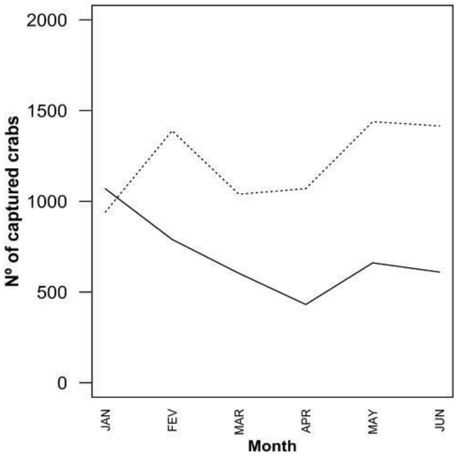


Figure 8 – Number of crabs captured per month.

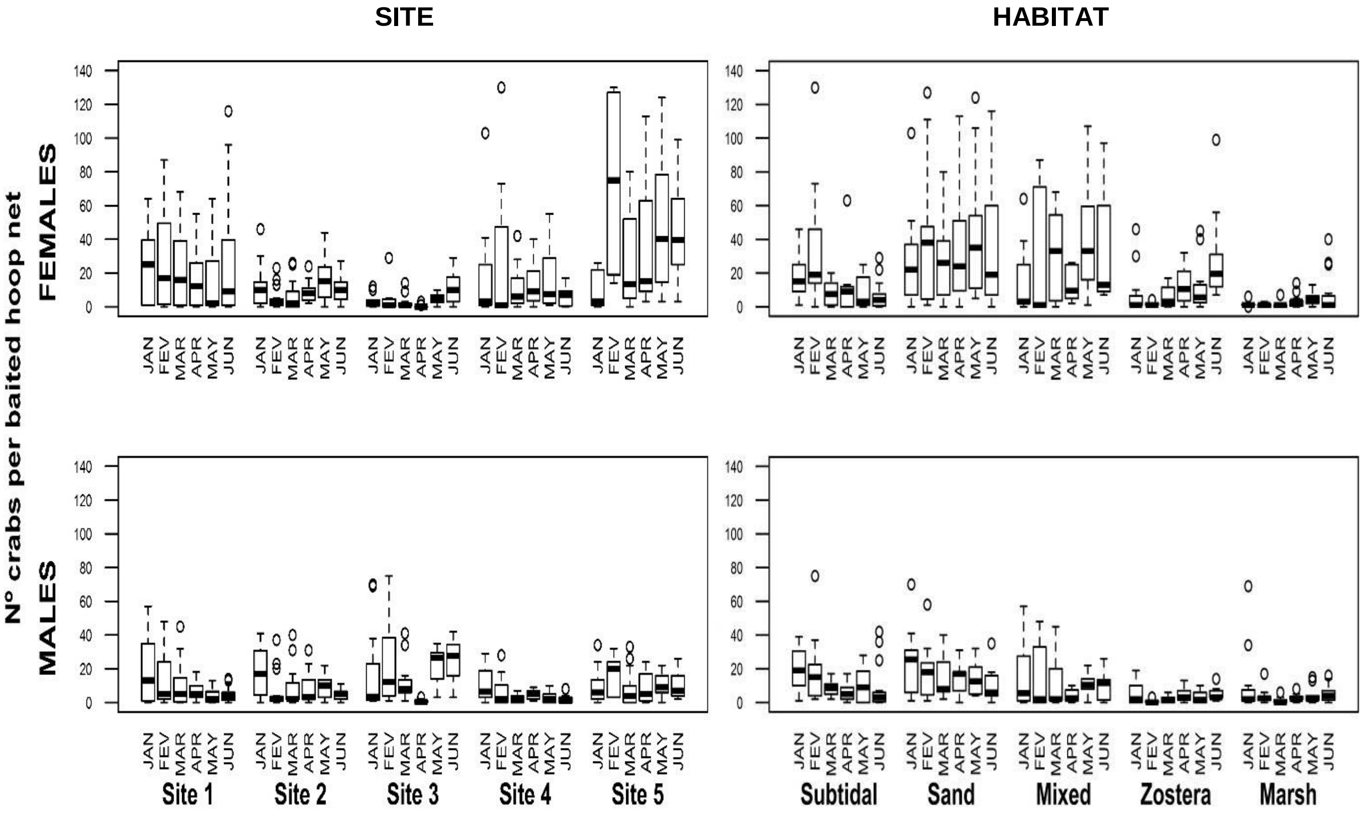


*Figure 9 – Medium boxplot of the number of female and male crabs captured per baited hoop net per **site** through time (on the left) and per **habitat** through time (on the right). Note that the habitats are ordered according to its depth in high tide from the highest,* Subtidal *habitat, to the lowest, salt* Marsh *habitat.*

Table 2 – Nº of crabs and its relation with Site | Habitat | Month factors using PERMANOVA statistical model.

	Type III Square Sum	df	Mean Square	Pseudo-F	Unique perms	p-value	
Month	5051.1	5	1010.20	0.84	9960	0.5593	
Habitat	57139.0	4	14285.00	5.02	9928	0.0004	***
Site	28333.0	3	9444.20	7.82	9957	0.0022	**
Habitat * Month	19513.0	20	975.67	1.18	9907	0.3069	
Site * Month	18585.0	15	1239.00	6.78	9910	0.0001	***
Habitat * Site	24681.0	12	2056.70	2.49	9936	0.0101	*
Habitat * Site * Month	47151.0	57	827.22	4.52	9856	0.0001	***
Res	41867	229	182.83				
Total	252230	345					

Month	3277.2	5	655.43	5.02	9945	0.0075	**
Habitat	6641.2	4	1660.30	2.33	9930	0.0269	*
Site	1509.1	3	503.03	3.86	9949	0.0348	*
Habitat * Month	3711.7	20	185.59	1.31	9921	0.2121	
Site * Month	2008.0	15	133.87	5.89	9928	0.0001	***
Habitat * Site	7119.9	12	593.32	4.19	9925	0.0001	***
Habitat * Site * Month	8084.4	57	141.83	6.24	9877	0.0001	***
Res	5202.5	229	22.718				
Total	38170	345					

PERMANOVA of the CW showed a highly significant (Table 2; p-value << 0.001) 3-way interaction among Site, Habitat and Month in both, indicating a complex pattern of habitat utilization (Fig. 9). The number of crabs captured per baited hoop net remained more or less constant over time in each site for both sexes. However, female crabs were consistently more abundant than males in sites 1, 4 and 5, being almost inexistent in site 3 until May. A large variation was observed through time and site in the case of females. By opposition, males seem fairly well distributed through the sites sampled in Ria de Aveiro (Fig. 9).

Concerning the number of crabs per habitat, higher number of crabs in subtidal, sand and mixed habitats were observed in both sexes (Fig. 9). Higher number of captures were verified in Sand, Mixed and Subtidal habitats (Subtidal: 2391, Sand: 4366, Mixed: 2529, Zostera: 1051, Marsh: 656). In spite of a large variability of crab numbers with time, general trends in abundances can be identified among the different habitats (Fig. 9). This matter will be further addressed in relation with the results of CW analysis.

3.2. CRABS SIZE AND ITS RELATION WITH SITE, HABITAT AND MONTH

Crab size increased from January to June in both sexes, with males consistently larger than females on average (Fig. 10). The size of the females and the males' crabs captured during this 6-month experiment ranged from 4 to 61.5 mm and from 5 to 70 mm, respectively.

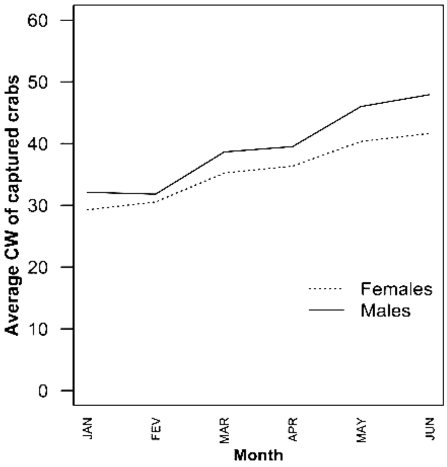


Figure 10 – Average CW of captured crabs per Month and per Sex.

This growth pattern resulted from non-proportional changes in the absolute abundances of the different size classes, which followed similar trends in both sexes, when pooling the catches from all sites and habitats per month (Fig. 11). Size classes 30-40 mm and 40-50 mm dominated the catches in January and February, with values above 200 individuals, and decreased in numbers from March onwards. The size class > 50 mm was captured in numbers usually below 100 individuals per month, with the smaller catches in March and April. Size classes < 20 mm and 20-30 mm had catches between 50 and 220 individuals in January and February, decreasing to less than 50 individuals from March onwards.

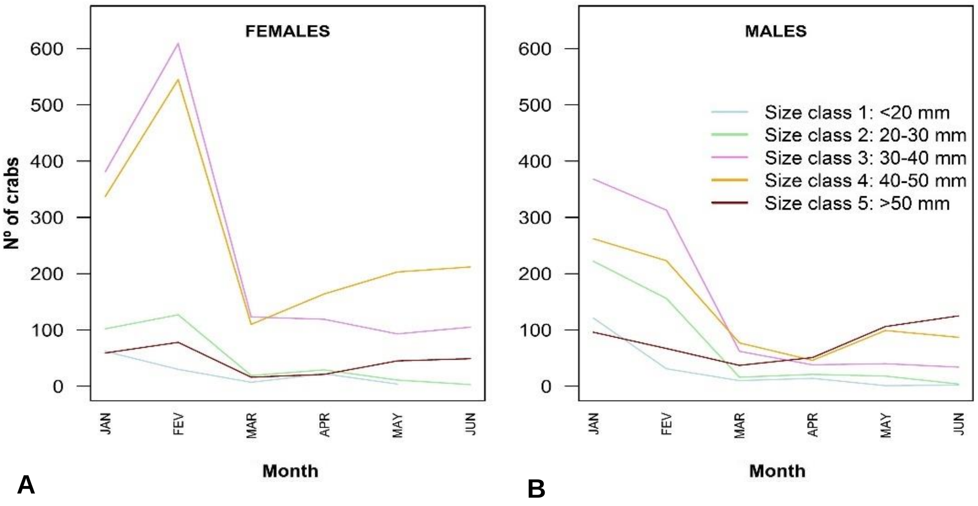


Figure 11 - Number of female (A) and male (B) crabs captured per month. Both variables presented are described according to their group of size (labels on the right).

Table 3 - CW and its relation with Site | Habitat | Month factors using ANOVA statistical model.

		Type III Square Sum	**df**	**Mean Square**	**Z**	**p-value**	
Females	Intercept	9794.56	1	9794.56	1182.25	0.0000	***
	Month	41.31	5	8.26	12.81	0.0000	***
	Habitat	22.09	4	5.52	4.12	0.0120	*
	Site	2.87	3	0.96	1.63	0.2314	
	Habitat * Month	30.10	20	1.50	3.58	0.0000	***
	Site * Month	6.46	15	0.43	1.25	0.2603	
	Habitat * Site	12.47	12	1.04	2.81	0.0041	**
	Habitat * Site * Month	37.81	55	0.69	20.56	0.0000	***
Males	Intercept	8575.45	1	8575.45	1126.30	0.0000	***
	Month	39.55	5	7.91	6.77	0.0016	**
	Habitat	25.25	4	6.31	4.93	0.0113	*
	Site	3.57	3	1.19	0.78	0.5230	
	Habitat * Month	21.57	20	1.08	1.46	0.1344	
	Site * Month	19.59	15	1.31	2.00	0.0312	*
	Habitat * Site	16.14	12	1.34	1.93	0.0505	
	Habitat * Site * Month	52.90	52	1.02	13.92	0.0000	***

ANOVA of CW indicated a highly significant (Table 3; p<< 0.001) 3-way interaction among Site, Habitat and Month in both sexes, reflecting a complex pattern of distribution of size classes among the factors (Annexes 6, 7 and 8). However, when the data from all Sites are pooled by Habitat and Month (Fig. 12) a clear pattern emerges in both sexes. Mixed, Zostera and Marsh habitats, which are those located in higher tidal levels and with higher structural complexity, had median sizes below 30 mm CW in the first months of the time series, while in the Subtidal and Sandy habitats the median sizes were always above 30 mm. Moreover, a clear size increase was observed in all habitats from January through June.

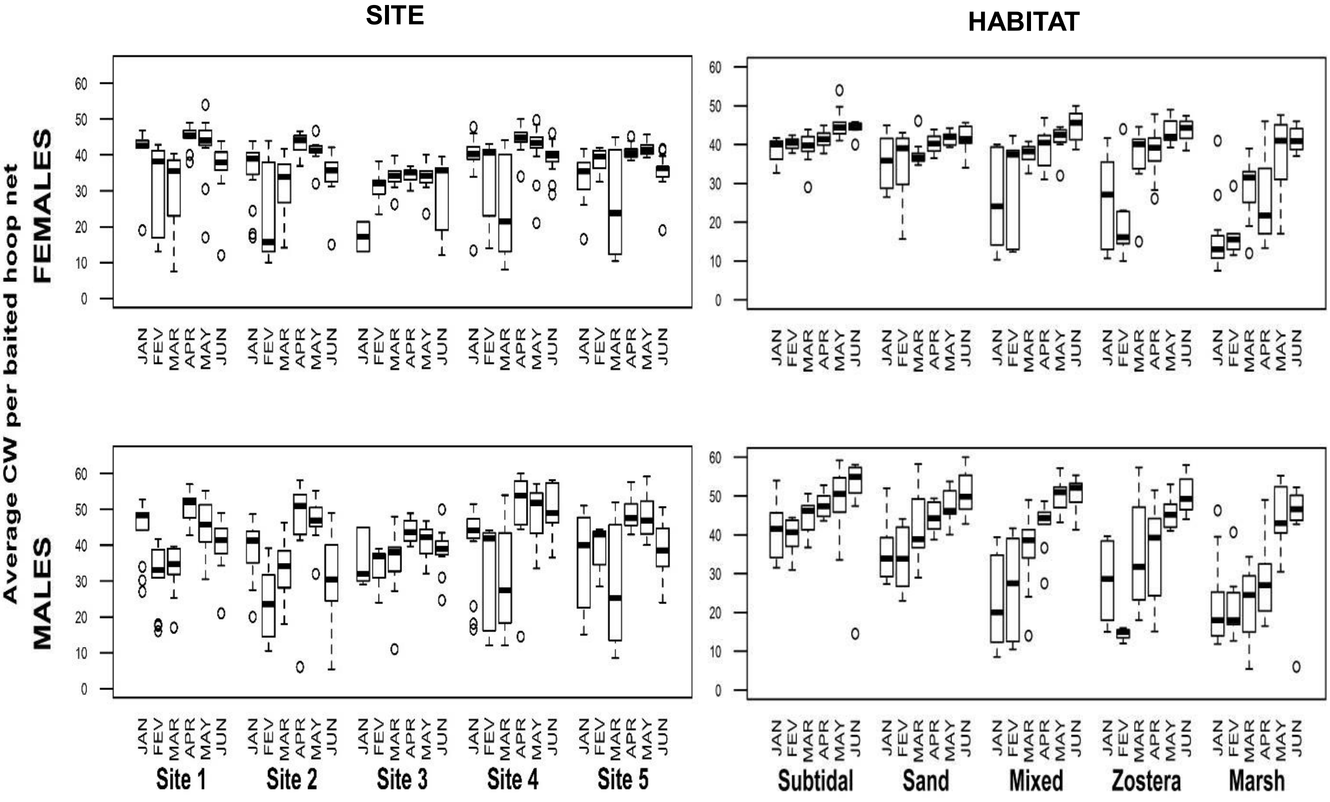


*Figure 12 - Median boxplot of the average carapace width of the crabs captured per baited hoop net per **site** through time (on the left) and per **habitat** through time (on the right). Note that the habitats are ordered according to its depth in high tide from the highest,* Subtidal *habitat, to the lowest, salt* Marsh *habitat.*

Regarding habitat use by the totality of crabs captured in this 6-month experiment, we found that the presence of size classes of immature crabs increases as the complexity of the habitat is higher and its depth at high tide is lower (Table 4). The presence of size classes characteristic of mature crabs has the exact opposite behavior, being higher in subtidal habitats, where its presence represents 90% of the individuals captured. There is in Annex 9 an illustration that resumes the information presented in Table 4 about each size class habitat use per crabs' sex.

Table 4 – Number of captures per Size class and Habitat. Comparison between Nº of captures of immature and mature crabs.

	Subtidal		**Sand**		**Mixed**		**Zostera**		**Marsh**			
Size class	Nº of captures	%	Nº of captures	%	Nº of captures	%	Nº of captures	%	Nº of captures	%	**Total per Size class**	**% per Size class**
Size class 1 (≤ 20 mm)	22	0.92	59	1.35	87	3.44	65	6.18	131	19.97	364	3.31
Size class 2 (20 - 30 mm)	203	8.49	484	11.09	231	9.13	89	8.47	91	13.87	1098	9.99
Immature crabs	225	9.41	543	12.44	318	12.57	154	14.65	222	33.84	1462	13.30
Size class 3 (30 - 40 mm)	927	38.77	1819	41.66	852	33.69	373	35.49	184	28.05	4155	37.80
Size class 4 (40 - 50 mm)	938	39.23	1583	36.26	1050	41.52	420	39.96	186	28.35	4177	38.00
Size class 5 (> 50 mm)	301	12.59	421	9.64	309	12.22	104	9.90	64	9.76	1199	10.91
Mature crabs	2166	90.59	3823	87.56	2211	87.43	897	85.35	434	66.16	9531	86.70
Total per Habitat	2391		4366		2529		1051		656			
% per Habitat	21.75		39.72		23.01		9.56		5.97			

3.3. BHATTACHARYA MODAL ANALYSIS

The decomposition of the size distributions into normal components, which can be interpreted as representing different age classes, is complicated by the presence of heterogeneity of size distributions among sites and habitats, and by the fact that the data so far obtained only represent part of the annual cycle. Taking this into account, different analyses are presented pooling the data into habitats (Fig. 13), pairs of months and habitats (Fig. 14) and months (Fig. 15), separately for females and males. Depending on month, two or three modal components could be identified. Other arrangements of this results can be found in Annexes 10 and 11.

The presence of smaller individuals with carapace lengths ranging up to 20 mm in structurally complex habitats (Mixed, Zostera and Marsh) occurs throughout the time series, but is more evident in females in January and February (Figs. 14 and 15). Crabs in the size classes ranging from 20 to 50 mm are present in all habitats throughout the year (Figs. 14 and 15). Bigger sized crabs, larger than 50 mm, were almost exclusively males and were mainly captured in the Subtidal and Sandy habitats. As a consequence, physically complex habitats showed a larger variety of size classes than non-vegetated and deeper habitats.

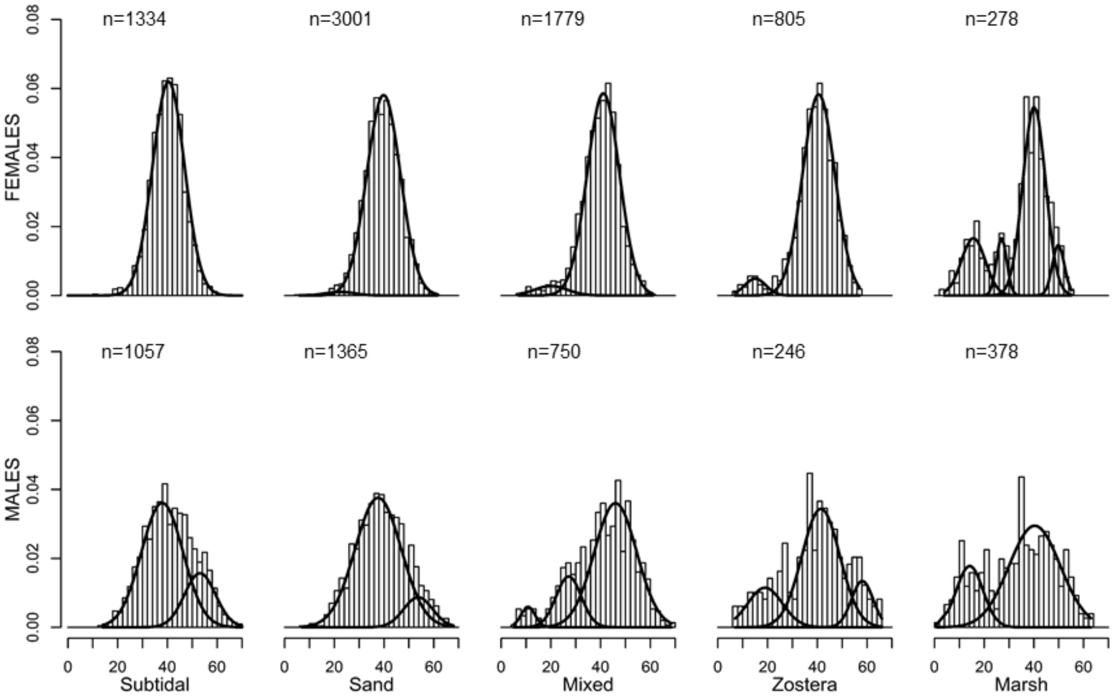


Figure 13 - Modal analysis of CW distribution per habitat

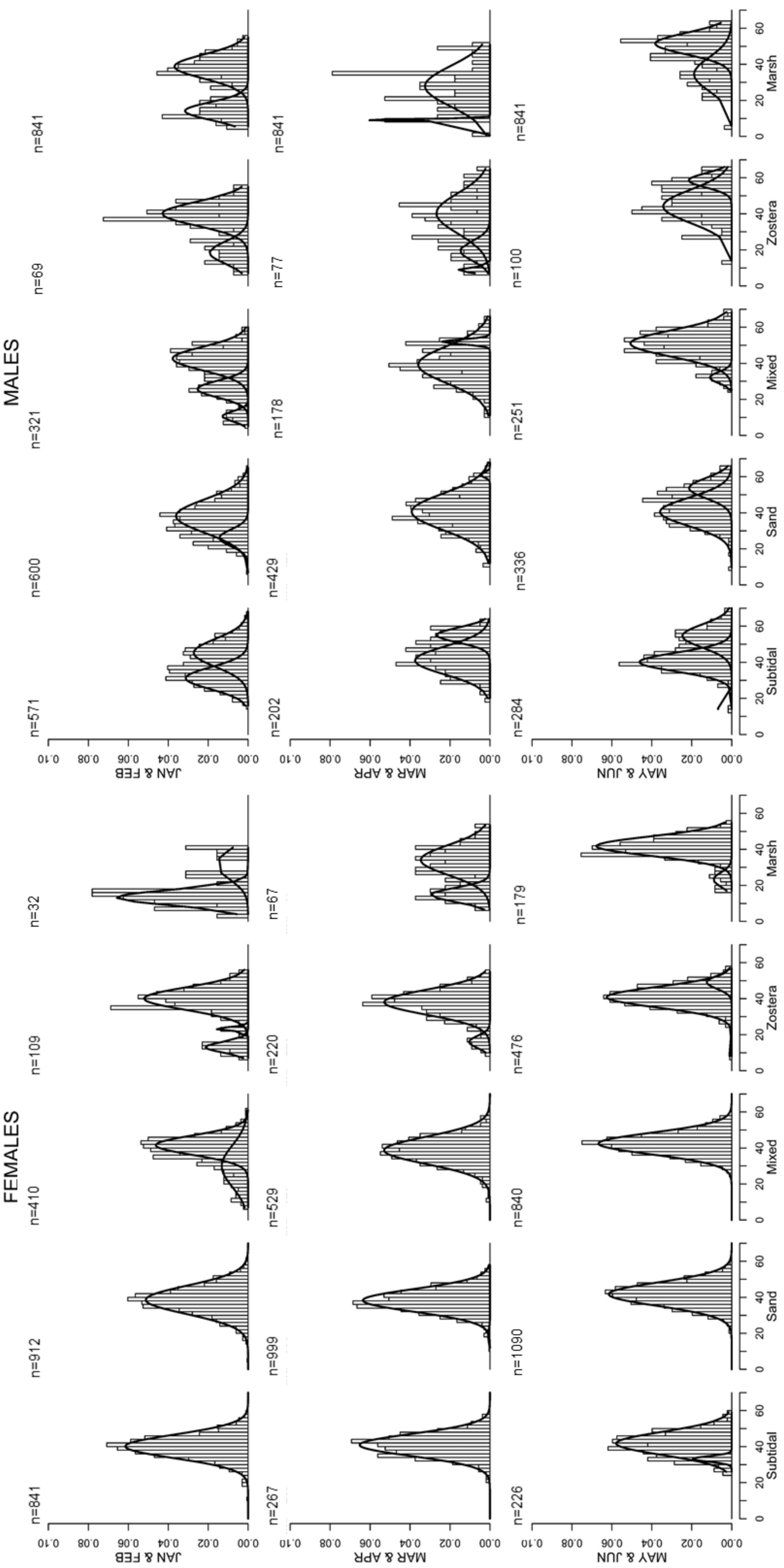


Figure 14 –Bi-mensal modal analysis of female and male crabs' carapace width per habitat.

Inspection of the size distributions pooled into months (Fig. 15) clearly indicates the presence of the smaller size classes (< 25 mm) in January to April and approximately constant average sizes of the remaining classes until March, for both sexes. Starting in April for males, and June for females, the data indicate that crabs larger than 30 mm entered a growing season, concurrently with the split of a larger component into two (or three) components, suggesting that the large normal component may be composed of different age classes.

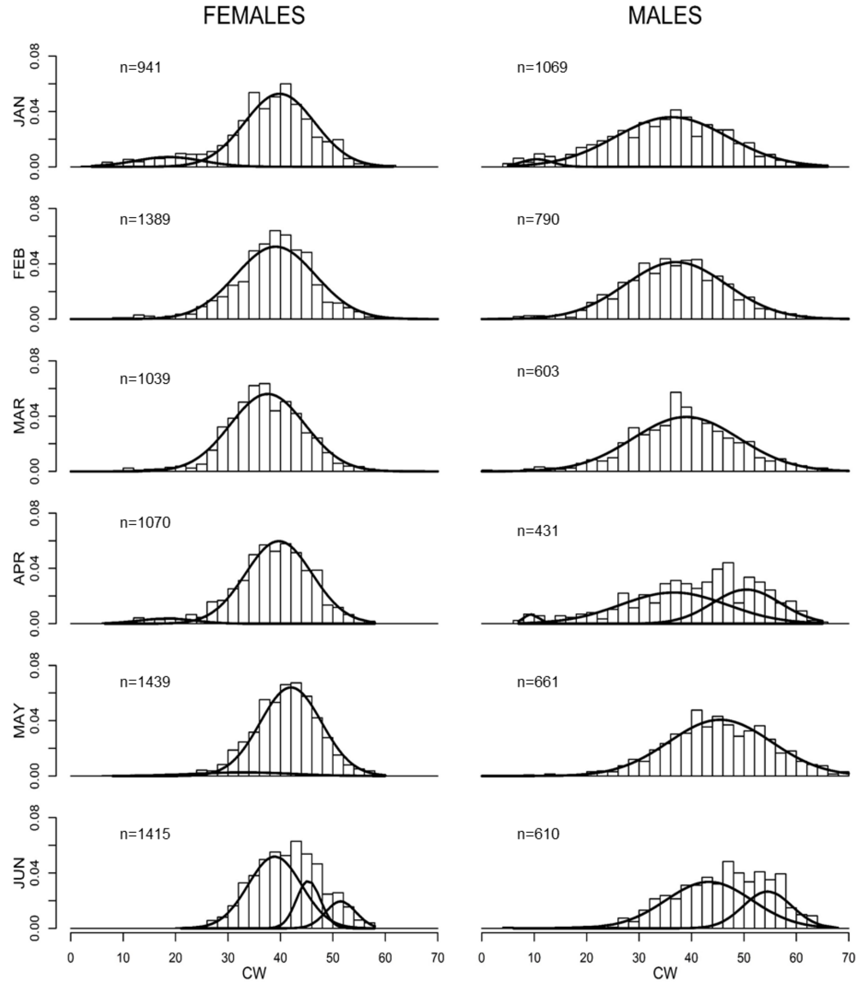


Figure 15 –Modal analysis of CW distribution per month.

3.4. ALLOMETRY ANALYSIS

The monthly regression lines between ln(weight) and ln(CW) (Table 5, Fig. 17 in the following page) were highly heterogeneous in both sexes, as indicated by ANCOVA analyses (Table 6; Month*CW interaction with p<< 0.001).

Table 5 - Regressions lines' slope(d) and intercept(b) per Sex | Month.

ln(weight)=**d**.ln(cw)+**b**

	Females		Males	
Month	d	b	d	b
JAN	2.80	-7.66	2.83	-7.75
FEV	2.63	-7.04	2.80	-7.63
MAR	2.70	-7.31	2.43	-6.29
ABR	2.85	-7.83	2.91	-8.04
MAI	2.73	-7.40	2.91	-8.04
JUN	2.65	-7.03	2.95	-8.17

Table 6 - ANCOVA metric showing that the homogeneity between the regression lines of the different months was rejected.

	Females						**Males**					
	Tipo III Sum of Squares	**df**	**Mean Square**	**Z**	**p-value**		**Tipo III Sum of Squares**	**df**	**Mean Square**	**Z**	**p-value**	
Corrected model	1936.20**a**	11	176.014	6017.326	0.0000	***	2884.00**b**	11	262.185	6022.177	0.0000	***
Intercept	359.094	1	359.094	12276.218	0.0000	***	445.202	1	445.202	10225.925	0.0000	***
Month	1.659	5	0.332	11.346	0.0000	***	7.168	5	1.434	32.928	0.0000	***
CW	683.162	1	683.162	23355.034	0.0000	***	894.101	1	894.101	20536.75	0.0000	***
Month * CW	1.628	5	0.326	11.132	0.0000	***	7.219	5	1.444	33.164	0.0000	***
Error	103.520	3539	0.029				86.159	1979	0.044			
Total	25767.552	3551					14454.54	1991				
Corrected Total	2039.671	3550					2970.198	1990				

a R Square = 0.949 (R Ajusted Square = 0.949)

b R Square = 0.971 (R Ajusted Square = 0.971)

The analysis of the temporal progression of 40 mm CW standard individuals (Fig. 16; see full pair-wise statistical analyses in Annexes 12 and 13) indicates that both females and males in June were significantly heavier than in the preceding months, with the standard female attaining a higher weight than the standard male (15.3 g for the female, 14.8 g for the male). March was the month when the condition was lower (14.2 g for the female, 14.6 g for the male). The average weights of standard female and male during the whole period were 14.5 g and 14.8 g, respectively.

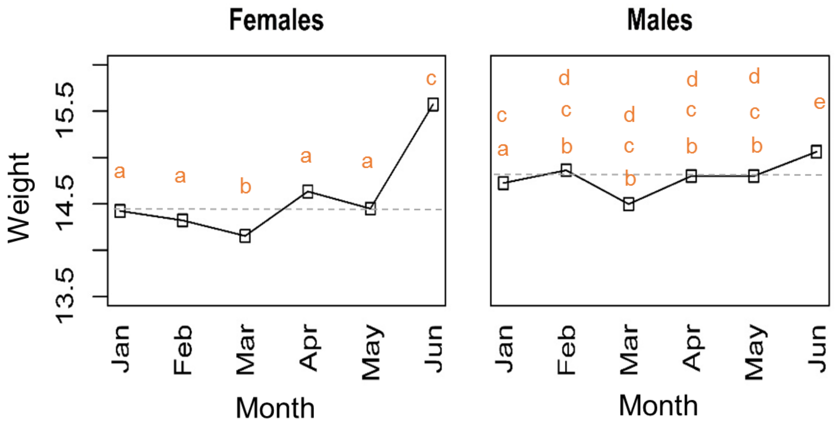


Figure 16 - Estimated weigh of a standardized individual (with 40 mm CW for both sex) for this 6-month time series using each month regression line's equation is presented in Table 4. Letters above each dot represent homogeneous groups of month (see full pair-wise statistical analyses in Annexes 11 and 12). In a dashed line is presented the average of the estimated weight for each sex (F:14.47; M: 14.76).

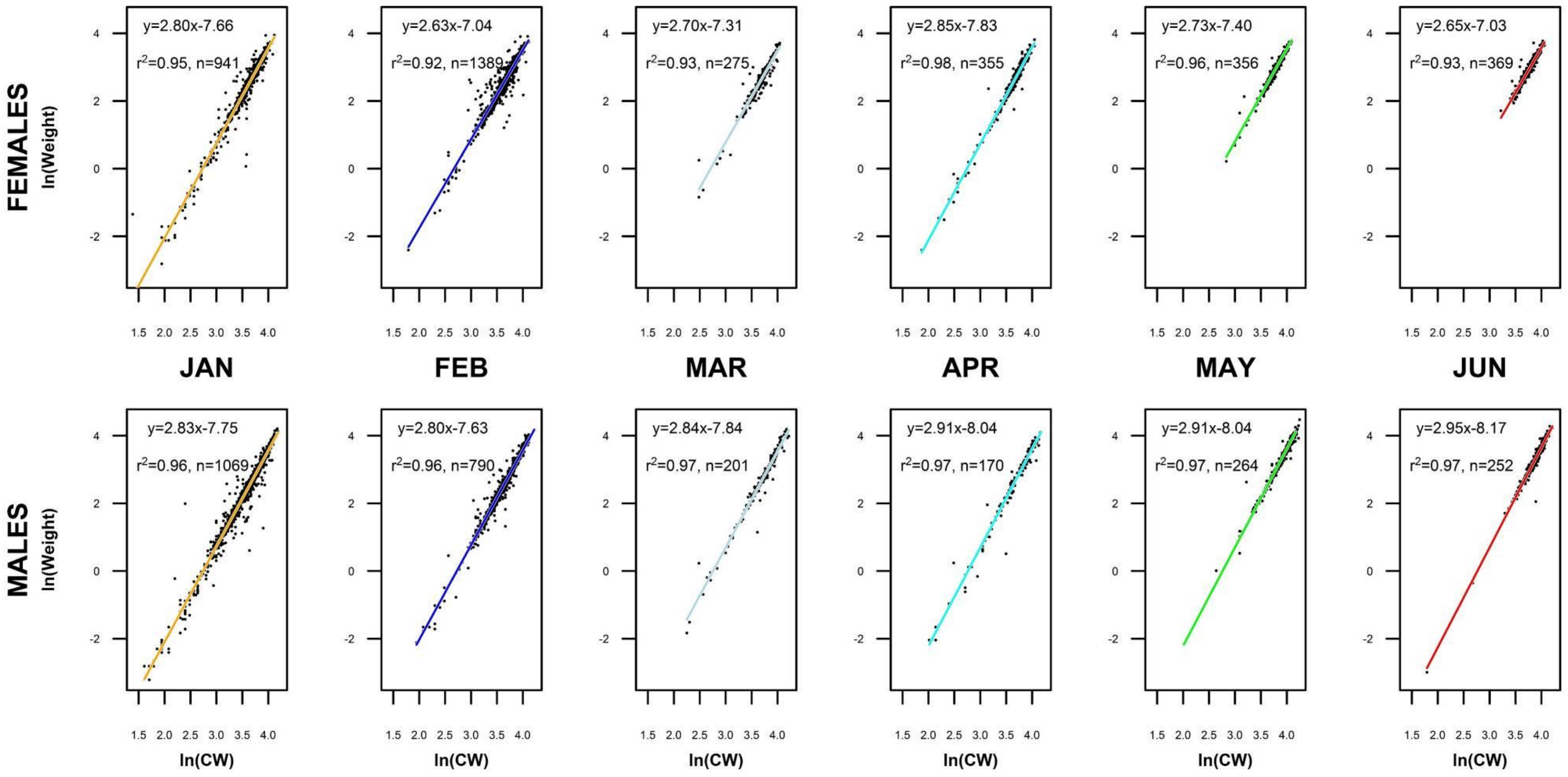


Figure 17 - Samples and correspondent regression lines between crabs' CW and Weight separated by month for ***a)*** *Females (on top);* ***b)*** *Males (on the bottom). In black for each figure is presented the regression line formula, its coefficient of determination (r^2) and the number of crabs sampled (n).*

3.5. GREGARIOUS BEHAVIOR MEDIATED BY SIZE

In both sexes, individuals in the size range from 25 to 55 mm were collected with netmates of lower average size ranges, between 35 and 45 in females and between 30 and 50 mm in males (Figs. 18A and 18D). In the 25 to 55 mm range, CW standard deviation tended to be lower than in size classes below and above this range, around 7 mm in females and 9 mm in males (Figs. 18B and 18E), indicating a relatively homogeneous composition of the catch. These catches tended also to be relatively large, composed of 20 to 45 females or 25 to 45 males (Figs. 18C and 18F). Females below 25 mm CW tended to occur in more homogeneous or more heterogeneous groups, as indicated by standard deviations around 4 and 12 respectively, while in the case of males in this size range the standard deviation of the size was very variable (Figs. 18B and 18E). Females tended also to be captured in groups larger than the groups of males (Figs. 18C and 18F).

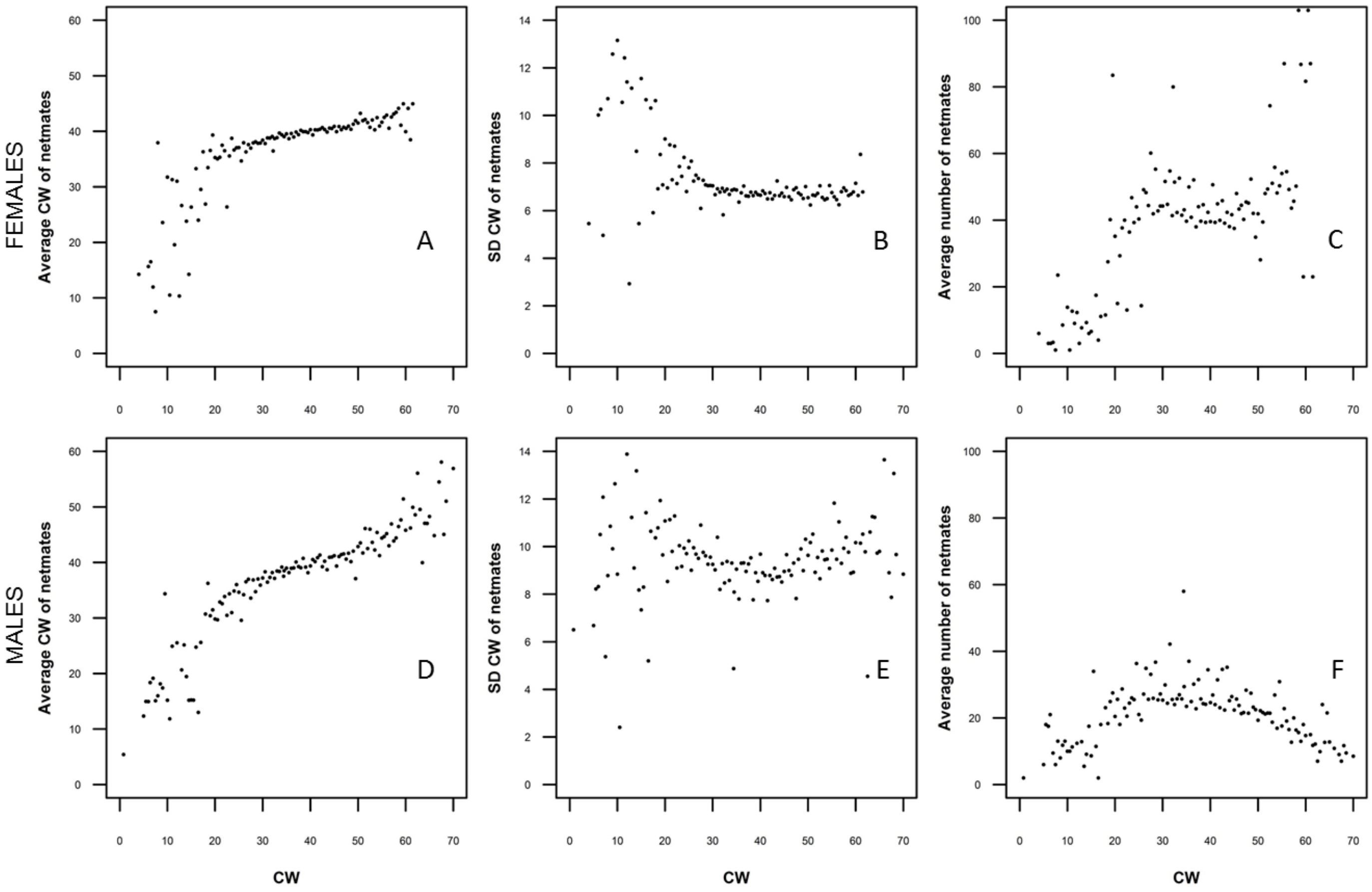


Figure 18 – Average CW of netmates (A, D), standard deviation of CW of netmates (B, E) and average number of netmates (C, F) as a function of CW size classes, separately for females (A, B, C) and males (D, E, F). Size classes in 1 mm intervals.

Females above 35 mm CW were sometimes collected in high numbers, with over 70 individuals in a single net, while the number of males in this size range collected per net was usually below 30 (Figs. 19A and 19B).

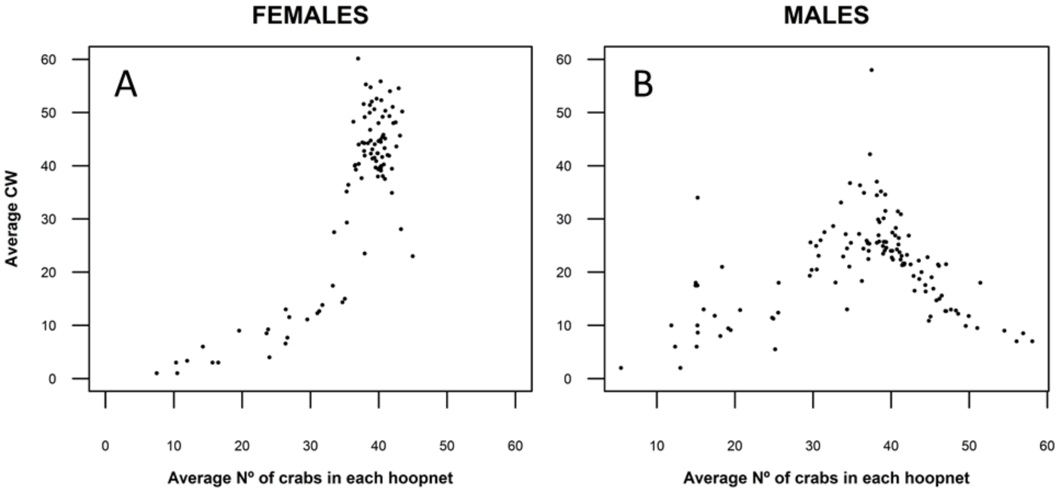


Figure 19 – Relation between the average number of female (A) and male(B) crabs captured in each net and its average size (mm).

PART II – TIDAL MIGRATIONS

3.6. MIGRATION STUDY

Recordings of migrating crabs were obtained for two day flood tides and two night flood tides (Fig. 20).

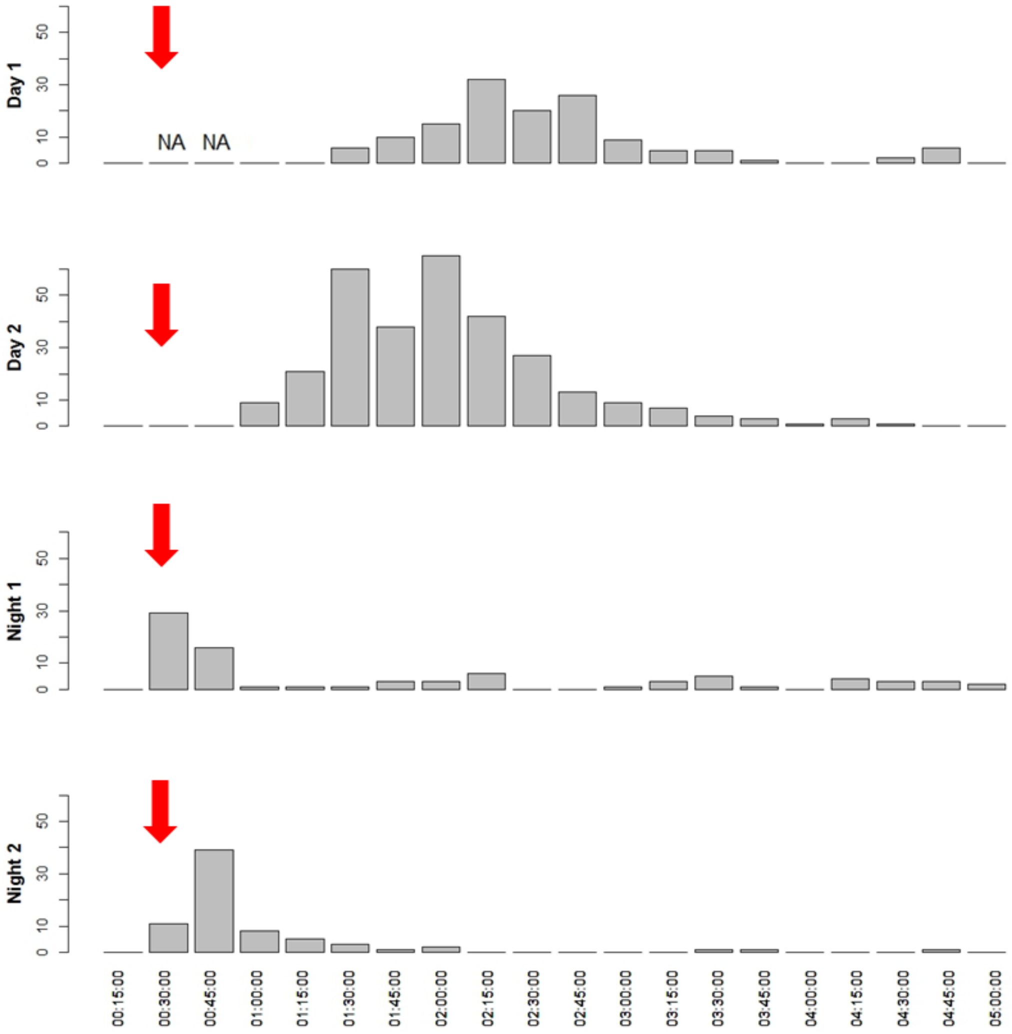


Figure 20 - Number of crabs filmed every 15-minute since low tide. The red arrows represent the moment that water reached the filming area.

Recordings started 15 min after low tide at the recording site. During Day 1 recordings only started 30 minutes after the water had reached the camera area, i. e., about 50 min after low tide at the site (See Table 1 - Methods). Day 2 had the highest number of migrating crabs with total count of 303 animals filmed, which corresponded to 50% of the total amount of crabs filmed in this experiment. During the day, the maximum rates of migration occurred 60 to 120 min after the start of the recordings, i. e., 75 to 135 min after low tide. Those peaks occurred after the immersion of the video camera lens, at about 90 to 105 min since low tide, which corresponded to a water height of 40 - 50 cm.

During the night, the maximum rates of migration occurred 15 to 30 min after the start of the recordings, i. e., 25 to 45 min after low tide. Therefore, the data indicate that the tidal migration started earlier during the night, while the flat was not yet covered by the tide and the water column height in the recording area was inferior to 40 cm. This was also noted visually before the start of the night recordings. The data also suggest that the night migration is undertaken by a smaller number of crabs. Due to worse recording conditions during the night, when the only light source were the camera infrared lights, some individuals could have escaped detection, especially at the edges of the recording area. The worse recording conditions were mainly noticed during the last two hours of the flood, when the velocity of the water was higher and the amount of IR light that reached the bottom was diminished. These periods occurred after the peaks of tidal migration and the number of individuals that were recorded still migrating were relatively small. Because of that, this underestimation is considered to be no more than 20%. Accounting for this source of error, the number of migrating crabs recorded during the night would still be less than half of those recorded during the day.

A qualitative inspection of the data decomposed by size class (Fig. 21) indicates that the crabs did not appear to be segregated by size during the migration

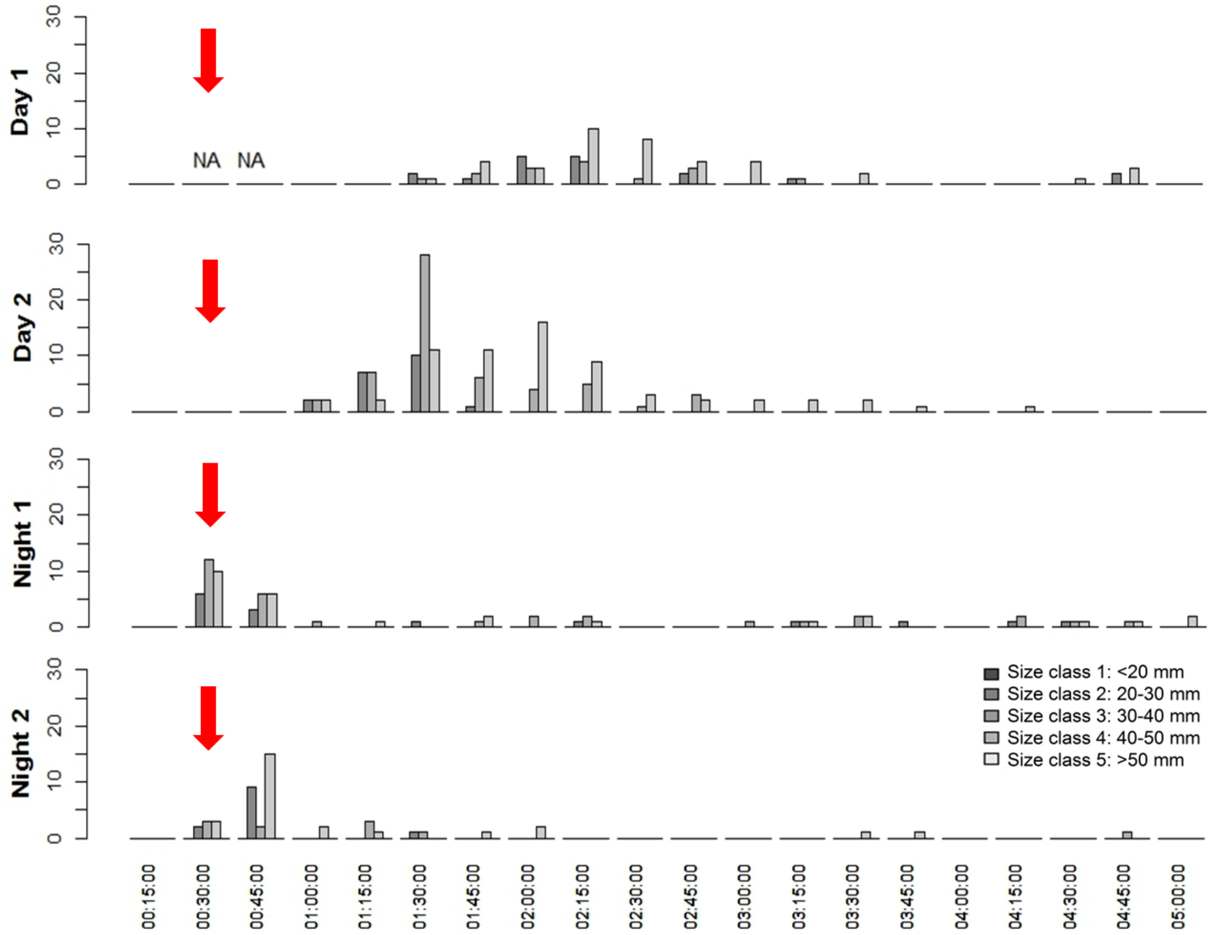


Figure 21 – Number of crabs, that fit different groups of size's criteria, filmed every 15-minute since low tide. The red arrows represent the moment that water reached the filming area.

4. DISCUSSION

PART I – DISTRIBUTION AND HABITAT USE

4.1. CRABS' DISTRIBUTION IN RIA DE AVEIRO: MAIN TEMPORAL TRENDS

We have found that female crabs represent the majority of the crabs collected (Fig. 8), and that their average size each month is always smaller than males (Fig. 10). We also verified that 50% of the crabs captured in this 6-month period had a CW within 35 to 45.5 mm (inter-quartile range's limits for all sizes distribution). In *C. maenas* species, males attain sexual maturity at a CW of 20 - 30 mm and females between 15 and 31 mm, depending on the season of the year and local environmental features (Broekhuysen 1936; Crothers 1968a). According to this, most crabs with CW larger than 30 mm are probably sexually mature. The number of mature crabs was dominant over the number of immatures, for both sexes and for time period studied (Fig. 11 and Table 4). However, we verified that the number of mature crabs in the baited hoop nets had been declining for males since late spring until June (Fig. 11B) and were smaller for females during the months of March and April (Fig. 11A). A decrease in the number of captured crabs over the sampling months was observed and it was coincident with the beginning of this species' reproduction season in the Ria de Aveiro, which reaches its highest picks during summer, from June to September (Domingues et al. 2011). It is possible that the warm weather and mild temperatures of 2016' late spring may have rushed the beginning of the mating period.

Mating in the shore crab coincides with females' moult (ecdysis), in which females feeding activity is low, and copulation usually occurs shortly after this event (Hartnoll 1969). Soft-bodied females pair with males to mate, whose predominant behavior is to guard the female under his abdomen (Crothers 1968a) protecting and defending the female against predators and other competitors (Hayden 2007). While matting, the search for food appears to be secondary for both sexes, being however ultimately dependent on the physiological state of the individual (McFarland 1976), and during copulation neither animal feeds (Spooner et al. 2007). Males are continuously searching for mates and during the reproduction season nearly all adult females are fertilized (Broekhuysen 1936; Crothers 1968a). This may be the possible reason why the frequencies of male crabs captured with baited hoop nets have continually dropped since this year early beginning of the reproduction season (Fig 11b). Females that are physically unfit to mate or that are not yet prepared to moult, which is when mature females are more receptive to mate, or even that already were fertilized (and been thought moult), showed, comparing with males, intense feeding activity during summer (Hayden et al 2007). Since the beginning of the summer season (May), the number of female crabs captured with baited hoop nets presented an increasing trend (Fig. 11A).

Hayden et al. (2007) measured in laboratory the relative intensity of feeding responses of intermoult *C. maenas* females and males over an annual cycle. Their results also showed sex-specific differences over the months. Males were also more active during winter and females during summer. This subject is highly linked to crabs' condition and weight, so this discussion will be continued during the Allometry study (Section 5.4). Another point of discussion is that, as our results are concordant with other studies, we could also say that the bait the we used, mussels' carrion, appears to be a good feeding stimulant to this carnivorous crab.

4.2. CRABS' DISTRIBUTION BY SITE: SPATIAL VARIATION

Site seems to be an important factor when analyzing the distribution of females in the Ria de Aveiro (Table 2). A great amount of females was captured in monthly sampling. A relatively high number of female crabs was always verified at the sites located near the mouth of the lagoon (Fig. 9, Sites 1 and 4). The average number of females captured by site ranged from 5 individuals (Site 3) to around 30 individuals (Site 5) per baited hoop net. For males, it seems that no pattern was found in the number of crabs per site, hence, site alone cannot be considered an important factor in the analysis of the number of males (Fig. 9 and Table 2).

However, interquartile ranges of the number of both female and male crabs captured per baited hoop net varied significantly from site to site and from month to month (Fig. 9). In this regard "Site proximity to the lagoon's mouth" could be a better factor to understand crabs' spatial distributions over time in Ria de Aveiro, especially for females. Sites 1 and 4 are located near to the mouth and share many environmental characteristics. For those sites, both females and males' distributions were similar over time (Fig. 9: median values and interquartile ranges), especially in the case of females. When comparing these distributions with the ones in the remaining sites, which were more distant to the mouth, the number of crabs captured by the baited hoop net method over time was consistently lower (Fig. 9).

One of the theoretical consequences of a species having sexual dimorphism is the ecological niche segregation between males and females (Shine 1989). However, studies support the idea that foraging activity in this species does not present significant differences when comparing both sexes (Spooner et al. 2007). So differences in the spatial distribution of both sexes is not probably related with different food supplies available through the sites sampled. Salinity gradients through the Ria de Aveiro channels, from the inlet to upstream, are possibly the main environmental feature that could influence crabs' spatial distributions. One possible hypothesis for this may be related to each sex tolerance to different salinity gradients. Other is that females probably migrate to downstream areas when they are about to reach maturity or ready to mate (Baeta et al. 2005). It is likely that they prefer locations near the estuary mouth in order to provide their offspring (free swimming planktonic larvae), before hatching, better chances of transportation to the sea by tide (Queiroga et al 1994, Queiroga et al 1997).

Regarding specifically sampling Site 1, there is a last factor that may affect the number of crabs captured: fishing pressure. Although fishery statistics regarding this species are not available, it is known that this species' fishery effort is mainly concentrated on the large and heavier crabs in this region of the Ria de Aveiro. Common knowledge attributes those characteristics to the red adult females. The harvesting of crabs is carried out evenly, but with unknown periodicity. Accordingly, the numbers of captured crabs in Site 1 could have been much higher if its fishery wouldn't happen so regularly.

In short, Site and the proximity to the lagoon's mouth appear to be important factors when comparing sex segregation data. Sex differential spatial distribution was also verified in the study of Baeta et al (2005) in the green crabs of the Mondego estuary, Portugal.

Regarding the trends of the medium values per month, it can be seen that for almost every site, with the exception of females in Site 3, higher number of crabs were captured during the first two months of the year (Fig. 9). In females' particular case, its numbers decreased in every site during March and April, rising again during the beginning of summer. The trends in data per site and per month are concordant with the trends showed by the total amount of crabs captured by month (Fig. 8), presented in the previous section. This shows that, there is a pattern through time of the number of captures for each sex, consistent in every site sampled in Ria de Aveiro.

4.3. CRABS' SEGREGATION PER HABITAT

The number of crabs and its size distributions per habitat showed clear patterns regarding habitats' depth and complexity (Figs. 9, 12 and Table 4). Those patters were also detected with the Bhattacharya modal analysis (Fig. 13, 14 and 15). However, due to the lack of data on a complete annual cycle, we do not attempt to interpreted growth rates from the modal analysis. When interpreting results of size distributions per habitat, we have to be especially cautious in matters regarding small sized crabs (< 20 mm) because of the selectivity of the sampling method. As referenced before, baited hoop nets are a sampling method that actively selects the most mobile and hungry crabs and because of that small, injured, paired, parasitized and berried crabs are less captured. That said, and to ease the presentation of this section's main conclusions, the results' interpretation will be presented: first (i), for adult crabs (sizes larger than 30 mm according to Crothers 1968a); second (ii), for juvenile crabs (sizes smaller than 30 mm according to Crothers 1968a); and third (ii), for the relation between all crabs' size distribution and the Habitat factor.

i) Adult crabs

Crabs Crabs with sizes larger than 30 mm represent 87% of the total captures in the Ria de Aveiro. They were found in almost every habitat in every month (Subtidal: 90.59%; Sand: 87.56%; Mixed: 87.43%; Zostera: 85.36%; Marsh: 66.16%), however reaching higher frequencies in the subtidal and in the middle and low intertidal sandy and mixed habitats (Table 4). Crabs' adult population were found to present a more or less similar size structure along the year, with a size increase in the last month (Fig. 15 and Subtidal and Sand habitats in Figs. 13 and 14).

Post-larval recruitment appear to be fairly seasonal in the Ria de Aveiro (Queiroga 1993; Domingues et al. 2011). If that is in fact true, the increase in size recorded for adult crabs as monthly average temperatures got higher (Fig. 12), could be explained by assuming that, once the species reach maturity, it only moults during summer (Queiroga 1993). One summer moult for the adult crabs' assumption has been referred by many authors as a typical behavior in crustaceans (see Queiroga 1993; Broekhuysen 1936; Demeusey 1958). However, we have to have always in consideration that European studies available that focuses this assumption in this species are mainly from the North-East region, where the climatic conditions are much different from ours, especially regarding the temperature rates throughout the annual seasons. Even so, "One summer moult" assumption may still support our findings. Broekhuysen (1937) studied Dutch crabs' growth in the North Sea. His findings showed that crabs' growth slowed down after puberty, so that by the end of the first year all young crabs were approximately the same size, regardless of its settlement time. Thereafter, this study could have shown the process how to achieve one summer moult. As so, Dutch crabs moult once a year until reaching terminal anecdysis in the third or fourth year. During adulthood, as it happens with *C. maenas* species, its increases in size are around 10 mm per moult. Assuming that *C. maenas* summer moult works in a similar way, our results using Bhattacharya modal analysis suggest that crabs with sizes larger than 30 mm remained with a CW more or less constant over the first 6 month of the year. Although, in our average size analysis, where the average size of captured individuals was measured in each replica per habitat and site, we could see that there was a small increase of size in the mature individuals (> 30 mm). This means that not all crabs moult during summer (see Subtidal and Sand habitats in Fig. 12 and Fig. 15), however it could be noticed that the higher picks happened during the summer.

The percentages of the total adult crabs' captures in subtidal habitat were 22.73%, whereas in low and medium intertidal habitats (Sand and Mixed) were 40.11% and 23.20%, respectively, and high intertidal habitats (*Zostera* and Marsh) were 13.96%. Based on previous evidence about tidal migration (Crothers 1968a; Hunter & Naylor 1989; Reid et al. 1997), we formulate an hypothesis that could explain our results regarding the patters of abundance of adult crabs (> 30 mm) in the different habitats. The number of adult crabs in subtidal habitats could represent the individuals that are not engaging in tidal migrations or that, at least, are no longer engaging in them regularly. Also, according to Reid et al. (1997), older individuals in prolonged intermoult (> 60 mm) migrate less frequently or discontinue migrations between habitats altogether and remain permanently below low-water mark (Crothers 1968a; Hunter & Naylor 1989). In fact, we could only find individuals with sizes larger than 60 mm in subtidal habitats, especially in the case of males (Fig. 14), since males seem to always reach bigger sizes than females (Fig.10). On the other hand, the great number of adult crabs captured in low and medium intertidal habitats (63.31%) could represent the individuals that actively engage in tidal migrations, foraging in intertidal habitats on the flood tide and retreating with the ebb (Crothers 1968a; Hunter & Naylor 1989). Thus, the food supply available in those habitats seems to be enough for the majority of the migrating crabs, which showed no need to reach high intertidal habitats in order to feed. The remaining medium and bigger sized individuals collected in high intertidal habitats could represent: 1) the individuals that may have begun migration earlier, and so, were foraging in (more distant) high intertidal complex habitats, or 2) the individuals that remain permanently hidden on the shore at low tide (Crothers 1968a). Tidal migration may as well be the main engine behind the habitat distribution of adult crabs. Our findings in the study of tidal migration (Discussion - Part II), executed in a sandy habitat located between subtidal and high intertidal habitats at Site 1, support this hypothesis.

ii) Juvenile Crabs

Our results showed that as habitat complexity increased and habitat depth decreased, the number of captured crabs with small sizes also increased (Subtidal: 9.41%; Sand: 12.44%; Mixed: 12.57%; Zostera: 14.65%; Marsh: 33.84%). Despite that, their presence was notorious particularly in *Zostera* beds and salt marshes, where 22% of the captures corresponded to crabs fitting this description (Table 4). The Bhattacharya modal analysis also detected higher presence of small individuals in complex habitats. In this analysis, we should take in consideration once more the deficiency in the capture of individuals with these size characteristics due to the use of baited hoop nets as sampling method. Using a different capture method, we would expect to have found, for juvenile crabs in complex habitats, a bottleneck distribution as size of juveniles increases (meaning that the large numbers of small post-settlement crabs that can be found in this habitats decrease as they grow). According to Beck (1997) and Almeida et al. (2011), this is mainly caused by predation. Literature suggests that there is a general trend of exponential decrease in abundance due to mortality during early juvenile life found in benthic invertebrates with limited dispersal (Moksnes 1998; Almeida et al. 2011).

In spite of the methods selectivity and most likely under-estimation of the abundance of smaller crabs, especially those under 20 mm, the methodology did detect increasing numbers of smaller crabs with an increase of habitat complexity. It was in these habitats that we could find not only the youngest of the year (0+), but also the late recruits of the year before (Fig. 14, “JAN & FEB”). In the Ria de Aveiro, higher intensities of recruitment events regularly happen from February to July (Domingues et al., 2011). In this study, in figures 12, 13 and 14, we can observe that higher frequencies of the number of small crabs with sizes from 10 to 20 mm were found in the first two months of the year for both sexes (January and February). We theorize that those represent the later recruits of the previous year, July and August of 2015, that are still growing, and have not yet reached the adult stage. Those are normally descendants of females physiologically unable to produce eggs at a “normal” time, usually the youngest matured female crabs (Demeusey 1958). Moksnes (1998; 2002) sugegsted that it is during crabs’ settlement that habitats choice as nursery is made and that crabs remain within the boundaries of such nursery (Almeida et al. 2008) for several weeks to a few months. There are some differences between types of available food items and abiotic conditions among habitats’. Also, different potential nursery habitats showed different ranges of habitat quality, in shelter value and opportunistic use by settling post-larvae and young juveniles (See Hedvall et al. 1998; Moksnes 2002, Amaral et al. 2009).

In fact, many studies report that smaller juveniles, (<20 mm), tend to remain in the high intertidal zone, whereas adults tend to perform vertical migrations during flood, withdrawing to subtidal zone during low tide (Crothers 1968a; Hunter & Naylor 1989; Warman et al. 1993). However, as a juveniles grow their behavior changes. We considered that small immature crabs ranged from 4 (minimum CW captured) to 30 mm. Within this range of sizes, we can find juvenile crabs with very different behaviors: from the new recruits that stay confined in complex habitats to the late juvenile stages that are already making tidal migrations. The majority of the crabs captured with sizes inferior to 30 mm were of crabs with sizes between 20-30 mm (Table 4). Because of that, while verifying juvenile distributions per habitat, we found that 15.39% of the crabs were captured in the subtidal habitat, 37.14% in the Sand habitat (low intertidal), 21.75 in the Mixed habitat (medium intertidal) and 25.72% in high intertidal habitats (*Zostera* and Marsh). The majority of the small crabs captured (Table 4), as they were between 20-30 mm, were found in low intertidal habitats. This could mean that: either they are dispersing from highly to less complex habitats (ontogenetic migration) or they are already doing tidal migrations. Our findings in the study of tidal migration that will be discussed above in Part II of this chapter, we found crabs with sizes between 20-30 mm actively doing tidal migration.

iii) Relation between all crabs' size distribution and factor Habitat.

Habitat is an important factor when comparing the number of crabs captured and their size, despite the sex, presenting spatial and temporal differences of its use by this species (See results in Table 2 and Table 3), meaning that crabs in different stages of life were caught using different habitats in similar patterns of size (Figs. 13 and 14; Table 4). Note that sometimes minimum distance between habitats in the same site can be of about 10 meters and sampling is done in high tide when migration between habitats is enhanced. In addition to local differences between habitats, geographic variation and seasonal life cycle changes may influence the use of habitats by *C. maenas*. Amaral et al. (2009) states that the quality of estuarine habitats and its use by this species is susceptible to vary between different latitudes. Justification for that may be closely related with particular characteristics in estuarine systems in different latitudes (Amaral et al. 2009). It has been established that habitat use by animals is determined by trade-offs between maximizing growth and minimizing mortality risk (Amaral et al. 2009). Habitats where growth and survival are enhanced and where reproductive potential is higher are considered high quality habitats (Amaral et al. 2009; Beck et al. 2001) and their use for a given species in different stages of life may vary a lot in temporal and spatial scales.

4.4. ALLOMETRY STUDY

Findings in the allometry study showed that changes in crabs' weight in Ria de Aveiro were not homogeneous through this 6-month time and that there were periods of stability, and that there were periods of decrease and increase in weight that were not synchronized across sexes. Life cycle events may very well be correlated with the results found. Our findings support the conclusions on seasonal spatial distributions, possibly associated with foraging, with the majority of the standardized individuals, despite the sex, reaching bigger weight during summer (Fig. 16).

Comparing both sexes, we observed that the estimated weight were higher during the beginning of the summer, especially in female' analysis. In this 6-month experiment we noticed an increasing trend of the estimated weights for both sexes from January to June (Fig. 16). Concordant results can also be found in Hayden et al. (2007). Their study agrees on the idea that feeding stimulation is greater in females than in males throughout the year and that summer is the period of the year where feeding activities for both sexes are the most different, being extremely high in females and extremely low in males. That can be noticed in our estimations of weight (Fig. 16). Spring is the season of the year with smallest differences in feeding responses between the sexes (Hayden et al. 2007). This is probably the period of the year were weight of the standardized individual (with 40 mm of size) for both sexes is most similar (also observed in Fig. 16).

Pearson et al. (1979) referred that seasonality of foraging behavior is potentially linked to chemical responses coordinating reproduction. In fact, marine crustaceans are known to have their behavior (such as feeding, mating, social interactions, hatching of young and larval settlement) intimately dependent upon chemoreception (Carr 1988; Eales 1974). However, chemosensitivity may be influenced by environmental factors (such as temperature, light intensity, photoperiod) and circadian rhythm (Atema 1987; Derby 2000). This complexity of co-dependent relations with a large number of factors makes the reasoning of such behaviors a very complex task.

In short, the crabs condition (relation between weight and size) appears to be better during the summer and that might be associated with seasonal variation of their feeding activities. The allometry study came to re-enforce the conclusions previously made in the section 5.2.

4.5. GREGARIOUS BEHAVIOR

The formation of animal aggregations is one of the most studied patterns in biology (Parrish & Edelstein-keshet 1999). According with our findings (Figs. 18 and 19), this species may present some gregarious behavior, fairly different for each sex. Females with sizes larger than 20 mm were captured always with other female individuals with sizes with standardized deviations around 7 mm from their own and in higher amounts (around 30 to 60 netmates). On the other hand, males appear to aggregate in smaller groups, with SD of size around 10 mm and the average CW of all individuals, in the same net, seems to be similar to the size of each individual (Figs. 18 and 19).

Aggregation has been seen regularly as an evolutionary advantageous state (Parrish & Edelstein-keshet 1999). Typical benefits of aggregation for the individuals are protection, mate choice and centralized information. The behavior in such aggregations is normally balanced by the resources available (Parrish & Edelstein-keshet 1999). Studies about crustaceans seem to have found the presence of gregarious behavior in, at least, one adult life cycle stage in a variety of crab and lobsters species: two species of porcelain crab (Jensen 1991; Donahue 2004); one species of tanner crabs (Stevens et al. 1994); one species of red king crabs (Stone et al. 1993); one species of spider crab (Sampedro & González-Gurriarán 2004); crabs of the genus Ocypode (Barrass 1962); Dungeness crab (Butler 1960); more than one number of species of spiny lobsters and western rock lobsters (Berrill 1975; Zimmer-Faust & Spanier 1987; Johnston et al. 2006).

Deriving benefit by virtue of other individual presence is the basis for intrinsic gregariousness on which is dependent the formation of social groups (Morse 1980). Both sexes of green crabs sampled by baited hoop net seem to appear associated to crabs with sizes not so different from their one. The shore crab is known for its agonistic and cannibalistic behaviors (Almeida et al. 2011; Januario & Navarrete 2013). We speculate that the co-occurrence of conspecifics of similar sizes does not present a threat since they would probably have similar chances of winning in a confrontation. Another possibility is that the presence of others may actually have positive outcome while foraging and that what we appear to be seeing is actually intrinsic gregariousness in this species. In our specific situation, it is also possible that aggregation might have arisen because these crabs exploit a shared but patchy resource and so, our findings may not necessarily reflect gregarious behavior (Zimmer-Faust & Spanier 1987) even though sizes within the groups were similar. The aggregations we seem to observe may also be in part related with tidal migration. For instance, dense aggregations of the spiny lobsters have been observed for migrating purposes (Berrill 1975).

Donahue (2004) studied intraspecific competition between individuals of one species of porcelain crab that is known to live in high-density and multi-size aggregations. Her study revealed, within aggregations, size-dependent agonistic interactions in feeding rate and, ultimately, on growth rate. In aggregations of porcelain crab, the smaller sized individuals are the ones that benefit less from it (Donahue 2004). Comparing this species with *C. maenas* as completely different food regime and food availability and so is very dependent upon its particular gregarious behavior to subsist. What we can withhold from this and apply to our study is that, even in such gregarious species, smaller and less capable individuals do not benefit as much as the bigger sized ones from aggregation. Thus, aggregation of similar sized individuals seems to be more beneficial in terms of intra-specific agonistic relations.

In short, even though the reason why is still unclear, our findings suggest that adult crabs may form aggregations with others with similar sizes (Fig. 18 and 19). One thing that appears to be clearer is that female crabs appear to demonstrate higher aggregation when comparing with males. For a clear observation of this species possible gregarious behavior, we suggest specific tracking of a population using, for instance, small GPS device glued to each individual carapace. We strongly suggest the separated analysis of females and males' data as they seem to have different behaviors in this matter.

PART II – TIDAL MIGRATION

Tidal migration is a behavior resulting from some crabs' need to come up the shore with the flood to feed, moving back on the ebb (Crothers 1968a). It is important to refer that not all individuals take part in this tidal migration. Natural variations in abiotic factors (such as hydrostatic pressure, salinity and temperature) may operate as entrainment factors in tidal migrations, even though studies report that green crabs are also perfectly capable of measuring the passage of time when this external cues are not present (Styrishave et al. 2004). Tidal rhythmicity is an inherited and adaptive feature of the behavioral physiology of the *Carcinus*' genus (Naylor 1996; Williams & Naylor 1967; Williams & Naylor 1969). From this study, two main results could be taken:

> First, invasion of intertidal banks was consistent in time from low tide within day and night trials. However, the phases when the highest migration rates occurred differed between day and night.

Our data showed that during both daylight experiments, the migration peaks only occurred after tide had reached 0.5 m in height in the recording area, 90 to 105 min after low tide. Note that we always started filming the tidal migration about 15 minutes after low tide, when water started invading the observation area and the camera was around 10 to 15 m distant from low water line. Dare & Edwards (1981) used underwater television during the daytime, also trying to understand how *C. maenas* performed tidal migrations. Their experiment was tested in an intertidal mudflat located > 25 m upshore from sublittoral zone, reaching their gate only 102-120 min after the flood started. Because of that their peaks of migration were also observed later in time, after 135 min from low tide. Despite that, their findings showed exactly the same pattern as ours regarding the depth of the water column with higher number of observations. We could say that both our and their results in the number of crabs migrating upshore, peaks of migration in time from low tide and depth of the water column in the recording area were much alike.

During both nighttime experiments, higher rates of migration occurred 25 to 45 min after low tide, while the lens of the video camera was not yet submerged and the water column in the recording area was less than 0.40 m in height. Despite the similar conditions, the numbers of migrating crabs caught on camera during the night were consistently lower than during day. Crustaceans are known for being mainly nocturnal. They usually spend the day hidden from visually directed predators, normally underwater, concealed under boulders, in rocky clefts or buried in substrate (Warner 1977; Dunham 1978; Zimmer-Faust 1993). Then, during night time when most of their predators are inactive or absent, they emerge to feed (Hayden et al. 2007) and must therefore seek their prey in the virtual absence of light, using other sensory stimuli like chemical cues to locate their prey (Zimmer-Faust 1989). This typical behavior in crustaceans may be the justification for our observations during night periods and the absence of light might induce earlier tidal migration in *C. maenas*.

Carcinus maenas' nighttime migrations using underwater video cameras or more or less similar technologies, e.g. underwater television (Dare & Edwards 1981) and acoustic telemetry (Holsman et al. 2006), is a subject which there is not still much information about. However, using different technics, Naylor (1958) and Kitching et al. (1959) found that the highest peak of locomotory activity and foraging intensity by this crabs were both more intense during the night than during the day. We hypothesized that, in our case the migration began during the night previously to low tide, during the last stages of the previous ebb and that what we could actually observe on camera were the moments shortly after the migration peaks.

Other important matter to be addressed is the importance of the direction of the experiment scheme in the number of migrating crabs observed. Due to the Mira Channel particular characteristics, direction of the water flow above the intertidal flats changes almost 90º during flood. It begins with a major flow perpendicular to low water line and, as time passes and water velocity increases, the direction of the main flow changes as well. Flood water flow finishes almost parallel to the low water line. In our experiments, it was chosen to redirect the 15 meter's mouth perpendicular to the low water line. However, higher picks of migration, in both day and night trials, were observed during the flood's first 2.5 hours. We consider that if our experimental setup was parallel instead of perpendicular to the low water line, we would had caught in video higher densities of migrating crabs. Dare & Edwards (1981) used a similar experimental setup with a net funnel shaped and a gate also to increase their area of observation. As their experimental setup was parallel to low water line, they could observe substantially higher number of crabs migrating up the shore. We strongly advise that the orientation of the funnel should be taken in consideration when planning future experiments related with crabs' tidal migration. That was also the advice given by Hunter & Naylor (1993), although they used a different technic to understand the migration flow during flood. Finally, as also stated by Dare & Edwards (1981) in their experiments, we noticed that the extent to which crabs escaped from, or perhaps entered, the directional nets by climbing over the guiding walls was unknown.

Second, migratory behavior in this experiments did not present segregation by size (Fig. 21).

In this experiment, we tried to understand if tidal migration could have been segregated by size through time. Since the main goal of the tidal migration by green crabs was the search for food supply in intertidal areas, we hypothesized that, due to this species' cannibalistic behavior, smaller crabs (≤ 30 mm) might migrate in different periods of time from low tide in order to avoid encounters with bigger sized crabs. Almeida et al. (2008) studied the locomotory activity of juvenile green crabs in different intertidal habitats. They found that there were significant differences between activity peaks of smaller and bigger juvenile crabs in all intertidal habitats sampled. Their findings suggested temporal segregation of activity windows between juvenile crabs with different sizes, which might be a key mechanism to avoid predation by cannibalism (Almeida et al. 2008). Our findings did not suggest the presence of such key mechanism in tidal migration and so the assumption that crabs migrating activity could be somehow segregated through time by crabs' size appear to be dismissed. Our observations, however, concluded that the majority of the migrating crabs had sizes superior to 20-50 mm carapace width (Fig. 21), similar results were found by Dare & Edwards (1981).

5. CONCLUSION

Regarding *Carcinus maenas* spatial and temporal distribution study in the subtidal and intertidal habitats in Ria de Aveiro (Part I), nearly 11 500 crabs were collected and analyzed, about 2 000 crabs per month. The great majority of the crabs collected during this 6-month experiment were adults, with sizes equal or superior to 30 mm. Females represented the larger amount of the individuals collected. From this study, we verified that Site seemed to be an especially relevant factor when comparing sex segregation data. Higher number of females were sampled in sites near the mouth of the lagoon every month, whereas males were relatively well spread through all the 5 sites samples. We found that Habitat presented spatial and temporal differences of its use. The number of crabs and its size distributions per habitat showed clear patterns regarding habitats' depth and complexity. Adults (CW > 30 mm) were present in every habitat sampled. Older individuals were found more frequently in subtidal habitats, particularly noticed in male crabs' analysis. The sampling method – the baited hoop net – was not the most suitable method to capture less mobile and hungry individuals, and because of that there was a most likely under-estimation of their numbers. Despite that, the numbers of the smaller crabs, especially those under 20 mm, had a general increasing trend with an increase of habitat complexity and a decrease in its depth. Seasonal life cycle changes in the distribution of this species might influence the results of the use of habitats with different degrees of structural complexity.

The study of condition could be sum up as the study of the relation between weight and size and, in this analysis, it was chosen the average size of the crabs' population (CW 40 mm) in order to evaluate the variation of weight through the months. With the exception of the last month sampled, June, we found that males were always heavier than females and that both sexes presented similar trends of condition through the months. For both sexes the condition was lower during March, probably a finding related with the begging of the reproductive season. We found that condition was higher during the summer months for both sexes, being however especially higher for females. This results are probably related with the higher abundance of food supply during the summer months.

Adult crabs seem to form aggregations with others with similar sizes, once more, a finding particularly notable in the females' analysis. We found that females with sizes between 35 and 55 mm were usually captured in large numbers (35 to 45 individuals) in the same net. Although with smaller sizes (between 20 to 40 mm), same amounts of males seemed to be normally captured in the same net. Further analysis may be required in order to understand if there is in fact gregarious behavior in the adult populations of *C. maenas*.

The study of tidal migrations (Part II) was executed using an underwater video camera settled near the low water mark in a sandy habitat of Ria de Aveiro. In this experiment the migrating crabs were counted and their CW was roughly measured to the centimeter. We found that peaks of migration were noticed between the first and second hour before low tide during the day and in the first hour before low tide during the night. In short, migration was performed later in time from low tide during the day than during night. Most of the migrating crabs were adults and had sizes superior to 25 mm. Finally, we verified that migratory behavior did not seemed to be segregated through time by size.

REFERENCES

Almaça, C. (1982). Note sur la biologie des populations *Carcinus maenas* (L.) de la zone intertidale du Portugal ocidental. *Quaderni Laboratorio Tecnologia Pesca, Ancona*, *3(2-5)*, 179–185.

Almeida, J. M., González-gordillo, J. I., Flores, A. A. V., & Queiroga, H. (2011). Cannibalism, post-settlement growth rate and size refuge in a recruitment-limited population of the shore crab *Carcinus maenas*. *Journal of Experimental Marine Biology and Ecology*, *410*, 72–79. http://doi.org/10.1016/j.jembe.2011.10.011

Almeida, M. J., Flores, A. A. V, Queiroga, H., & Joa, M. (2008). Effect of crab size and habitat type on the locomotory activity of juvenile shore crabs, *Carcinus maenas*. *Estuarine, Coastal and Shelf Science*, *80*, 509–516. http://doi.org/10.1016/j.ecss.2008.09.006

Almeida, M. J., González-gordillo, J. I., Flores, A. A. V, & Queiroga, H. (2011). Cannibalism , post-settlement growth rate and size refuge in a recruitment-limited population of the shore crab *Carcinus maenas*. *Journal of Experimental Marine Biology and Ecology*, *410*, 72–79. http://doi.org/10.1016/j.jembe.2011.10.011

Amaral, V., Cabral, H. N., Jenkins, S., Hawkins, S., & Paula, J. (2009). Comparing quality of estuarine and nearshore intertidal habitats for *Carcinus maenas*. *Estuarine, Coastal and Shelf Science*, *83*, 219–226. http://doi.org/10.1016/j.ecss.2009.03.029

Araújo, I. B., Dias, J. M., & Pugh, D. T. (2008). Model simulations of tidal changes in a coastal lagoon, the Ria de Aveiro (Portugal). *Continental Shelf Research*, *28*(8), 1010–1025. http://doi.org/10.1016/j.csr.2008.02.001

Ashton, E. C., Hogarth, P. J., & Macintosh, D. J. (2003). A comparison of brachyuran crab community structure at four mangrove locations under different management systems along the Melaka Straits-Andaman Sea Coast of Malaysia and Thailand. *Estuaries*, *26*(6), 1461–1471. http://doi.org/10.1007/BF02803654

Atema, J. (1987). Tuning and noise in chemoreception—the acoustic analogy. *Chem. Senses*, *12*, 638–639.

Azevedo, A., Sousa, A. I., Lencart e Silva, J. D., Dias, J. M., & Lillebø, A. I. (2013). Application of the generic DPSIR framework to seagrass communities of Ria de Aveiro: a better understanding of this coastal lagoon. *Journal of Coastal Research*, *65*, 19–24. http://doi.org/10.2112/SI65-004.1

Baeta, A., Cabral, H. N., Neto, J. . M., Marques, J. C., & Pardal, M. A. (2005). Biology, population dynamics and secondary production of the green crab *Carcinus maenas* (L .) in a temperate estuary. *Estuarine, Coastal and Shelf Science*, *65*, 43–52. http://doi.org/10.1016/j.ecss.2005.05.004

Barrass, R. (1962). Aggregations of crabs of the genus Ocypode (Crustacea, Ocypodidae). In *Proceedings of the association for the study of Animal Behaviour, Animal Behaviour, XI, 2-3* (pp. 404 – 405).

Bartolini, F., Penha-Lopes, G., Limbu, S., Paula, J., & Cannicci, S. (2009). Behavioural responses of the mangrove fiddler crabs (*Uca annulipes* and *U. inversa*) to urban sewage loadings: Results of a mesocosm approach. *Marine Pollution Bulletin*, *58*(12), 1860–1867. http://doi.org/10.1016/j.marpolbul.2009.07.019

Beck, M. W., Heck, K. L., Able, K. W., Childers, D. L., Eggleston, D. B., Gillanders, B. M., Halpern, B., Hays, C. G., Hoshino, K., Minello, T. J., Orth, R. J., Sheridan, P. F., Weinstein, M. P. (2001). The Identification, Conservation, and Management of Estuarine and Marine Nurseries for Fish and Invertebrates. *BioScience*, *51*(8), 633. http://doi.org/10.1641/0006-3568(2001)051[0633:TICAMO]2.0.CO;2

Bhattacharya, C. G. (1967). A simple method of resolution of a distribution into Gaussian components. *Biometrics*, (23), 115–135.

Broekhuysen, G. J. (1936). On Development, Growth and Distribution of Carcinides Maenas (L.). *Archives Néerlandaises de Zoologie*, *2*(1), 257–400. http://doi.org/10.1163/187530137X00104

Cannicci, S., Bartolini, F., Dahdouh-Guebas, F., Fratini, S., Litulo, C., Macia, A., Mrabu, E. J., Penha-Lopes, G., Paula, J. (2009). Effects of urban wastewater on crab and mollusc assemblages in equatorial and subtropical mangroves of East Africa. *Estuarine, Coastal and Shelf Science*, *84*(3), 305–317. http://doi.org/10.1016/j.ecss.2009.04.021

Carr, W. E. (1988). The molecular nature of chemical stimuli in the aquatic environment. In *Sensory biology of aquatic animals* (pp. 3-27). Springer New York.

Cohen, A., Carlton, J., & Fountain, M. (1995). Introduction, dispersal and potential impacts of the green crab *Carcinus maenas* in San Francisco Bay, California. *Marine Biology*, *122*(2), 225-237. Retrieved from http://link.springer.com/article/10.1007/BF00348935

Conhen, A. N., Carlton, J. T., & Fountain, M. C. (1995). Introduction, dispersal and potential impacts of the green crab *Carcinus maenas* in San Francisco Bay, California. *Marine Biology*, *122*, 225–237.

Crothers, J. H. (1968a). The biology of the shore crab *Carcinus maenas* (L.) 1.The background - Anatony, Growth and Life History. *Field Studies Council*, *2*(5), 579–614.

Crothers, J. H. (1968b). The biology of the shore crab *Carcinus maenas* (L.) 2. The Life of the adult crab. *Field Studies Council 2*(5), 579-614.

Dare, P. J., & Edwards, D. B. (1981). Underwater television observations on the intertidal movements of shore crabs, *Carcinus maenas*, across a mudflat. *Journal of the Marine Biological Association of the United Kingdom*, 61, 107–116.

Demeusey, N. (1958). Recherches sur la mue de puberté du dacapode Brachyoure *Carcinus maenas* Linné. A*rchives de Zoologie Experimentale et Generale.*, *95*, 253–492.

Derby, C. D. (2000). Learning from spiny lobsters about chemosensory coding of mixtures. *Physiology & Behavior.*, *69*, 203–209.

Dias, J. M. (2001). *Contribution to the study of the Ria de Aveiro hydrodynamics*. Universidade de Aveiro, Portugal.

Domingues, C. P., Almeida, M. J., Dubert, J., Nolasco, R., Cordeiro, N., Waap, S., Sequeira, A.,Tavares, S., Queiroga, H. (2011). Supply of crab larvae to an estuary in the eastern Atlantic upwelling system exhibits predictable and haphazard variation at different temporal scales. *Marine Ecology Progress Series*, *425*, 113–124. http://doi.org/10.3354/meps08992

Donahue, M. J. (2004). Size-dependent competition in a gregarious porcelain crab *Petrolisthes cinctipes* (Anomura: Porcellanidae). *Marine Ecology Progress Series*, *267*, 219–231. http://doi.org/10.3354/meps267219

Dumas, J. V., & Witman, J. D. (1993). Predation by herring-gulls (*Larus argentatus Coues*) on 2 rocky intertidal crab species (*Carcinus maenas* [L.] and *Cancer irroratus Say*). *Journal of Experimental Marine Biology and Ecology*, *169*, 89–101.

Eales, A. J. (1974). Sex pheromone in the shore crab *Carcinus maenas*, and the site of its release from females. *Mar. Behav. Physiol.*, *2*, 345–355.

Grosholz, E. D., & Ruiz, G. M. (1995). Spread and potential impact of the recently introduced European green crab, *Carcinus maenas*, in central California. *Marine Biology*, *122*(2), 239–247. http://doi.org/10.1007/BF00348936

Hartnoll, R. G. (1969). Mating in the Brachyura. *Crustaceana*, *16*, 161–181.

Hayden, D., Jennings, A., Müller, C., Pascoe, D., Bublitz, R., Webb, H., Breithaupt, T., Watkins, L., Hardege, J. (2007). Sex-specific mediation of foraging in the shore crab, *Carcinus maenas*. *Hormones and Behavior*, *52*, 162–168. http://doi.org/10.1016/j.yhbeh.

Hedvall, O., Moksnes, P., & Pihl, L. (1998). Active habitat selection by megalopae and juvenile shore crabs *Carcinus maenas*: a laboratory study in an annular flume. *Hydrobiologia*, 375, 89–100.

Holsman, K. K., McDonald, P. S., & Armstrong, D. A. (2006). Intertidal migration and habitat use by subadult Dungeness crab *Cancer magister* in a NE Pacific estuary. *Marine Ecology Progress Series*, *308*, 183–195. http://doi.org/10.3354/meps308183

Hunter, E., & Naylor, E. (1989). Intertidal migration by the shore crab *Carcinus maenas*. *Marine Ecology Progress Series*, 101, 131–138. http://doi.org/10.1017/CBO9781107415324.004

ICNF. (2014). *Rede Natura 2000*. Retrieved from http://www.icnf.pt/portal/naturaclas/rn2000

Ingle, R. W. (1983). *Shallow-water Crabs.* London, Brithish Museum, Department of Zoology: The Linnean Society of London, The Estuarine and Brackish-water Sciences Association & Cambrigde University Press.

Januario, S. M., & Navarrete, S. A. (2013). Cannibalism and inter-specific predation in early stages of intertidal crab species that compete for refuges. *Journal of Experimental Marine Biology and Ecology*, *446*, 36–44. http://doi.org/10.1016/j.jembe.2013.04.017

Jensen, G. C. (1991). Competency, settling behavior, and postsettlement aggregation by porcelain crab megalopae (Anomura : Porcellanidae). *Journal of Experimental Marine Biology and Ecology*, *153*(1), 49–61. http://doi.org/10.1016/S0022-0981(05)80005-X

Johnston, D., Melville-Smith, R., Hendriks, B., Maguire, G. B., & Phillips, B. (2006). Stocking density and shelter type for the optimal growth and survival of western rock lobster *Panulirus cygnus* (George). *Aquaculture*, *260*, 114–127. http://doi.org/10.1016/j.aquaculture.2006.05.057

Kitching, J. A., Sloane, J. F., & Ebling, F. J. (1959). The ecology of Lough Ine: VIII. Mussels and their predators. *Journal of Animal Ecology*, *28*, 331–341.

Kristensen, E., Bouillon, S., Dittmar, T., & Marchand, C. (2008). Organic carbon dynamics in mangrove ecosystems: A review. *Aquatic Botany*, *89*(2), 201–219. http://doi.org/10.1016/j.aquabot.2007.12.005

Lebour, M. V. (1928). The larval stages of Plymouth Brachyura. *Proceedings of the Zoological Society of London*, *34*, 473–560.

Leignel, V., Stillman, J. H., Baringou, S., Thabet, R., & Metais, I. (2014). Overview on the European green crab *Carcinus spp*. (Portunidae, Decapoda), one of the most famous marine invaders and ecotoxicological models. *Environmental Science and Pollution Research*, *21*(15), 9129–9144. http://doi.org/10.1007/s11356-014-2979-4

Lillebø, A. I., Pereira, E., Queiroga, H., Dias, J. M., Keizer, J., & Cleary, D. (2009). *Long Term Ecosystem Research in a Southern European Estuary: Ria de Aveiro*, Portugal, CESAM publications. http://www.cesam.ua.pt/subsites/files/miranda_et_al_cesam_highlights_2010_1_.pdf

Lindley, J. (1987). Continuous plankton records: the geographical distribution and seasonal cycles of decapod crustacean larvae and pelagic post-larvae in the north-eastern Atlantic Ocean and the North Sea. *Journal of the Marine Biological Association of the United Kingdom*, *3*(67), 145–167.

Lopes, J. F., Silva, C. I., & Cardoso, A. C. (2008). Validation of a water quality model for the Ria de Aveiro lagoon, Portugal. *Environmental Modelling and Software*, *23*(4), 479–494. http://doi.org/10.1016/j.envsoft.2007.07.001

Lowe, S., Browne, M., Boudjelas, S., & De Poorter, M. (2000). *100 of the world's worst invasive alien species: a selection from the Global Invasive Species database.* Contribution to the Global Invasive Species Programme (GISP) Published by the IUCN/SSC Invasive Species Specialist Group. http://www.issg.org/pdf/publications/worst_100/english_100_worst.pdf

Macintosh, D. J., Ashton, E. C., & Havanon, S. (2002). Mangrove Rehabilitation and Intertidal Biodiversity: a Study in the Ranong Mangrove Ecosystem, Thailand. *Estuarine, Coastal and Shelf Science*, *55*(3), 331–345. http://doi.org/10.1006/ecss.2001.0896

Marôco, J. (2014). *Análise Estatística com o SPSS Statistics (6ª Edição)*. ReportNumber, Lda. Portugal. ISBN: 9789899676343

McFarland, D. (1976). *Form and function in the temporal organisation of behaviour.* Growing Points in Ethology. Eds.: Bateson, P.P.G., Hinde, R.A.. Cambridge University Press, Cambridge. (pp. 55–93).

Moksnes, P. (2002). The relative importance of habitat-specific settlement, predation and juvenile dispersal for distribution and abundance of young juvenile shore crabs *Carcinus maenas* L. *Journal of Experimental Marine Biology and Ecology*, *271*, 41–73.

Moksnes, P.-O. (2004). Interference competition for space in nursery habitats: density-dependent effects on growth and dispersal in juvenile shore crabs *Carcinus maenas*. *Marine Ecology Progress Series*, *281*, 181–191.

Moksnes, P.-O., Pihl, L., & van Montfrans, J. (1998). Predation on postlarvae and juveniles of the shore crab *Carcinus maenas*: importance of shelter, size and cannibalism. *Marine Ecology Progress Series*, *166*, 211–225.

Morgado, F., Antunes, C., & Pastorinho, R. (2003). Distribution and patterns of emergence of suprabenthic and pelagic crustaceans in a shallow temperate estuary (Ria de Aveiro, Portugal). *Acta Oceologica*, *24*(SUPPL. 1), 205–217. http://doi.org/10.1016/S1146-609X(03)00035-3

Morse, D. H. (1982). *Behavioral mechanisms in ecology*. Harvard University Press.

Naylor, E. (1958). Tidal and diurnal rhythms of locomotory activity in *Carcinus maenas* (L.). *Journal of Experimental Biology*, *35*, 602–610.

Naylor, E. (1996). Crab clockwork: the case for interactive circatidal and circadian oscillators controlling rhythmic locomotor activity of *Carcinus maenas*. *Chronobiology International*, *13*(3), 153–161. http://doi.org/10.3109/07420529609012649

Nordhaus, I., Hadipudjana, F. A., Janssen, R., & Pamungkas, J. (2009). Spatio-temporal variation of macrobenthic communities in the mangrove-fringed Segara Anakan lagoon, Indonesia, affected by anthropogenic activities. *Regional Environmental Change*, *9*(4), 291–313. http://doi.org/10.1007/s10113-009-0097-5

Parrish, J. K., & Edelstein-keshet, L. (1999). Complexity, Pattern and Evolutionary Trade-Offs in Animal Aggregation. *Science*, *284*(April), 99–101.

Paula, J. (1989). Rhythms of larval release of decapoda crustaceans in the Mira Estuary, Portugal. *Marine Biology*, *100*, 309–312.

Pearson, W. H., Sugarman, P. C., & Woodruff, D. L. (1979). Thresholds for detection and feeding behavior in the Dungeness crab, *Cancer magister* (Dana). *Journal of Experimental Marine Biology and Ecology, 39*, 65–78.

Penha-Lopes, G., Bartolini, F., Limbu, S., Cannicci, S., Kristensen, E., & Paula, J. (2009). Are fiddler crabs potentially useful ecosystem engineers in mangrove wastewater wetlands? *Marine Pollution Bulletin*, *58*(11), 1694–1703. http://doi.org/10.1016/j.marpolbul.2009.06.015

Queiroga H, 1993. An analysis of the size structure of *Carcinus maenas* (L.) in Canal de Mira (Ria de Aveiro,Portugal), using the probability paper method. *Bios*, 1(1): 89-106

Queiroga H, 1995. *Processos de dispersão e recrutamento das larvas do caranguejo Carcinus maenas na Ria de Aveiro.* Ph. D. Thesis. Universidade de Aveiro, 268 pp.

Queiroga, H. (1996). Distribution and drift of the crab *Carcinus maenas* (L.) (Decapoda, Portunidae) larvae over the continental shelf off northern Portugal in April 1991. *Journal of Plankton Research*, *18*(11), 1981–2000. http://doi.org/10.1093/plankt/18.11.1981

Queiroga, H., Costlow, D., & Moreira, M. H. (1994). Larval abundance patterns of *Carcinus maenas* (Decapoda, Brachyura) in Canal de Mira (Ria de Aveiro , Portugal). *Marine Ecology Progress Series*, *111*, 63–72.

Reid, D. G., Abelló, P., Kaiser, M. J., & Warman, C. G. (1997). Carapace Colour, Inter-moult Duration and the Behavioural and Physiological Ecology of the Shore Crab *Carcinus maenas*. *Estuarine Coastal and Shelf Science*, (44), 203–211.

Reid, D. G., & Aldrich, J. C. (1989). Variation in responses to environmental hypoxia of different colour forms of the shore crab *Carcinus maenas* (L.). *Comparative Biochemistry and Physiology*, *92A*, 535–539.

Richard Shine. (1989). Ecological causes for the evolution of sexual dimorphism: A Review of the evidence. *The Quarterly Review of Biology*, *64*(4), 419–461.

Rodrigues, A. M., Quintino, V., Sampaio, L., Freitas, R., & Neves, R. (2011). Benthic biodiversity patterns in Ria de Aveiro, Western Portugal: Environmental-biological relationships. *Estuarine, Coastal and Shelf Science*, *95*(2-3), 338–348. http://doi.org/10.1016/j.ecss.2011.05.019

Rodrigues, M., Oliveira, a., Queiroga, H., & Brotas, V. (2012). Seasonal and diurnal water quality and ecological dynamics along a salinity gradient (Mira channel, Aveiro lagoon, Portugal). *Procedia Environmental Sciences*, *13*(2011), 899–918. http://doi.org/10.1016/j.proenv.2012.01.084

Sampedro, M., & González-Gurriarán, E. (2004). Aggregating behaviour of the spider crab *Maja squinado* in shallow waters. *Journal of Crustacean Biology*, *24*(1), 168–177. http://doi.org/10.1651/C-2404

Sheaves, M. (2005). Nature and consequences of biological connectivity in mangrove systems. *Marine Ecology Progress Series*, *302*, 293–305. http://doi.org/10.3354/meps302293

Sousa, L. P., Lillebø, A. I., Gooch, G. D., Soares, J. A., & Alves, F. L. (2013). Incorporation of Local Knowledge in the Identification of Ria de Aveiro Lagoon Ecosystem Services (Portugal). *Journal of Coastal Research*, (65), 1051–1056. http://doi.org/10.2112/SI65-178.1

Sousa, L. P., Sousa, A. I., Alves, F. L., & Lillebø, A. I. (2016). Ecosystem services provided by a complex coastal region: challenges of classification and mapping. *Scientific reports*, *6*.

Spooner, E. H., Coleman, R. A., & Attrill, M. J. (2007). Sex differences in body morphology and multitrophic interactions involving the foraging behaviour of the crab *Carcinus maenas*. *Marine Ecology*, *28*, 394–403. http://doi.org/10.1111/j.1439-0485.2007.00186.x

Stevens, B. G., Haaga, J. A., & Donaldson, W. E. (1994). Aggregative mating of Tanner crabs, *Chionoecetes bairdi*. *Canadian Journal of Fisheries and Aquatic Sciences*, *51*, 1273–1280. http://doi.org/10.1139/f94-127

Stone, R. P., Clair, C. E. O., & Shirley, T. C. (1993). Aggregating Behavior of Ovigerous Female Red King Crab, *Paralithodes camtschaticus*, in Auke Bay, Alaska. *Canadian Journal of Fisheries and Aquatic Sciences, 50*, 750–758.

Styrishave, B., Rewitz, K., & Andersen, O. (2004). Frequency of moulting by shore crabs *Carcinus maenas* (L .) changes their colour and their success in mating and physiological performance. *Journal of Experimental Marine Biology and Ecology*, *313*, 317–336. http://doi.org/10.1016/j.jembe.2004.08.013

Teixeira, S. (1994). *Dinâmica Morfossedimentar da Ria de Aveiro (Portugal).* Ph. D. Thesis. Faculdade de Ciências da Universidade de Lisboa, Portugal.

Thresher, R., Proctor, C., Ruiz, G. M., Gurney, R., MacKinnon, C., Walton, W. C., Rodriguez, L., Bax, N. (2003). Invasion dynamics of the European shore crab, *Carcinus maenas*, in Australia. *Marine Biology*, *142*, 867–876. http://doi.org/10.1007/s00227-003-1011-1

Underwood, A. J. (1997). *Experiments in Ecology: Their Logical Design and Inter- pretation Using Analysis of Variance.* Cambridge University Press. Cambridge, 504 pp.

Vasconcelos, R. M. P. (2009). *Contribution of estuarine nursery areas to the structure and biocontamination of stocks of commercially important fish species along the Portuguese coast.* Ph. D. Thesis, Faculdade de Ciências da Universidade de Lisboa, Portugal. 219 pp.

Vermeiren, P., & Sheaves, M. (2014). Predicting habitat associations of five intertidal crab species among estuaries. *Estuarine, Coastal and Shelf Science*, *149*, 133–142. http://doi.org/10.1016/j.ecss.2014.08.008

Warman, C. G., Reid, D. G., & Naylor, E. (1993). Intraspecific variation in the tidal migratory behaviour & associated rhythmic responsiveness to directional light in the shore crab *Carcinus maenas*. *Journal of the Marine Biological Association of the United Kingdom*, *73: in press*.

Warner, G. (1977). *The Biology of Crabs.* Elek Science, London. 202 pp.

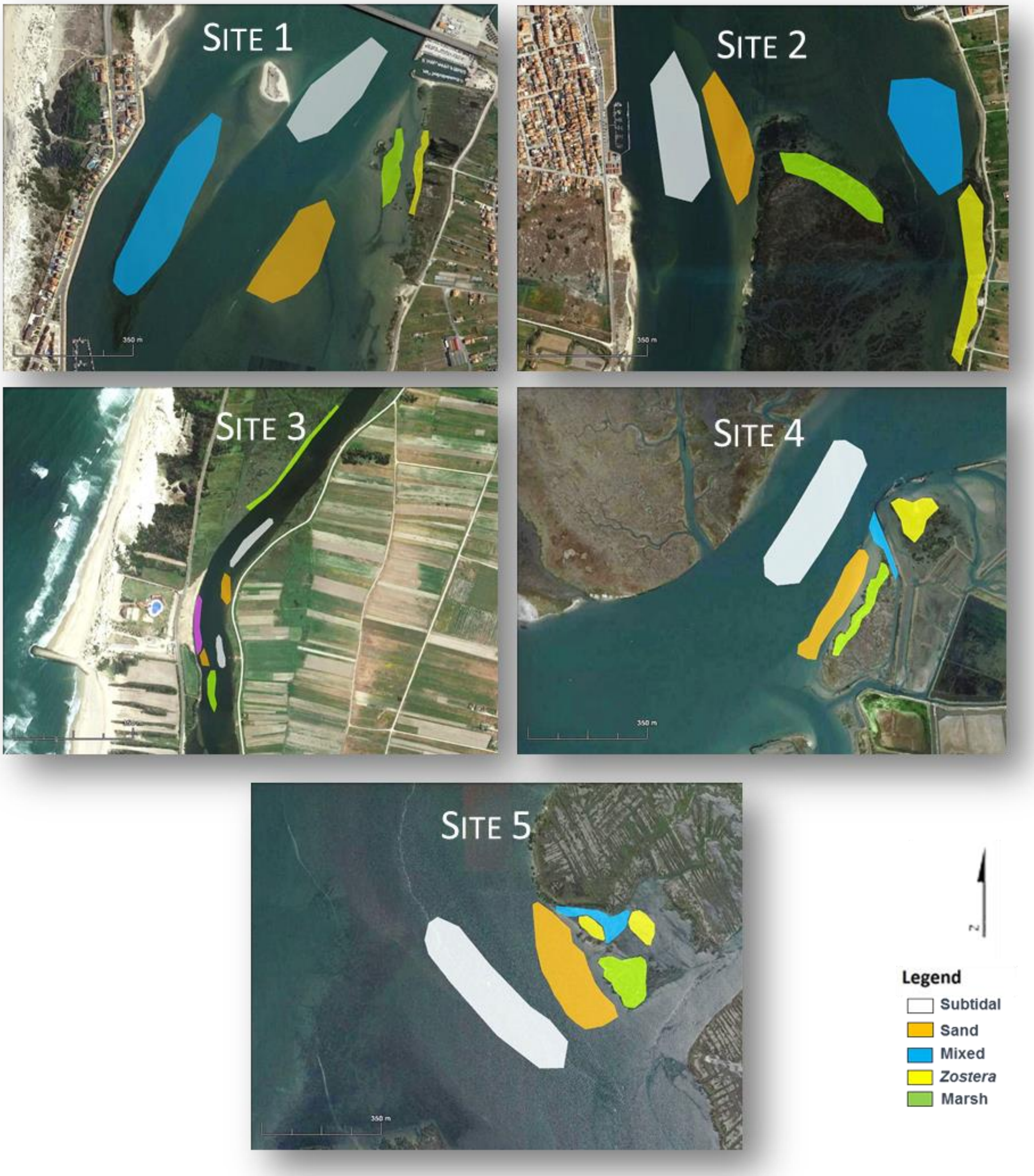
Williams, B. G., & Naylor, E. (1967). Spontaneously induced rhythm of tidal periodicity in laboratory-reared Carcinus. *Journal of Experimental Biology*, *47*(2), 229–234. http://doi.org/10.1016/j.marenvres.2007.12.002

Williams, B., & Naylor, E. (1969). Synchronization of the locomotor rhythm of Carcinus. *Journal of Experimental Biology*, *51*, 715–725. Retrieved from http://jeb.biologists.org/content/51/3/715.short

Zimmer-Faust, R. K., & Spanier, E. (1987). Gregariousness and sociality in spiny lobsters: implications for den habitation. *Journal of Experimental Marine Biology and Ecology*, *105*(1), 57–71. http://doi.org/10.1016/S0022-0981(87)80029-1

ANNEXES

Annex 1 - Habitats sampled per Site.



Annex 2 – Normality, homogeneity and homoscedasticity test of Number of female and male crabs' variables. This analysis supported the use PERMANOVA model test.

Normality test
Variable: Ln(Nº of Crabs)

	Kolmogorov-Smirnov[a]				Shapiro-Wilk			
	Statistics	df	p-value		Statistics	df	p-value	
Females	0,090	306	0,000	***	0,949	306	0,000	***
Males	0,099	282	0,000	***	0,966	282	0,000	***

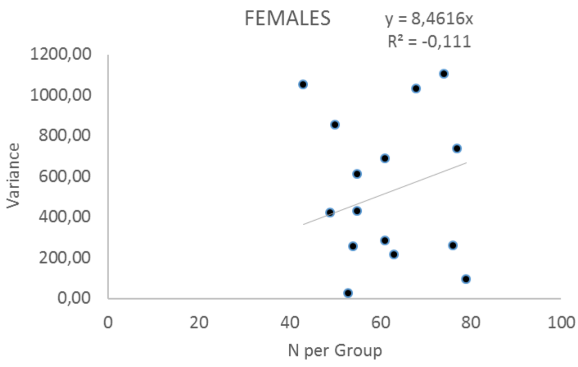
Levene test
Variable: Ln(Nº of Crabs)

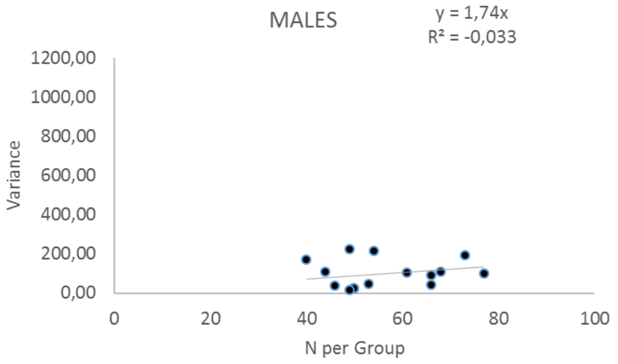
	Z	df1	df2	Sig.	
Females	4,114	114	191	0,000	***
Males	2,892	111	170	0,000	***

Descriptive statistics
Variable: Ln(Nº of Crabs)

		Females			Males		
	Groups	**Mean**	**Variance**	**n**	**Mean**	**Variance**	**n**
Month	**Jan**	13,72	255,34	54	16,37	224,78	49
	Feb	25,77	1053,09	43	12,28	170,72	40
	Mar	17,80	423,00	49	8,34	109,16	44
	Apr	16,27	430,24	55	7,45	48,14	53
	May	20,05	610,53	55	7,41	37,67	46
	Jun	23,50	855,97	50	5,88	25,70	50
Site	**Site 1**	22,95	737,42	77	12,00	192,47	73
	Site 2	10,28	94,23	79	9,08	100,70	77
	Site 4	13,84	262,75	76	6,14	41,42	66
	Site 5	28,50	1105,54	74	10,74	89,83	66
Habitat	**Sand**	35,41	1032,10	68	13,59	107,26	68
	Mixed	26,80	689,99	61	12,22	215,23	54
	Marsh	3,92	27,49	53	3,96	25,75	50
	Subtidal	15,30	218,09	63	11,15	104,13	61
	Zostera	11,13	283,72	61	4,65	17,98	49

Homoscedasticity test





Annex 3 - Normality, homogeneity and homoscedasticity test of female and male crabs' CW variables. This analysis supported the use ANOVA model test.

Normality test
Variable: Ln(CW)

	Kolmogorov-Smirnov[a]				Shapiro-Wilk			
	Statistics	df	p-value		Statistics	df	p-value	
Females	0,122	6983	0,000	***	0,831	6983	0,000	***
Males	0,110	2969	0,000	***	0,078	2969	0,000	***

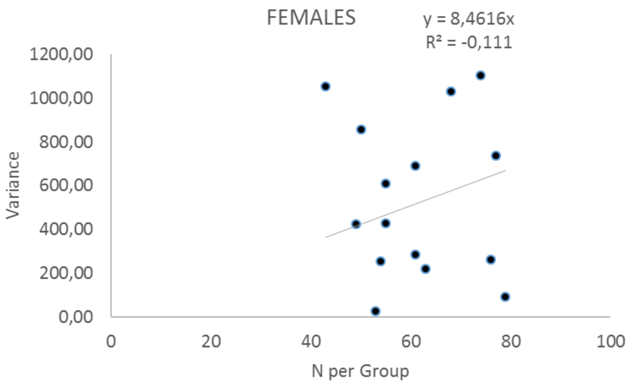
Levene test
Variable: Ln(CW)

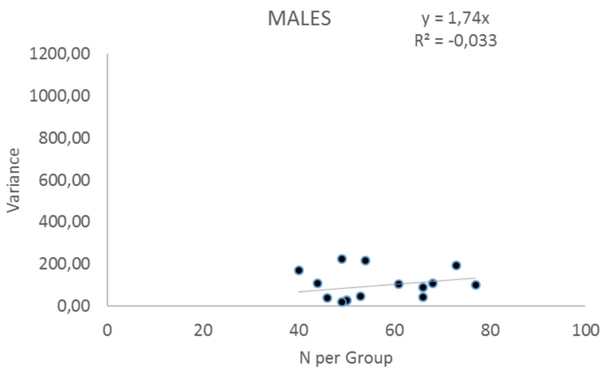
	Z	df1	df2	Sig.	
Females	10,697	114	6868	0,000	***
Males	6,743	111	2857	0,000	***

Descriptive statistics
Variable: Ln(CW)

		Females			Males		
	Groups	**Mean**	**Variance**	**n**	**Mean**	**Variance**	**n**
Month	**Jan**	3,46	0,20	865	3,58	0,12	903
	Feb	3,56	0,12	518	3,65	0,05	1342
	Mar	3,62	0,13	457	3,61	0,04	1007
	Apr	3,67	0,15	422	3,63	0,06	1066
	May	3,84	0,06	395	3,72	0,03	1382
	Jun	3,88	0,05	312	3,74	0,02	1283
Site	**Site 1**	3,65	0,09	930	3,70	0,05	2029
	Site 2	3,59	0,15	843	3,61	0,09	857
	Site 4	3,64	0,24	424	3,70	0,06	1313
	Site 5	3,63	0,20	772	3,64	0,05	2784
Habitat	**Sand**	3,64	0,10	1005	3,67	0,04	2921
	Mixed	3,65	0,16	750	3,67	0,06	1779
	Marsh	3,24	0,48	207	3,50	0,21	253
	Subtidal	3,70	0,08	761	3,70	0,03	1225
	Zostera	3,59	0,20	246	3,64	0,08	805

Homoscedasticity test





Annex 4 - Normality, homogeneity and homoscedasticity test of female and male crabs' Weight and CW variables. This analysis supported the use ANCOVA model test.

Both variables raw data were tested with Shapiro-Wilk normality test and was found that neither of them had a normal distribution for both females (Weight: W = 0.97573, p-value < 2.2 x 10^{-16}; CW: W=0.82155, p-value < 2.2 x 10^{-16}) and males' samples (Weight: W = 0.90586, p-value < 2.2 x 10^{-16}; CW: W=0.93031, p-value < 3.096 x 10^{-10}).

Normality test
Variables: Ln(weight) and Ln(CW)

			Kolmogorov-Smirnov[a]				Shapiro-Wilk			
	Variable	Month	Statistics	df	p-value		Statistics	df	p-value	
Females	Ln(weight)	Jan	0,170	903	0,0000	***	0,809	903	0,0000	***
		Feb	0,108	1342	0,0000	***	0,854	1342	0,0000	***
		Mar	0,084	265	0,0001	***	0,877	265	0,0000	***
		Apr	0,178	351	0,0000	***	0,760	351	0,0000	***
		May	0,092	343	0,0000	***	0,895	343	0,0000	***
		Jun	0,041	347	0,2000		0,989	347	0,0095	**
	Ln(CW)	Jan	0,183	903	0,0000	***	0,799	903	0,0000	***
		Feb	0,134	1342	0,0000	***	0,840	1342	0,0000	***
		Mar	0,097	265	0,0000	***	0,885	265	0,0000	***
		Apr	0,205	351	0,0000	***	0,742	351	0,0000	***
		May	0,110	343	0,0000	***	0,885	343	0,0000	***
		Jun	0,076	347	0,0001	***	0,985	347	0,0012	**
Males	Ln(weight)	Jan	0,092	865	0,0000	***	0,915	865	0,0000	***
		Feb	0,066	518	0,0000	***	0,918	518	0,0000	***
		Mar	0,178	141	0,0000	***	0,775	141	0,0000	***
		Apr	0,157	161	0,0000	***	0,825	161	0,0000	***
		May	0,109	152	0,0001	***	0,885	152	0,0000	***
		Jun	0,152	154	0,0000	***	0,639	154	0,0000	***
	Ln(CW)	Jan	0,100	865	0,0000	***	0,913	865	0,0000	***
		Feb	0,087	518	0,0000	***	0,915	518	0,0000	***
		Mar	0,204	141	0,0000	***	0,642	141	0,0000	***
		Apr	0,166	161	0,0000	***	0,821	161	0,0000	***
		May	0,110	152	0,0001	***	0,872	152	0,0000	***
		Jun	0,165	154	0,0000	***	0,632	154	0,0000	***

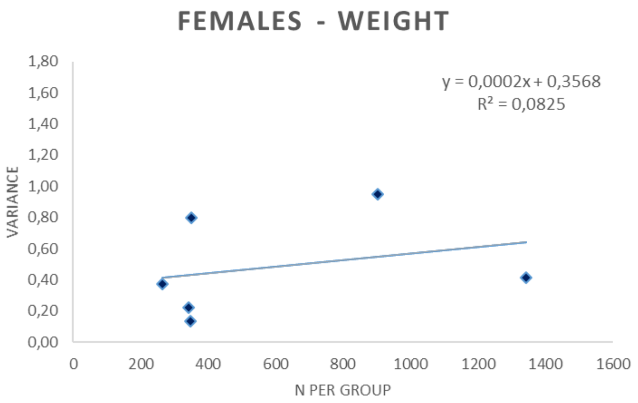
Levene test

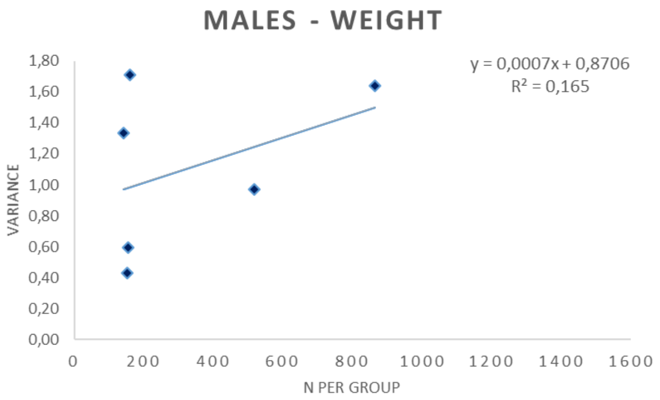
		Z	df1	df2	Sig.
Females	ln(CW)	31,79	5,00	3545,00	0,00
	Ln(Weight)	38,25	5,00	3545,00	0,00
Males	ln(CW)	22,07	5,00	1985,00	0,00
	Ln(Weight)	22,50	5,00	1985,00	0,00

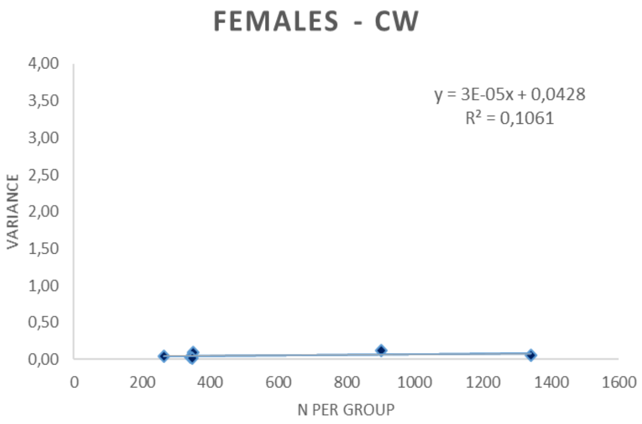
Descriptive statistics
Variables: Ln(weight) and Ln(CW)

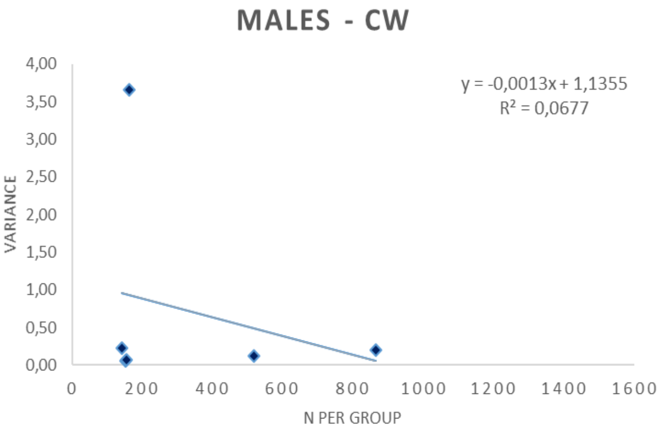
	Groups of Months	Females Mean	Females Variance	Females n	Males Mean	Males Variance	Males n
Ln (Weight)	Jan	2,38	0,95	903	2,01	1,64	865
	Feb	2,57	0,41	1342	2,35	0,97	518
	Mar	2,53	0,37	265	2,67	1,33	141
	Apr	2,55	0,80	351	2,60	1,71	161
	May	2,88	0,22	343	3,33	0,43	152
	Jun	2,98	0,14	347	3,41	0,59	154
Ln (CW)	Jan	3,58	0,12	903	3,46	0,20	865
	Feb	3,65	0,05	1342	3,56	0,12	518
	Mar	3,65	0,05	265	3,68	0,23	141
	Apr	3,64	0,10	351	3,66	3,66	161
	May	3,76	0,03	343	3,91	0,05	152
	Jun	3,78	0,02	347	3,92	0,07	154

Homoscedasticity test









X

Annex 5- Underwater video camera: A low budget solution for benthic estuarine studies underwater.

In order to do the tidal migration experiment, we needed an underwater camera that fulfilled this criteria: it had to have InfraRed light (night vision), also at least 6h of autonomy, water resistance (to at least 5 m depth), plus, if possible, some quality of resolution and high memory space. Those criteria where only totally fulfilled by highly expensive cameras. A low budget investigation always requires some level of creation, so we decided to create a hybrid of what already exists, using an IR low cost street camera, and fulfill the other needed criteria with a inox water resistant case.

For the construction of the waterproof case we used the following methodology which should be read while consulting Fig. 4:

In one side (see Fig. 4 – left side of Side view), this waterproof case has a top border in where, with screws, are fixed some necessary components such as: a double inox and rubber o'rings, a cylindrical silica glass with 20 cm of diameter and another rubber o'ring. These components can be removed when camera need to be put in position for a dive, however it's necessary to verify that those components are screwed back in the same original order on the waterproof case. This way the camera will be completely sealed from water. This side is headed down during the experiments.

On the other side (see Fig. 4 – right side of Side view), is a completely sealed and non-removable area of the inox case. A net cable was passed through a small perforation in the inox, being the remaining area completely sealed with silicone glue. This net cable is the bridge between the camera inside the waterproof case and the remaining equipment outside the waterproof case. This remaining equipment is responsible for the power source and for storing the video image information, which the camera by itself is not able to do.

After the waterproof case setup, we placed the IR camera inside connected to the net cable in its interior. The camera was powered by a switch poE which is linked to a portable computer where the camera's software was installed. The storage of the live video in made in the computer so it also had to have a power source in order to remain active during all the experiment duration. Both, the switch poE and the portable computer are linked to a cyclic 33Ah battery by an inverter. All this secondary equipment during the experiment is assembled at the surface in an anchored stationary boat near the experiment site. Its markets reference and model are described in the following Table 7.

Table 7

	Material description	Markets Reference or Model No.
1	Infrared Bullet Network Camera (IP BULLET 2MP) Ambrella S2	DS-SUN-BULLET-2MA
2	Inox AISI 316L 114,3 x 2 x 6000 mm	P4 114.3x2
3	Desktop PoE Switch 8- port 10/100M	TL-SF1008P
4	300 WATT DC to AC modified sinewave inverter with USB output (12VDC P/230VAC)	HQ-INV300WU-12
5	Cyclic Battery 12V 33Ah S Yuasa	SLAYPC12330YUA

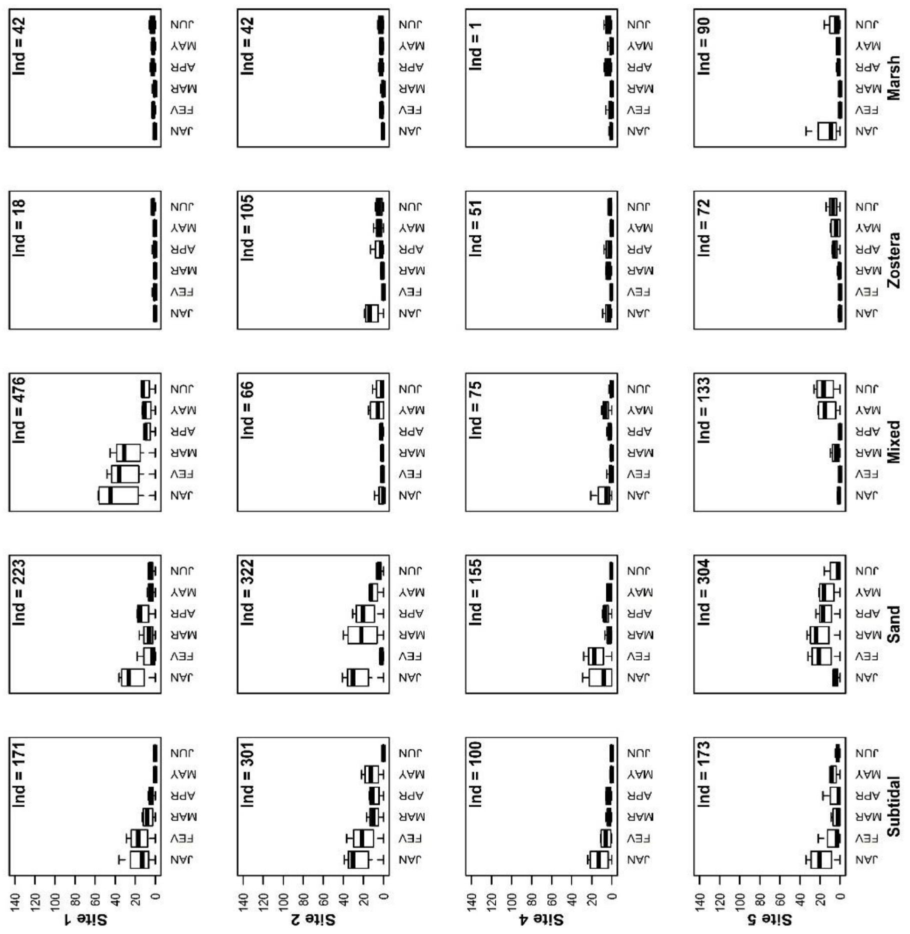
Finally, the “inox arms” presented in Fig. 4 (Front and Back view) allowed the stationary placement of the waterproof case underwater, with the camera directed to the recording area. The “inox arms” are connected to a metallic structure that is fixed in the field to the bottom surface (see Fig. 5).

Some problems that should be taken in consideration before using this methodology in the field:

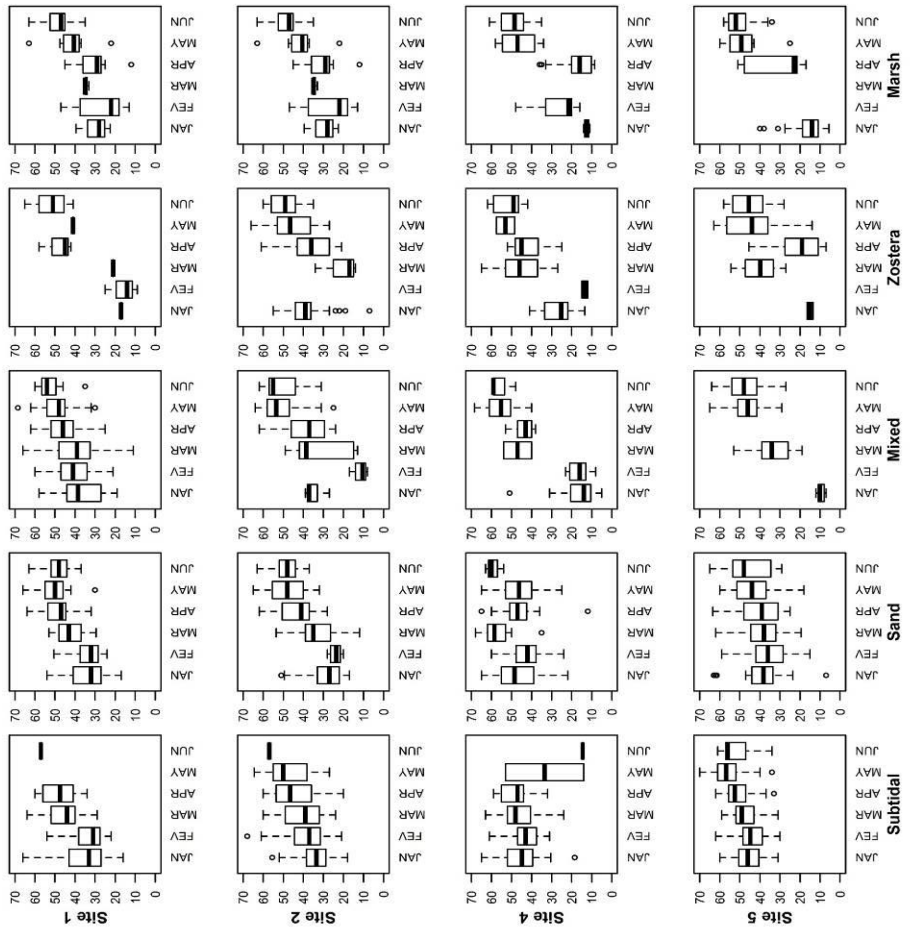
During the equipment pre-tests, we confronted with a major fogging issue in the camera’s lens protecting glass and that made entirely impossible the video image display. We resolved it by changing the existing double glass of the camera (manufactured made by origin) for a simple glass. That way, when heated, all the glass components were disposed to the same temperature. Then, we applied an anti-fogging product (Sea Gold Anti-fog gel & cleaner, 37 ml, McNETT - ref. RP2030) on both sides of the simple glass. Because all the field tests were highly time consuming, we needed them to be very cost effective. So, to make sure that the fogging issue ceased, we placed both the camera and the inox waterproof case in a dehumidifier during one day, previously to the pre-tests experiments. Later, still under the dehumidifier, we set up the camera inside the waterproof inox case and sealed it. Using this unique protocol, test in the field had always positive results.

Annex 6

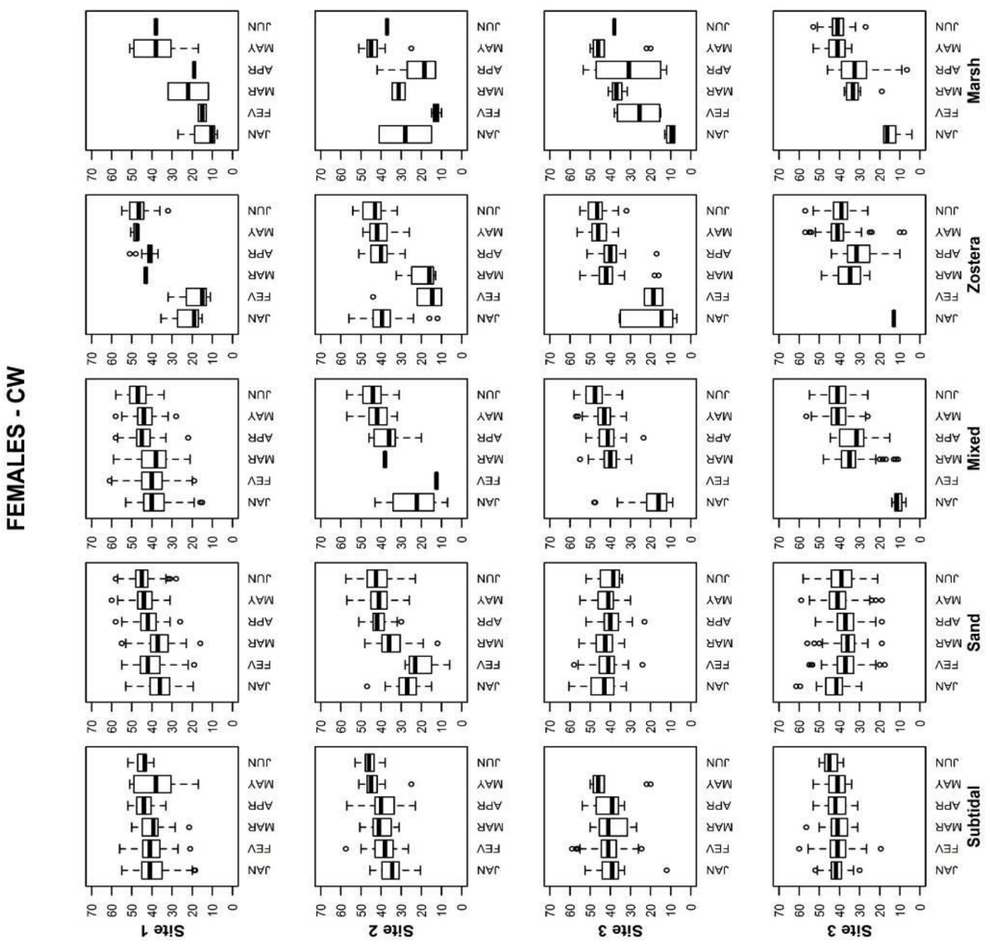
Nº of crabs - MALES

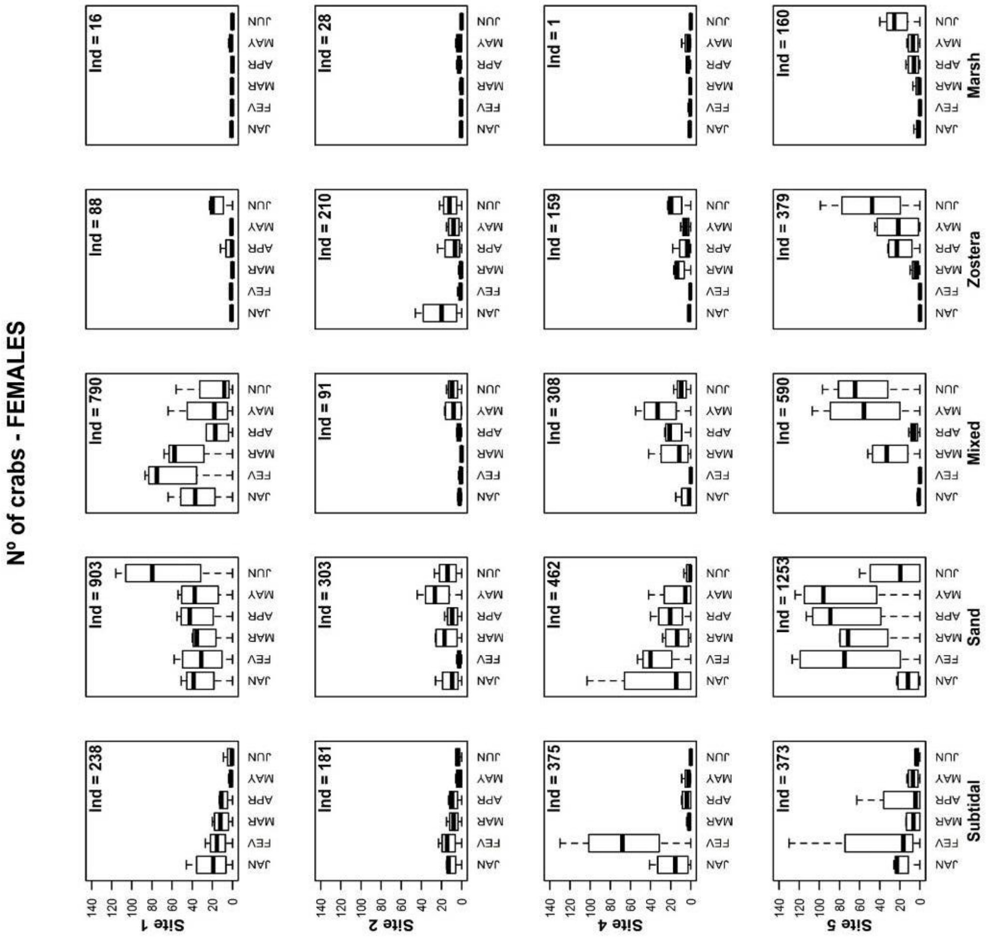


MALES - CW

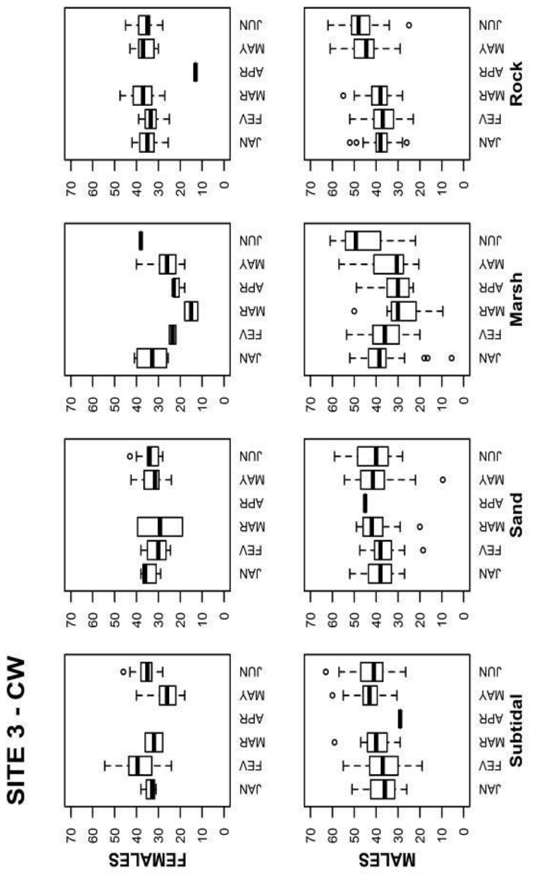


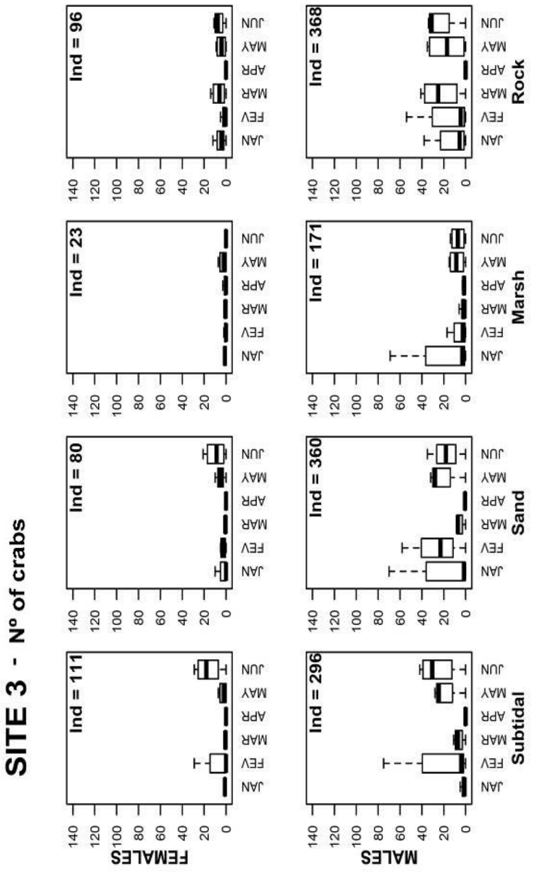
Annex 7


FEMALES - CW
Site 1
Site 2
Site 3
Site 3
Subtidal
Sand
Mixed
Zostera
Marsh
JAN
FEV
MAR
APR
MAY
JUN
70
60
50
40
30
20
10
0

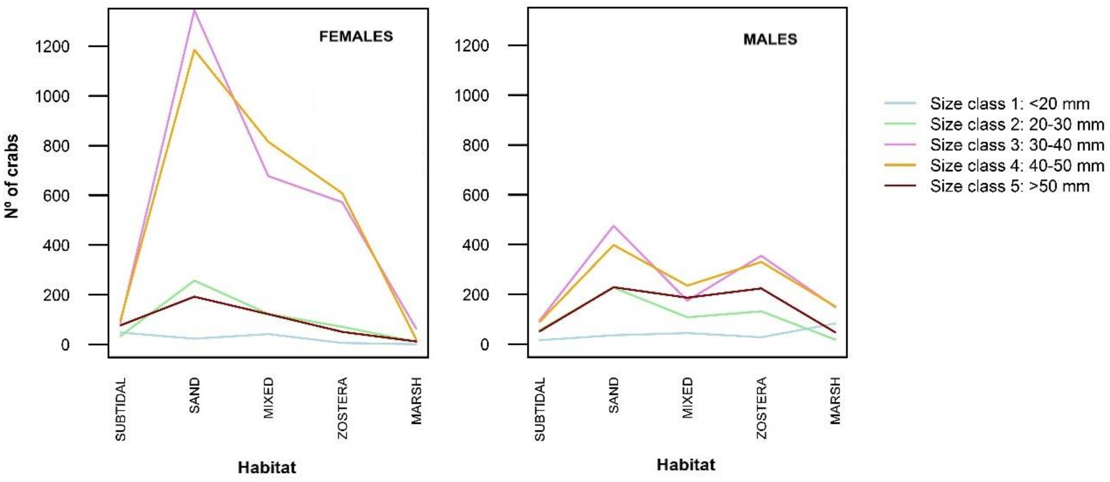

Nº of crabs - FEMALES
Site 1
Site 2
Site 4
Site 5
Subtidal
Sand
Mixed
Zostera
Marsh
Ind = 238
Ind = 903
Ind = 790
Ind = 88
Ind = 16
Ind = 181
Ind = 303
Ind = 91
Ind = 210
Ind = 28
Ind = 375
Ind = 462
Ind = 308
Ind = 159
Ind = 1
Ind = 373
Ind = 1253
Ind = 590
Ind = 379
Ind = 160
JAN
FEV
MAR
APR
MAY
JUN
140
120
100
80
60
40
20
0

Annex 8

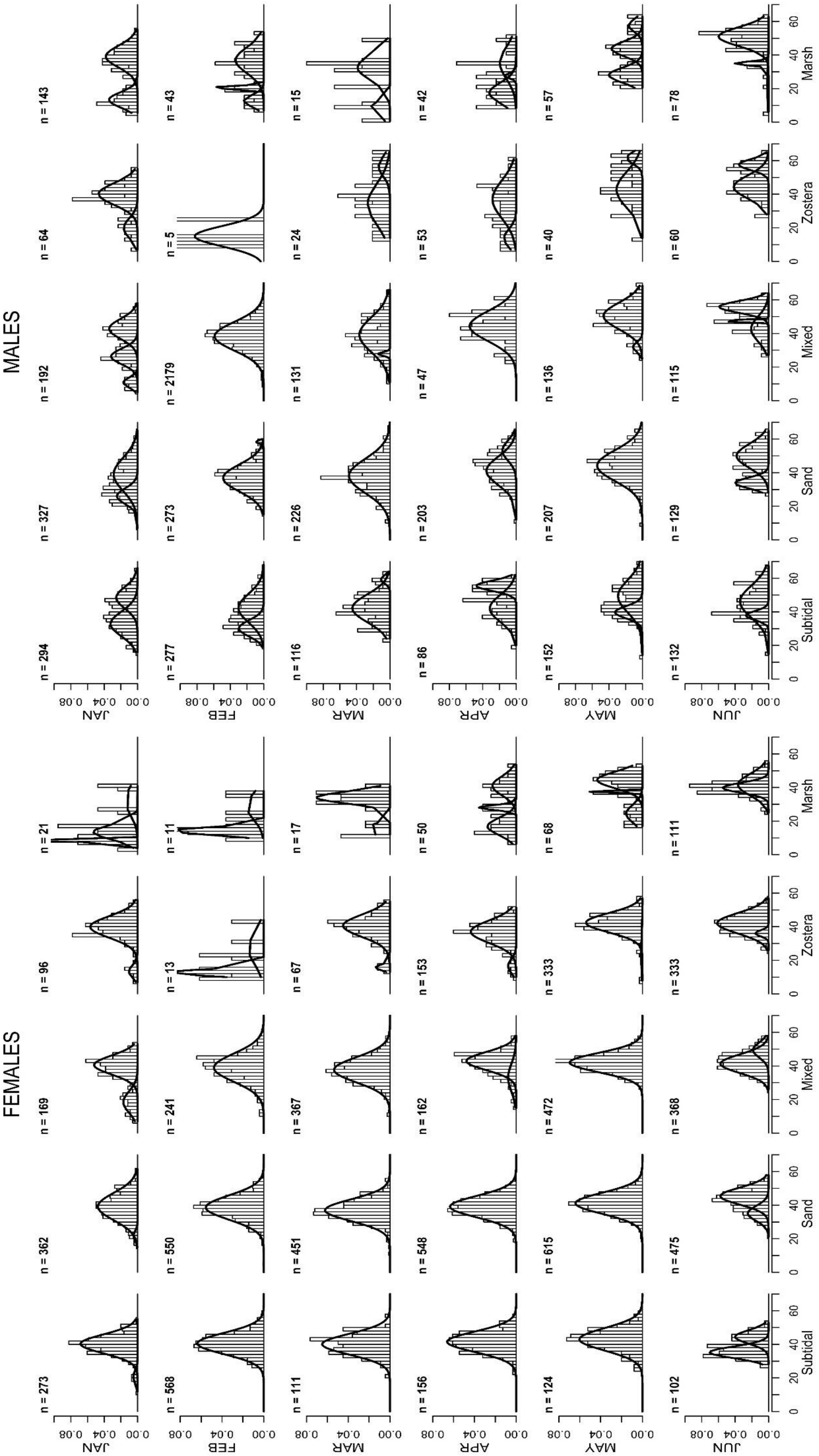

SITE 3 - CW
FEMALES
MALES
70
60
50
40
30
20
10
0
JAN
FEV
MAR
APR
MAY
JUN
Subtidal
Sand
Marsh
Rock


SITE 3 - Nº of crabs
FEMALES
MALES
140
120
100
80
60
40
20
0
JAN
FEV
MAR
APR
MAY
JUN
Ind = 111
Ind = 80
Ind = 23
Ind = 96
Ind = 296
Ind = 360
Ind = 171
Ind = 368
Subtidal
Sand
Marsh
Rock

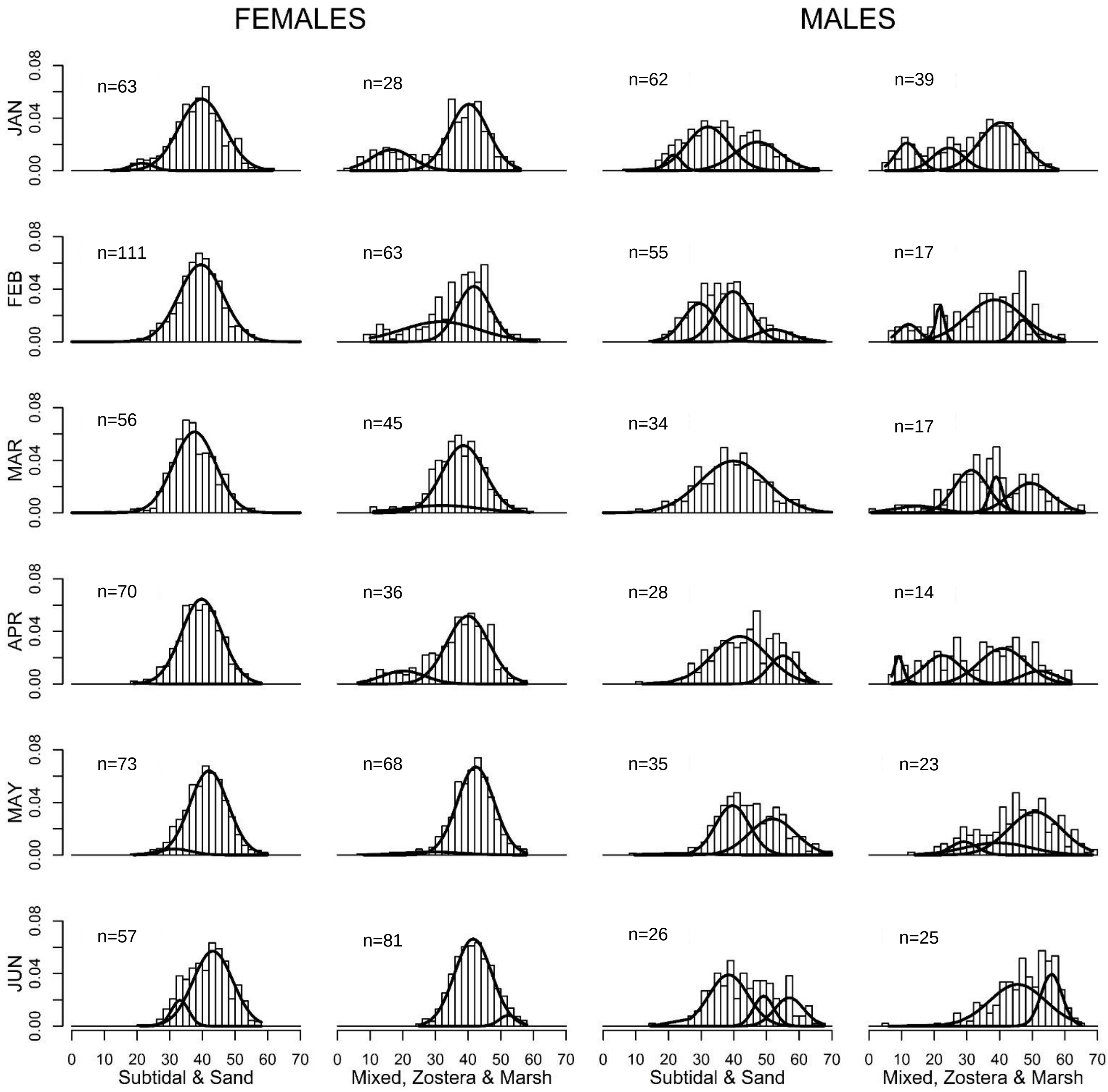
Annex 9


FEMALES
MALES
Nº of crabs
1200
1000
800
600
400
200
0
SUBTIDAL
SAND
MIXED
ZOSTERA
MARSH
Habitat
Size class 1: <20 mm
Size class 2: 20-30 mm
Size class 3: 30-40 mm
Size class 4: 40-50 mm
Size class 5: >50 mm

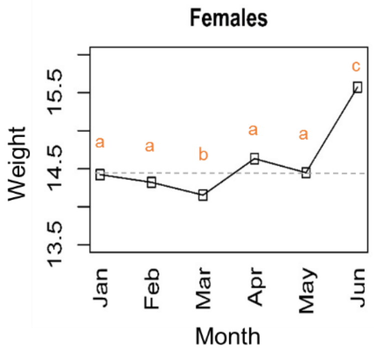
Annex 10


FEMALES
MALES
JAN
FEB
MAR
APR
MAY
JUN
0.00 0.04 0.08
Subtidal
Sand
Mixed
Zostera
Marsh
0 20 40 60
n = 273
n = 362
n = 169
n = 96
n = 21
n = 568
n = 550
n = 241
n = 13
n = 11
n = 111
n = 451
n = 367
n = 67
n = 17
n = 156
n = 548
n = 162
n = 153
n = 50
n = 124
n = 615
n = 472
n = 333
n = 68
n = 102
n = 475
n = 368
n = 333
n = 111
n = 294
n = 327
n = 192
n = 64
n = 143
n = 277
n = 273
n = 2179
n = 5
n = 43
n = 116
n = 226
n = 131
n = 24
n = 15
n = 86
n = 203
n = 47
n = 53
n = 42
n = 152
n = 207
n = 136
n = 40
n = 57
n = 132
n = 129
n = 115
n = 60
n = 78

Annex 11

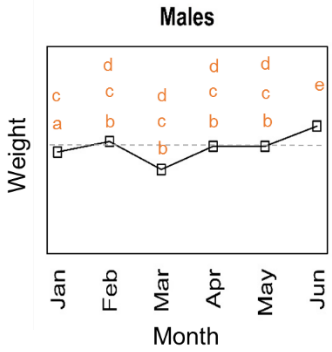

FEMALES
MALES
JAN
FEB
MAR
APR
MAY
JUN
0.00
0.04
0.08
n=63
n=28
n=62
n=39
n=111
n=63
n=55
n=17
n=56
n=45
n=34
n=17
n=70
n=36
n=28
n=14
n=73
n=68
n=35
n=23
n=57
n=81
n=26
n=25
0 10 20 30 40 50 60 70
Subtidal & Sand
Mixed, Zostera & Marsh
Subtidal & Sand
Mixed, Zostera & Marsh

Annex 12



	Month		Mean Difference	Standardized error	p-value	Difference's Confidence Interval of 95%	
						Inferior limit	Superior limit
FEMALES	JAN	FEB	-0,0086	0,0074	0,2501	-0,0232	0,0060
		MAR	0,0390	0,0121	0,0012 **	0,0155	0,0628
		APR	-0,0173	0,0109	0,1113	-0,0386	0,0040
		MAY	-0,0097	0,0111	0,3828	-0,0315	0,0121
		JUN	-0,0630	0,0111	0,0000 ***	-0,0849	-0,0414
	FEB	JAN	0,0086	0,0074	0,2501	-0,0060	0,0232
		MAR	0,0480	0,0116	0,0000 ***	0,0250	0,0704
		APR	-0,0087	0,0103	0,3987	-0,0290	0,0115
		MAY	-0,0011	0,0105	0,9146	-0,0217	0,0195
		JUN	-0,0550	0,0105	0,0000 ***	-0,0752	-0,0341
	MAR	JAN	-0,0390	0,0121	0,0012 ***	-0,0628	-0,0155
		FEB	-0,0480	0,0116	0,0000 ***	-0,0704	-0,0250
		APR	-0,0560	0,0140	0,0001 ***	-0,0839	-0,0289
		MAY	-0,0490	0,0141	0,0006 ***	-0,0766	-0,0211
		JUN	-0,1020	0,0141	0,0000 ***	-0,1300	-0,0746
	APR	JAN	0,0173	0,0109	0,1113	-0,0040	0,0386
		FEB	0,0087	0,0103	0,3987	-0,0115	0,0290
		MAR	0,0560	0,0140	0,0001 ***	0,0289	0,0839
		MAY	0,0076	0,0132	0,5638	-0,0182	0,0334
		JUN	-0,0460	0,0131	0,0005 ***	-0,0716	-0,0201
	MAY	JAN	0,0097	0,0111	0,3828	-0,0121	0,0315
		FEB	0,0011	0,0105	0,9146	-0,0195	0,0217
		MAR	0,0490	0,0141	0,0006 ***	0,0211	0,0766
		APR	-0,0076	0,0132	0,5638	-0,0334	0,0182
		JUN	-0,0530	0,0131	0,0000 ***	-0,0792	-0,0278
	JUN	JAN	0,0630	0,0111	0,0000 ***	0,0414	0,0849
		FEB	0,0550	0,0105	0,0000 ***	0,0341	0,0752
		MAR	0,1020	0,0141	0,0000 ***	0,0746	0,1300
		APR	0,0460	0,0131	0,0005 ***	0,0201	0,0716
		MAY	0,0530	0,0131	0,0000 ***	0,0278	0,0792

Annex 13



	Month		Mean Difference	Standardized error	p-value	Difference's Confidence Interval of 95%	
						Inferior limit	Superior limit
MALES	JAN	FEB	-0,0360	0,0121	0,0030 **	-0,0598	-0,0123
		MAR	-0,0324	0,0199	0,1032	-0,0715	0,0066
		APR	-0,0284	0,0188	0,1306	-0,0652	0,0084
		MAY	-0,0570	0,0199	0,0043 **	-0,0958	-0,0179
		JUN	-0,1120	0,0198	0,0000 ***	-0,1506	-0,0730
	FEB	JAN	0,0360	0,0121	0,0030 **	0,0123	0,0598
		MAR	0,0036	0,0207	0,8619	-0,0369	0,0441
		APR	0,0076	0,0196	0,6974	-0,0308	0,0461
		MAY	-0,0208	0,0205	0,3089	-0,0609	0,0193
		JUN	-0,0760	0,0204	0,0002 ***	-0,1157	-0,0358
	MAR	JAN	0,0324	0,0199	0,1032	-0,0066	0,0715
		FEB	-0,0036	0,0207	0,8619	-0,0441	0,0369
		APR	0,0040	0,0250	0,8720	-0,0450	0,0531
		MAY	-0,0244	0,0255	0,3389	-0,0745	0,0256
		JUN	-0,0790	0,0255	0,0018 **	-0,1293	-0,0295
	APR	JAN	0,0284	0,0188	0,1306	-0,0084	0,0652
		FEB	-0,0076	0,0196	0,6974	-0,0461	0,0308
		MAR	-0,0040	0,0250	0,8720	-0,0531	0,0450
		MAY	-0,0284	0,0247	0,2502	-0,0769	0,0201
		JUN	-0,0830	0,0247	0,0007 **	-0,1318	-0,0350
	MAY	JAN	0,0570	0,0199	0,0043 **	0,0179	0,0958
		FEB	0,0208	0,0205	0,3089	-0,0193	0,0609
		MAR	0,0244	0,0255	0,3389	-0,0256	0,0745
		APR	0,0284	0,0247	0,2502	-0,0201	0,0769
		JUN	-0,0550	0,0248	0,0268 *	-0,1036	-0,0063
	JUN	JAN	0,1120	0,0198	0,0000 ***	0,0730	0,1506
		FEB	0,0760	0,0204	0,0002 ***	0,0358	0,1157
		MAR	0,0790	0,0255	0,0018 **	0,0295	0,1293
		APR	0,0830	0,0247	0,0007 **	0,0350	0,1318
		MAY	0,0550	0,0248	0,0268 *	0,0063	0,1036