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Edited by

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Preface

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This volume includes the proceedings of the 8th Colloquium Crustacea Decapoda Mediterranea, which was held at the Ionian University on Corfu Isl., Greece, from 2 to 6 September 2002 and was organized by the National and Kapodistrian University of Athens. The meeting is the oldest European carcinological event held regularly at a 3-year interval. Since 1972, institutions from Mediterranean countries organized Colloquia in Rovinj, Ancona, Barcelona, Thessaloniki, Paris, Florence and Lisbon. Although the meeting is devoted to the Mediterranean area, contributions worldwide are mostly welcome, providing, thus, a forum for fruitful contact and scientific exchange.

In Corfu, 120 delegates from 21 countries representing all continents met and presented 8 plenary talks, 46 oral and 74 poster presentations. The themes included covered all issues of decapod biology: systematics, phylogeny, genetics, ethology, morphology, biogeography, reproduction, ecology, physiology and fisheries. A special session was devoted to deep-water shrimps and related research projects.

The Scientific Committee of the 8th Colloquium Crustacea Decapoda Mediterranea was composed of K. Anger (Germany), G. Charmantier (France), P. Dworschak (Austria), A. Eleftheriou (Greece), W. Emmerson (South Africa), D. L. Felder (USA), C. H. J. M. Fransen (The Netherlands), C. Froglia (Italy), B. Galil (Israel), J. E. García Raso (Spain), R. G. Hartnoll (UK), A. Koukouras (Greece), P. K. L. Ng (Singapore), P. Y. Noël (France), J. Paula (Portugal), K. Sakai (Japan), F. Sardà (Spain), R. Serjidi (Algeria), E. Spanier (Israel), Zd. Števčić (Croatia), M. Thessalou-Legaki (Greece), M. Türkay (Germany) and M. Vannini (Italy).

The Organizing Committee, based at the Department of Zoology–Marine Biology of the University of Athens, was composed of M. Apostolopoulou, A. Chilari, K. Kapiris, K. Kevrekidis, A. Merakou, S. Papaspyrou, J. Paula and Zd. Števčić and M. Thessalou-Legaki as coordinator.

Many thanks are due to all the institutions and persons who helped in the organization of the meeting and the publication of the proceedings: the Ionian University, especially Prof. A. Verganelakis and Mrs. E. Kourkoulou, for providing the facilities in Corfu; the University of Athens, the Greek Ministry of Education, the Region of the Ionian Islands and the Municipality of Corfu for supporting the meeting financially or providing us with facilities and services. I would like to express my gratitude to *Hydrobiologia* as well as its previous and present Editors-in-Chief, Profs. H. Dumont and K. Martens, for the collaboration, to the referees for their thorough reviews and to Mrs. A. Chilari for the support in the preparation of the proceedings. Thanks are also due to the members of the Organizing Committee, students, family and friends for their effective efforts, to the members of the Scientific Committee for their valuable suggestions and to all participants and authors of the present volume.

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Discrimination between the Mediterranean spiny lobsters *Palinurus elephas* and *P. mauritanicus* (Crustacea: Decapoda) by mitochondrial sequence analysis

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Key words: spiny lobster, *Palinurus elephas*, *Palinurus mauritanicus*, genetics, COI

Abstract

The Mediterranean spiny lobsters, the common spiny lobster *Palinurus elephas* (Fabricius, 1758) and the pink spiny lobster *P. mauritanicus* Gruvel, 1911, are important target species for commercial fisheries. In this study, we focus our attention on the DNA sequence variation of the mitochondrial cytochrome oxydase I gene (COI) in the two species of *Palinurus*. Spiny lobster DNA samples from four Mediterranean localities were analysed to examine the genetic variability at both the intra- and interspecific level. Furthermore, the phylogenetic relationships within the family Palinuridae (among the two species of *Palinurus*, most of the species of *Panulirus* and all the species of *Jasus*) are examined.

Introduction

In the Mediterranean Sea, two species of spiny lobsters are commonly found, both belonging to the genus *Palinurus* Weber, 1795: *Palinurus elephas* (Fabricius, 1787) and *Palinurus mauritanicus* Gruvel, 1911. The genus *Palinurus* comprises three more species (*P. charlestoni* Forest & Postel 1964, *P. delagoae* Barnard 1926, *P. gilchristi* Stebbing, 1900) distributed in the eastern Atlantic and off southeast Africa. All species of *Palinurus* are of present or potential future commercial interest (Holthuis, 1991).

In the last decades, there has been increasing fishing pressure on these Mediterranean species reaching overexploitation with marked declines in catches (Ceccaldi & Latrouite, 2000) that show the need for a new approach to management strategies based on a clear identification of genetic stocks.

The genetics at molecular level of the genus *Palinurus* is poorly investigated. The only known molecular data for *P. elephas* and *P. mauritanicus* refer to the analysis of the nuclear 28S rDNA with the description of species-specific markers but they do not refer to intraspecific nucleotide variability (Cannas et al., 2001).

Molecular characters could aid in clarifying the arguable phylogenetic position of the genus *Palinurus* since different results are obtained depending on whether larval morphology, adult morphology or paleontological data are considered in phylogenetic reconstructions (George & Main, 1967; Baisre, 1994). In recent years, molecular phylogenies with hypotheses about the evolution, the speciation mechanism, and the correlation of species radiation with paleoclimatic and paleogeographic events have been published for the two genera *Jasus* and *Panulirus*, (see Ovenden et al., 1997; Ptacek et al., 2001).

In this work, we focus our attention on the DNA sequence variation of the mitochondrial cytochrome oxydase I gene (COI) in two *Palinurus* species. In order to examine the genetic variability at both the intra- and interspecific level, spiny lobster DNA samples from different Mediterranean localities were analysed.

In addition, the same mitochondrial region was used to examine the phylogenetic relationships between the two species of *Palinurus* and other species of two genera belonging to the family Palinuridae, *Panulirus* and *Jasus*.

Materials and methods

Adult specimens of *P. elephas* and *P. mauritanicus* were collected by trammel nets and trawls from diverse locations of their distribution area during 1997–2002. In particular, for the common spiny lobster we analysed 10 individuals from the Sardinian Sea (both from the western and the southern coasts), five individuals from the Ligurian Sea and two from the Ionian Sea. In the case of the pink spiny lobster, we examined five specimens that were collected off the Sardinian coasts and two specimens from the Atlantic (off the Portuguese coasts).

For each sample, the leg muscle was dissected and stored in an excess of 90% ethanol. Total DNA was isolated using the standard phenol/chloroform extraction techniques (Sambrook et al., 1989).

A region of the COI gene (~750 bp, size estimated by agarose gel electrophoresis) was amplified by the polymerase chain reaction (PCR) using a pair of primers (LCO 5′-GGTCAACRAATCAYAARGATATTGG-3′, HCOI 5′-TAAACTTCAGGGTGRCCAAAAAATCA-3′) modified from those given in Folmer et al., (1994). A MJ Research PTC-100 thermal cycler was used with the following cycle profiles according to the TOUCHDOWN-PCR approach (Don et al., 1991): 5 min at 95 °C; 3 cycles of 30 s at 95 °C, 30 s at 52 °C, 1 min at 72 °C; 3 cycles of 30 s at 95 °C, 30 s at 50 °C, 1 min at 72 °C; 3 cycles of 30 s at 95 °C, 30 s at 48 °C, 1 min at 72 °C; 30 cycles of 30 s at 95 °C, 30 s at 46 °C, 1 min at 72 °C; 1 min at 72 °C.

COI PCR-amplified fragments were purified from TAE agarose gel electrophoresis by the QIAquick gel extraction kit (Promega, Wisconsin, USA). Sequences of the *Palinurus* specimens were obtained by the CEQ Dye Terminator Cycle Sequencing Kit (Beckman Instruments Inc., Fullerton, CA, USA) and LCO and HCOI primers, both directions for all sequences. The sequences, typically one almost complete strand for each reaction, were obtained by an automatic sequencer, CEQ 2000 sequencer (Beckman Instrument Inc.). The sequences were aligned with ClustalX (Thompson et al., 1997). Homologous mtCOI sequences from *Panulirus* and *Jasus* species, obtained by Ptacek et al. (2001) and Ovenden et al. (1997) respectively (see GenBank accession numbers therein), were also included in the phylogenetic analysis. *Pagurus longicarpus* Say, 1817 (Crustacea, Decapoda, Brachyura) sequence was used as outgroup (Genbank accession number NC 003058). A homologous mtCOI region (469 bp) of 25 taxa, including 217 variable sites and 187 parsimony-informative sites, was analysed by neighbour joining, maximum parsimony and maximum likelihood. Neighbour joining trees derived from the Kimura two-parameter distances were calculated with the Molecular Evolutionary Genetics Analysis (MEGA2) software package (Kumar et al., 2001). Support for nodes was assessed by bootstrap values (1000 replicates).

For parsimony analysis the min-mini heuristic search algorithm in MEGA2 was employed, with confidence levels evaluated using 100 bootstrap replicates. All parsimony-uninformative sites were ignored. Maximum likelihood analyses were performed using Tree-Puzzle 5.0 software package (Schmidt et al., 2002) using the fast tree search algorithm quartet puzzling (QP) that allows analysis of large data sets (more than 10–15 sequences) and automatically assigns estimations of support to each internal branch. We tested all the three substitution models implemented in Tree-Puzzle: the Tamura-Nei model (Tamura & Nei, 1993), the F84 model (Felsenstein, 1984) and the HKY85 model (Hasegawa et al., 1985), obtaining concordant results. In this paper only the tree obtained with the HKY85 model is shown.

Results

Nucleotide sequences for 659 bp of the mt-COI gene were determined through direct sequencing of amplified DNA for both species (EMBL

Nucleotide Sequence Database Accession Numbers AJ889577, AJ889578).

No intraspecific variation was found even when comparing sequences obtained from samples from different locations.

However, the alignment of the COI sequence permitted to determine several differences between the two species. The *P. elephas* and *P. mauritanicus* sequences present 67 single-base-pair substitutions (10% of the nucleotide positions); there is no evidence of insertions or deletions. Nucleotide transitions (A to G and C to T = 56) greatly outnumber transvertions (A to T and A to C = 11). Third positions in codons were the most variable (83%) followed by first positions (13.8%) and only two changes were observed in second positions. The COI region was 59% A + T rich, which is similar to the 59.5% and the 57% reported among *Jasus* and *Panulirus* species respectively (Ovenden et al., 1997; Ptacek et al., 2001). Nucleotide content averaged 27.1% A, 31.9% T, 18.2% G, 22.8% C. The sequence divergence between the two Mediterranean species was comparatively low (10%), lower than that reported for *Panulirus* species (12.4–31.8%) by Ptacek et al. (2001), similar to that calculated between *J. caveorum* and *J. frontalis*, but higher than that calculated for other recognised *Jasus* species by Ovenden et al. (1997).

To investigate the relationships of the Mediterranean species with other spiny lobster species, phylogenies were produced from mitochondrial sequence variations using our original data and published sequence data (Ovenden et al., 1997; Ptacek et al., 2001). Maximum likelihood (QP) and neighbour-joining (NJ) analyses produced trees with the same overall topology: four major lineages are clearly found; they correspond to the three genera of *Jasus*, *Palinurus* and *Panulirus* (this genus with two sub-lineages corresponding to morphological groups I–II and III–IV of George & Main, 1967). All these four internal branches were supported by moderate to high bootstrap values (73–100%) in NJ analysis and well supported in ML quartet puzzling analysis. Unweighted maximum parsimony analysis (MP), after 100 bootstrap replicates, yielded two most parsimonious trees with a length of 1070 steps (consistency index = 0.347664, retention index = 0.488645, rescaled consistency index = 0.160689). The consensus parsimony tree is similar to the NJ and ML trees, but with lower values of bootstrap for the clade of *Jasus* and *Panulirus* groups III–IV (Fig. 1).

Discussion and conclusions

The taxonomic status within the family Palinuridae has been recently questioned (Baisre, 1994) stressing the importance of acquiring genetic information on these species in order to address such issues.

In this paper, DNA nucleotide sequence analysis from a region of the mitochondrial genome (the cytochrome oxidase subunit I) was used to determine the extent of genetic differentiation within and between the two species *P. elephas* and *P. mauritanicus*.

Comparing nucleotide sequences, no intraspecific differences were observed within our sample size, not even among individuals collected from separate geographic locations. Absence of within species variation of the mt-COI gene in *Palinurus* species would not exclude the presence of partially

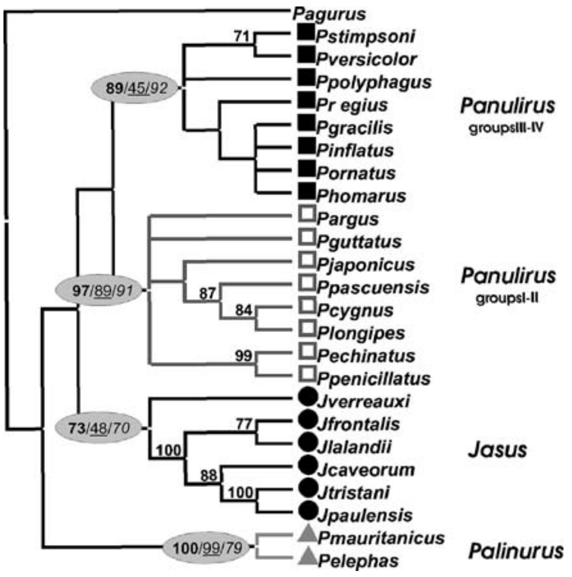

Figure 1. Neighbour joining consensus tree based on COI partial sequences for the three genera of spiny lobsters: *Palinurus*, *Panulirus* and *Jasus*. NJ bootstrap values, in bold, are indicated only for nodes > 70%. The same branching pattern was obtained with MP and ML quartet puzzling (QP). In circles at the principal nodes, the bootstrap support for NJ (bold, 1000 replicates) and MP (underlined, 100 replicates), as well as the ML QP support values (italic) are given.

or fully reproductively isolated populations. Mechanisms may be operating within the species to maintain complete or partial barriers to larval exchange between geographical localities as described for several marine Atlanto-Mediterranean species (Borsa et al., 1997).

The classical phylogenetic scheme of the Palinuridae family was essentially based on external adult morphology and paleontological data (George & Main, 1967). In recent years phylogenetic analyses were performed for the two genera *Jasus* and *Panulirus* using molecular characters, that is the sequence variation in mitochondrial gene regions (Ovenden et al., 1997; Ptacek et al., 2001).

In this study, sequences of cytochrome oxidase subunit I gene confirm that they are trustable phylogenetic indicators for spiny lobsters. All methods of phylogenetic reconstruction used consistently recovered the same branching pattern: four major evolutionary lineages were found. The topology of the trees obtained by NJ, MP and ML agrees with the accepted classification, showing a clear separation of the three genera *Palinurus, Panulirus* and *Jasus*. The weak support of some clades in MP analyses does not mean that this phylogenetic tree is worthless, but this was the best tree obtainable under the principle of reconstruction used. Actually, computer simulations have shown that many branching patterns of an inferred tree are correct even if they are not supported by high bootstrap values (Nei & Kumar, 2000).

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Short Research Note

Study of the nucleolar organizer regions in *Palinurus elephas* (Crustacea: Decapoda)

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Key words: Crustacea Decapoda, *Palinurus elephas*, ribosomal genes, FISH, B chromosomes, chromosome banding

Abstract

Using fluorescence *in situ* hybridization (FISH), chromomycin (CMA_3) staining and silver staining, we studied the nucleolar organizer regions in the spiny lobster *Palinurus elephas* in order to extend our knowledge on the karyology of this commercially important species. Multiple NORs have been detected by FISH, and CMA_3 showed a good correspondence between the localization of GC-rich heterochromatin and the ribosomal genes mapped by FISH. In contrast, the number of Ag-positive regions was higher than the number of FISH and CMA_3 signals, which may be explained by silver staining of the kinetochores. A variability in the number of FISH and CMA_3 signals has been detected in metaphases I and II which is probably due to the occurrence of rDNA cistrons on B chromosomes.

Introduction

The genes coding for the 18S, 5.8S and 28S rRNA are organized in tandemly-arrayed units clustered at specific chromosomal sites, the nucleolar organizer regions (NORs). The number and localization of NORs are important cytological data, which are widely utilized in phylogenetic studies, as in studies on chromosomal evolution of mosquito genera (Marchi & Pili, 1994) and cyprinid fishes (Amemiya et al., 1992). NORs are among the most intensely studied portions of the eucaryotic genome and silver staining is to date the most widely used technique for its localization. This technique detects transcriptionally active rDNA in interphase nuclei and in metaphase chromosomes. Moreover, NORs can be studied by rDNA FISH, which directly localizes ribosomal genes, and by fluorescence banding with CMA_3 which binds preferentially to GC-rich DNA that is typically present in the rDNA of many species (Sumner, 1990).

In this work we applied all these techniques to chromosomes of *Palinurus elephas* (Fabricius, 1787) in order to study the nucleolar organizer regions. In Crustacea Decapoda, the identification of suitable chromosomal markers is especially important because the large number, small size and peculiar structure of the chromosomes, together with technical constraints in obtaining good chromosomal preparations, hamper the determination of the karyotype of decapods.

Materials and methods

Five males of *P. elephas* captured in the sea of Southern Sardinia were analyzed. Chromosome preparations were obtained from the testis and digestive gland with an air-drying technique described by Deiana et al. (1996). Silver staining was performed according to Rufas et al. (1983).

Fluorescence *in situ* hybridization (FISH) was performed with a 45S rDNA biotinylated probe

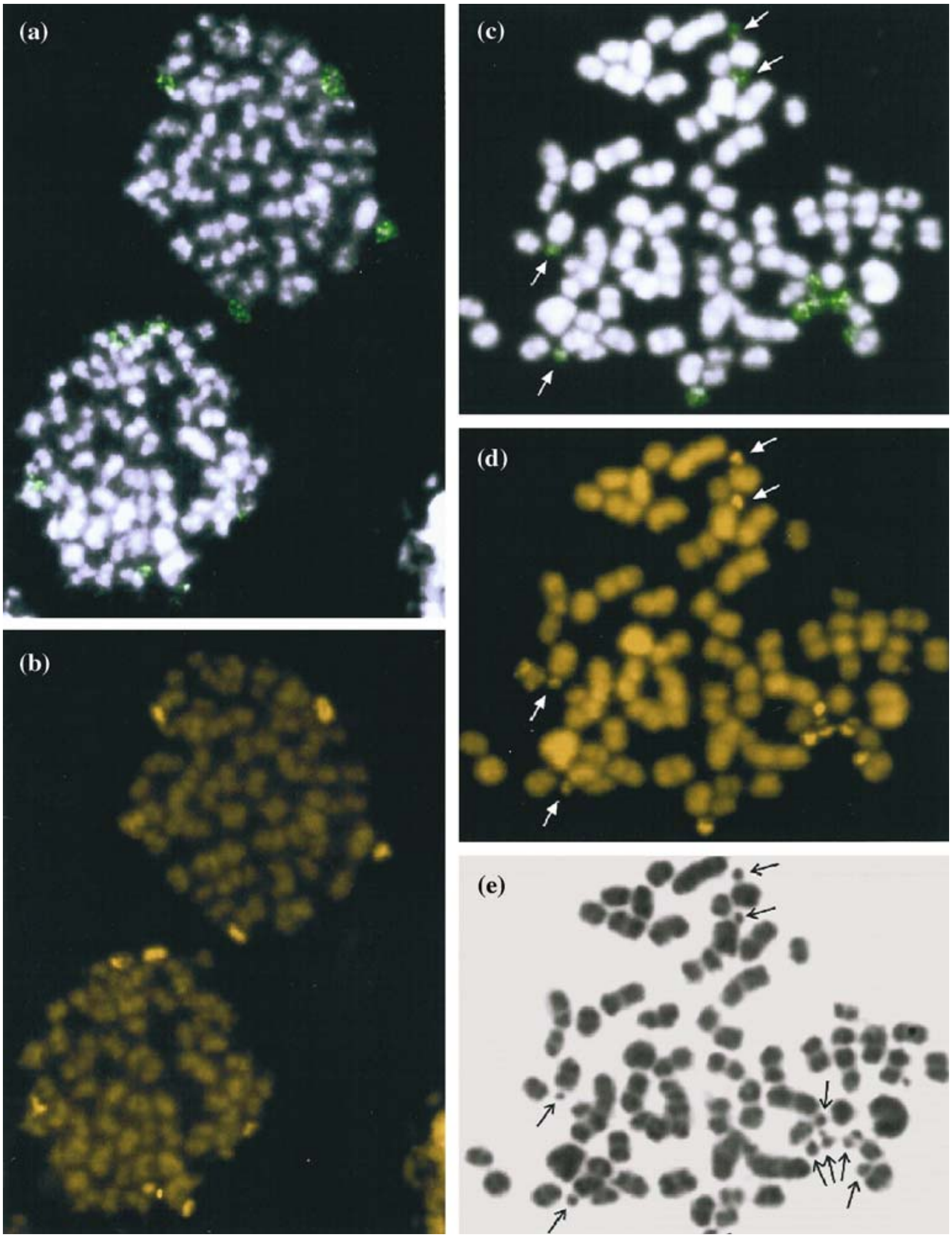

Figure 1. *Palinurus elephas*: sequential staining of a meiotic metaphase II (a, b) and of a meiotic metaphase I (c, d, e) with 45S rDNA FISH (a, c), CMA_3 (b, d) and Wright's staining (e). Arrows in (e) indicate the chromosomes labelled by FISH and CMA_3. Different numbers of signals are found in metaphases II from the same meiocyte and also in metaphase I, which is due to small, unpaired chromosomes entirely labelled after FISH and CMA_3. Such small chromosomes are clearly seen in the depicted metaphase I (arrows in c, d).

using fluorescein-conjugated avidin to detect the signals and counterstaining with DAPI (4,6-diamidino-2-phenylindole) according to Salvadori et al. (1995a). The slides were subsequently stained with chromomycin (CMA_3) according to Schweizer (1976) to detect the GC-rich heterochromatic regions and finally stained with Wright's stain according to Deiana et al. (1996) to sharply identify the chromosome morphology.

Results

The signals produced by FISH were localized on medium sized chromosomes and especially on small chromosomes, which were entirely labelled. In particular, ten signals in mitotic metaphases (not shown) and five signals both in meiotic metaphases I and II were always recorded (Fig. 1a, c). In addition, a variable number of signals on small chromosomes were recorded in some metaphases (Fig. 1a, c). The subsequent CMA_3 staining of the same metaphases produced bright fluorescent signals which corresponded in number and localization to the FISH signals, showing a GC-richness of these regions (Fig. 1b, d). The chromosomes labelled by FISH and CMA_3 could be morphologically identified after subsequent Wright's staining (Fig. 1e).

After silver staining a maximum of 6 nucleoli was detected in interphase nuclei (Fig. 2a). On metaphase plates Ag-positive spots were detected in most of the chromosomes, mainly in the centromeric regions (Fig. 2b).

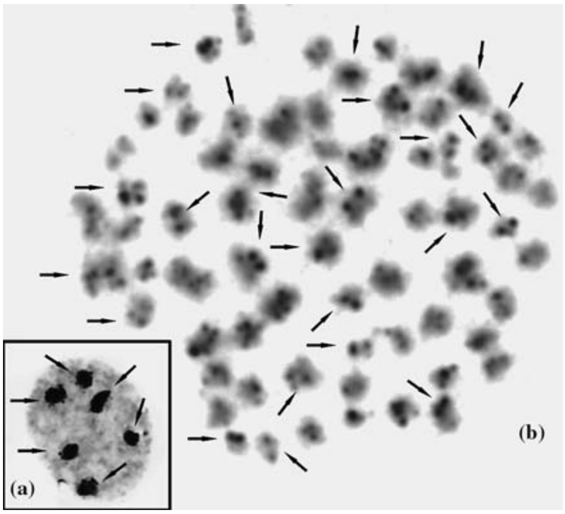

Figure 2. Palinurus elephas: silver staining of an interphase nucleus (a) showing 6 nucleoli (arrows) and metaphase chromosomes (b) showing a large number of Ag-positive signals mainly in centromeric regions (arrows).

Discussion

Our knowledge on the karyology of the Palinuridae family and the Decapoda in general is still very poor and is mostly restricted to the estimation of the chromosome number (reviewed in Lécher et al., 1995). Among the Palinuridae, *P. elephas* is the most intensively studied species with respect to its karyotype. A previous study of the mitotic and meiotic metaphases showed a variability of the chromosome number from 138 to 150, which is probably due to the presence of supernumerary B chromosomes. B chromosomes have been reported to be variable in number, smaller than the other chromosomes and completely composed of heterochromatin (Salvadori et al., 1995b).

In this work we studied the nucleolar organizer regions (NORs) by rDNA FISH, CMA_3-fluorescence and silver staining, aiming at the detection of markers to determine the karyotype of this species, to be able to compare it with that of the congeneric species *Palinurus mauritanicus* Gruvel, 1911 and other Palinuridae. In *P. elephas* multiple NORs were found by *in situ* hybridization. Sequential staining with FISH and CMA_3 showed a good correspondence between the localization of GC-rich heterochromatin and ribosomal genes, while the number of Ag-positive signals was higher than that of FISH and CMA_3-positive sites. In decapods, a high number of Ag-positive regions has been reported for *Homarus americanus* H. Milne Edwards, 1837 and it has been hypothesized for this species that the kinetochores were stained by Ag in addition to the NORs (Salvadori et al., 2001). In fact, besides staining NORs, silver staining has been proved to stain different proteinaceous chromosomal structures such as kinetochores, the synaptonemal complexes, chromosome cores and histones (Sumner, 1990). In *P. elephas* the presence of a higher number of Ag-positive regions compared to FISH signals is presumably also due to the presence of silver stainable centromeric proteins. The tendency of silver staining to stain the centromeric regions of chromosomes has been reported for other arthropods as well (Rufas et al., 1994).

The detection of a variability in the number of signals after using FISH and CMA_3-fluorescence may be explained by the occurrence of rDNA cistrons on B chromosomes. This idea is supported by different numbers of signals in metaphases II from the same meiocyte and by the presence of small, unpaired chromosomes entirely labelled after FISH and CMA_3 staining in metaphase I. The localization of NORs on B chromosomes has been described in mammals, plants and arthropods (Stitou et al., 2000).

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The constructional properties of the exoskeleton of homarid, palinurid, and scyllarid lobsters

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Abstract

Lobsters, as members of the Arthropoda, are already endowed with a laminated exoskeleton due to the mineralization of their cuticle. Mineralized laminate structures are found throughout animal phyla and convey, through their multiple surfaces, matrix planes that act as crack-blunting mechanisms. In addition, spiny and slipper lobsters, but not clawed lobsters, add a surface tubercle system that reflects a ventral surface pit system in the carapace. The architecture of these systems coincides with known strategies for crack blunting in composite materials. The division of vertical and horizontal crack blunting systems corresponding to laminate and tubercular systems and pit systems, respectively, may not be so easily separable in a functional sense. Although there is overlap in crack-blunting ability in both systems, separation into horizontal and vertical crack-blunting systems is a convenient way to discuss how skeletons of lobsters resist failure. While laminate structures dissipate forces horizontally in each layer, tubercle and pit systems serve to increase the surface area available to dissipate forces. These systems may represent evolutionary solutions to predation, particularly by predators that strike, crush or bite holes, rather than those that engulf.

Introduction

The strength of a skeleton is dependent upon its ability to resist stress and failure through breakage (Currey, 1980). Skeletons often are constructed in ways to increase their ability to resist stress, both on microscopic and macroscopic levels (Wainwright et al., 1976; Vermeij, 1987; Wilmot, 1990). On the macroscopic level, there are skeletal construction similarities that are repeated across phyla. Such construction includes arches that may represent the cross-section profiles of exoskeletons (Wainwright et al., 1976; Oxnard & Yang, 1981; Wilmot, 1990) and that convey resistance to applied stress. Other macroscopic features may include sculpturing with pocket formation seen in crocodilian osteoderms, the internal surfaces of crustacean carapaces, and layering of skeletal material – these are better understood at the microscopic level. On the microscopic level, there are two strategies employed in the construction of mineralized skeletons that aid in the prevention of fractures or cracks running through them. The first strategy is the formation of a layered skeleton seen in both vertebrates and arthropods (Dennell, 1960; Bouligand, 1971; Taylor & Layman, 1972; Joffe et al., 1975; Wainwright et al., 1976; Mutvei, 1977; Currey, 1984; Dalingwater, 1985; Märkel et al., 1986; Simkiss & Wilbur, 1989; Dalingwater & Mutvei, 1990; Smith, 1990). In higher vertebrates, layered skeletons are further strengthened with the subsequent development of a high surface area internal architecture, examples of which include the formation of Haversian or osteon systems in compact bone. The second strategy involves the formation of porous structures (Wainwright et al., 1976; Gordon, 1980; Oxnard & Yang, 1981), examples of which are cancellous bone, walls of corals, or blind-ended openings in the walls of skeletons, such as those found in crustaceans. Porosity is a complex matter (Anderson, 1977; Cocks & Ashby, 1982; Currey, 1986; Schaffler & Burr, 1988; Tvergaard, 1990) because the size and distribution of the pockets influence how the material reacts to stress. Moreover, much of the work on porosity and strength comes from ceramics whose material is uniform in contrast to the complex composite seen in exoskeletons. However, when solid structures become too porous, cracks can easily run through them (Dalingwater, 1985; Tvergaard, 1990), particularly in the case of crustaceans where pore canals run vertically through the exoskeleton. We consider this situation to differ from the architecture of cancellous bone, which forms an interconnected structure that resists stress by force dissipation through an increase in surface area and transference of stress to thicker portions of the bone (Goldstein, 1987). In addition, porosity developed because of stress is different from that formed as an integral part of mineralization process of the skeleton. In the former case, the skeleton has lost strength due to openings that reduce the material's ability to resist stress because chemical bonding has been broken and the material in the area of the voids can no longer function to absorb stresses (Currey, 1986; Tvergaard, 1990). In the latter case, the mineralization of the crustacean cuticule requires pore canals for delivery of calcium (Travis & Frieberg, 1963; Neville et al., 1969; Rohr & Dillaman, 1984; Compére & Gofinent, 1987). This porosity (via pore canals) is a source of skeletal weakness, and may be compensated for by the formation of internal sculpturing and thickening of the exoskeleton.

Cracks running in a material can be blunted or their force dispersed by establishing matrix planes within a material having elastic fibers (Cook & Gordon, 1964; Wainwright et al., 1976; Dalingwater, 1985; Currey, 1984, 1980; Hutchinson & Suo, 1992; He et al., 1994). Skeletons, therefore, typically have a laminate construction with layers of tensile matrix lying between (and incorporated into) a mineral-crystalline framework. Cracks running in such a composite material will encounter fibers that are pliable and likely to delaminate from the crystals that have either grown around them or have actually grown through them (Glimcher, 1976; Currey, 1984; Fleck, 1997). By delaminization of the fibers from the crystals and deformation of the elastic fibers under stress, the force of a crack is dissipated (Fig. 1, Cook & Gordon, 1964). Moreover, as the crack energy relies upon the crack tip diameter to be as small as possible, the opening of space between the fiber and crystalline matrix may increase the effective crack tip diameter, thereby reducing its overall energy. In addition, the fibers may serve to re-direct a crack and consequently dissipate the crack energy. For a more extensive review of the role of fiber matrices see Cook & Gordon (1964), Wainwright et al. (1976), and Currey (1980, 1984).

While it is enticing to hypothesize that laminate structures have been selected to act as crack blunting devices, it is actually the method of mineralization and thickening of a skeleton (for example, the case of crustaceans) that requires the skeleton to be laid down in layers (Horne et al., 2002) – any advantage in resisting cracking is incidental. Recent studies (Weiner & Hood, 1975; Giraud, 1981; Weiner & Traub, 1984; Simkiss & Wilbur, 1989; Lucas & Knapp, 1996; Miyamoto et al., 1996; Kono et al., 2000; Horne et al., 2002) have shown that the chitin and protein matrices of coral, molluscan, crustacean, and echinoderm exoskeletons contain the enzyme carbonic anhy-

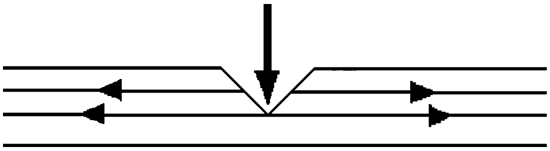

Figure 1. Diagrammatic representation of a laminate structure illustrating how layering a structure can blunt cracks. Force is dissipated by each successive lamellae.

drase. This enzyme is postulated to indirectly raise carbonate levels via maintenance of equilibrium concentrations of bicarbonate to begin precipitation of calcium carbonate on or in the matrix (Horne et al., 2002). The matrix proteins contain negatively charged amino acids that attract calcium ions for bicarbonate attraction (Simkiss & Wilbur, 1989; Dalingwater & Mutvei, 1990). As the mineralization process occurs, the enzyme becomes entombed (Horne et al., 2002). It may then be necessary for a new matrix layer, with carbonic anhydrase, to be exported by the hypodermis via cytoplasmic extensions (pore canals) that are hypothesized to supply calcium ions (Rohr & Dillaman, 1984). In this way, layering becomes a consequence of the mineralization process and automatically conveys a crack-blunting characteristic to the skeleton. The same may be true of bone containing proteins that carry phosphates in the matrix (Franzen & Heinegard, 1985; Dalingwater & Mutvei, 1990). Fiber matrix, including enzymes, then becomes necessary to attract calcium ions, acts a nucleating site, and raises phosphate or carbonate levels to high enough concentrations to allow precipitation to occur (Simkiss & Wilbur, 1989; Dalingwater & Mutvei, 1990).

In crustaceans, as in molluscs, a sheet of tissue is exporting the matrix and enzymes. In vertebrates and echinoderms, separate amoeboid cells do this (Simkiss & Wilbur, 1989; Dalingwater & Mutvei, 1990). Thus, there may be more flexibility in the internal construction of the skeletal walls in the latter forms. Furthermore, in vertebrates, the mineralization process may be more discontinuous (because the matrix fibers are short), which may lead to a stronger skeleton in terms of toughness. In the case of crustaceans, a fiber matrix composed of both proteins and chitin creates a composite structure because the fibers act as tensile elements embedded in a crystalline structure that provides compressive resistance. The fiber arrangements are complex (Bouligand, 1971) and are long; accordingly, they have a more uniform stress along their lengths. Recent work on the arrangement of the fibers indicates that flexible chitin rods (microfibrils) are embedded in a less flexible protein matrix, which stiffens further after calcium salts impregnate the protein matrix (Neville, 1993). Each layer of microfibrils is arranged helically, and the angle of one layer is shifted slightly with respect to that of the previous layer (Neville, 1993). These angular shifts result in distinctly visible laminar layers.

Openings or porosity in skeletons represent areas that may act to prevent a crack front from proceeding through the material (Cook & Gordon, 1964). The openings may increase the crack tip diameter, thereby reducing the ability of the crack to break the material. Neville et al. (1969) and Rohr & Dillaman (1984) have reported numbers of pore canals in the cuticles of crabs ranging from 250 000 per mm^2 to over 900 000 per mm^2. Such high numbers translate into the cuticle having a porosity of 20% or more of the cuticle volume (Waddy et al., 1995). Cracks running in materials that have fluid-filled canals or chambers may have an advantage over more solid materials, provided that the porosity is not so high as to make failure easier simply because there is less material volume and fewer bonds to break. When the openings are fluid-filled, the force of the crack can be dissipated by creating motion in the fluid pockets. Another advantage that openings provide includes the redirection of the crack around the opening (Fig. 2), which forces the crack to run through further crystals that not only may dissipate the energy necessary to break the crystals, but also may reduce crack energy while redirecting the crack. Such openings positioned throughout the mineralized portion of the skeleton are seen in diverse phyla, such as Cnidaria, Arthropoda, and Chordata (Dalingwater & Mutvei, 1990). In cnidarians, corals exist that have an open meshwork system resembling cancellous bone (Wendt et al., 1990). Among arthropods, crustaceans have openings in the carapace, as the result of spine, pit, or tubercle formation.

In this study, we examined the constructional properties of three diverse families of lobster: Nephropidae (clawed lobsters), Palinuridae (spiny lobsters), and Scyllaridae (slipper or shovel-nosed lobsters). Genera within these families show differences in shell construction, from very small pockets in the internal wall of the carapace to numerous deep pockets. Previous predation studies (Barshaw et al., 1996, 2003) show that these lobsters employ different behavioral strategies to resist the subduction phase of predation and

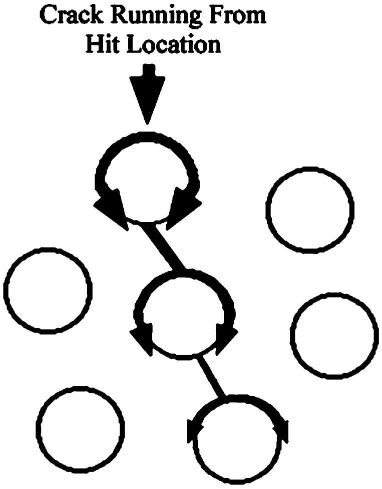

Figure 2. Diagrammatic representation of how holes in structure may serve to blunt cracks, by having to run around the openings/holes.

Barshaw et al. (2003) suggest that some of these behavioral strategies may be correlated to differences inherent in the properties of the shell of each morphological 'type' of lobster.

Methods

Specimens of nephropid (*Homarus americanus* (H. Milne-Edwards, 1837) and *Homarus gammarus* (Linnaeus, 1758)), palinurid (*Panulirus argus* (Latreille, 1804)), and scyllarid (*Scyllarides latus* (Latreille, 1802) and *Scyllarides aequinoctialis* (Lund, 1793)) lobsters were dissected to examine the ventral surfaces of the carapace and internal membranes. In addition, the carapaces of adult specimens of *H. americanus*, *P. argus*, and *S. latus* were histologically sectioned after being decalcified. Decalcification using 10% formic acid was applied to five pieces of carapaces for each species in 10-min increments beginning with 15 min, to determine optimal decalcification vs. ability to cut the material. Carapace pieces, approximately 1 cm^2 were then sectioned and stained using Wright's triple stain, or were treated unstained with antibodies for carbonic anhydrase. Antibody staining was carried out using a Sigma Quik–3 staining kit.

'Punch' tests on the shells of intermolt, size matched (60–90 mm carapace length) representatives from each of the three families of lobsters *(H. gammarus*, *Palinurus elephas* (Fabricius, 1787), and *S. latus*) were conducted in order to compare the differences in the strength of their carapace. Lobsters were preserved by freezing (to prevent tissue decay) and then placed into sealed plastic bags. They were thawed immediately prior to testing and placed into dissecting pans with hard wax bottoms; while the probe was punching the exoskeletons, the lobsters were held in place in the pans by the experimenters. All lobster species and replicates were tested on the same day. The punch tests were conducted with the MTS servo-controlled, hydraulic testing machine at the Materials Laboratory in the Department of Engineering at the Technion Institute for Technology in Haifa, Israel. This machine applied an increasing force onto a probe, designed to emulate the shape and sharpness of the mouth and teeth of a piscine predator, which eventually punctured the exoskeleton of the animal being tested (see Barshaw et al., 2003 for further information). The shells were tested in areas which represented typical points of attack as noted in field studies (Barshaw et al., 1996, 2003): (1) the first puncture location was immediately posterior to the dorsal cervical groove (DCG); (2) the second puncture location was between the eyes and anterior to the gastric chamber of the stomach (BE); and (3) the third puncture location was along the lateral edge near the gill chambers (LAT). Representative pieces of carapace at these same points were measured for shell thickness (to 100th of a mm) using calipers. Both the force to break the designated points and thickness of the shell were compared across the three families of lobsters with 1-factor ANOVAs and *post-hoc* tests (Bonferoni/Dunn) with Statview 4.01.

Results

Results on the macroscopic level

Gross anatomical examination of the internal structure of representatives of the three families of lobsters revealed that clawed lobsters lacked a well-developed pit system (Fig. 3a) compared to the other lobster families. Pits occur only in patches near the anterio-ventral surface of the carapace at a density of 28 pits per cm^2 where there are small external tubercles with fairly uniform diameters (Figs. 3a and 3b). In contrast to this limited internal pitting, clawed lobsters have pits on the external surface of their carapaces due to the presence of sensory hairs, which emanate from the pits. Spiny lobsters have a well-developed internal pit system with most pits having a small diameter. However, spiny lobsters may, in fact, have the largest diameter pits among lobsters (Figs. 4a and 6). These large pits are shallow and have no internal struts (Fig. 4b). Pit density is 55–65 pits per cm^2. Slipper lobsters have the best developed internal pit systems among lobsters (Fig. 5a,b) with larger pits having an extremely complex internal construction due to the thickness of the shell. Pits vary greatly in diameter (Fig. 6), but in those that attain a diameter of 0.4 mm or more, trabecular struts extend from one side of the pit to the other (Fig. 5c). These trabeculae are not as thick as the pit is deep, which creates subdivisions within the pit that may further prevent cracks from running through the carapace as a whole. Because of the size of these pits, their density is reduced to 41–56 pits per cm^2.

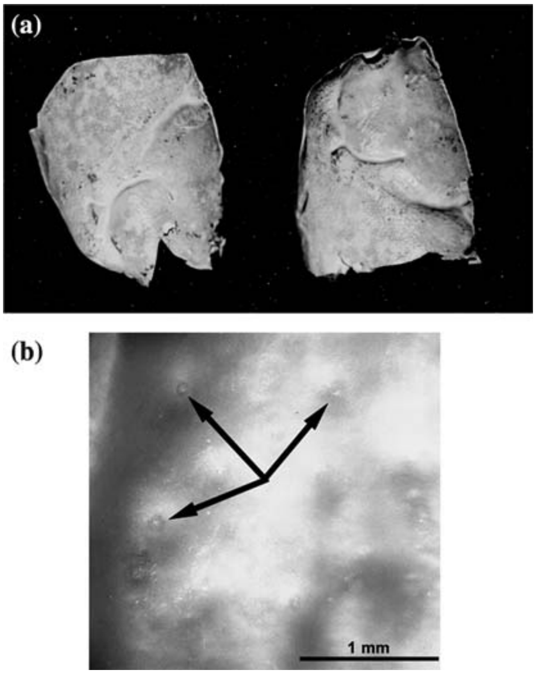

Figure 3. (a) The internal surface of the carapace of the clawed lobster, *Homarus gammarus*, illustrating the lack of any definitive pit system. (b) Rudimentary, small swellings present in the internal shell of *H. americanus* due to sensory hairs on the external carapace surface.

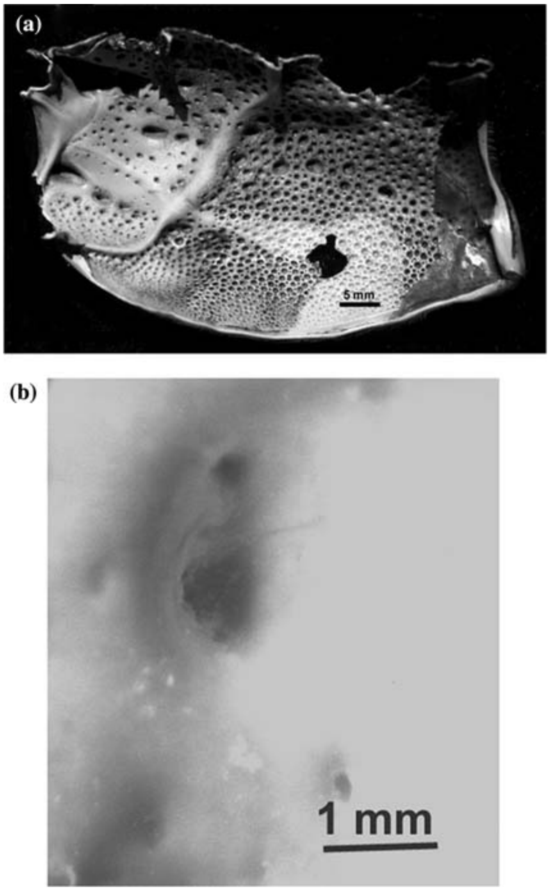

Figure 4. (a) The internal surface of the carapace of spiny lobster, *Palinurus elephas* illustrating the development of the pit system. (b) In *Panulirus argus*, the pits are simple without struts, but may be larger than those found in other lobster families. These pits will provide horizontal crack blunting properties to the carapace.

Results on the microscopic level

The sectioning of the lobster carapaces revealed the classic laminations of the cuticle described for arthropods (Simkiss & Wilbur, 1989). At low magnification (Fig. 7), these calcified layers are clearly visible and are arranged in horizontal layers when tubercles are not present. On the microscopic level (Fig. 7), the laminations and their fiber compositions are more evident. In addition, staining of histological sections of spiny lobster carapace with antibodies for carbonic

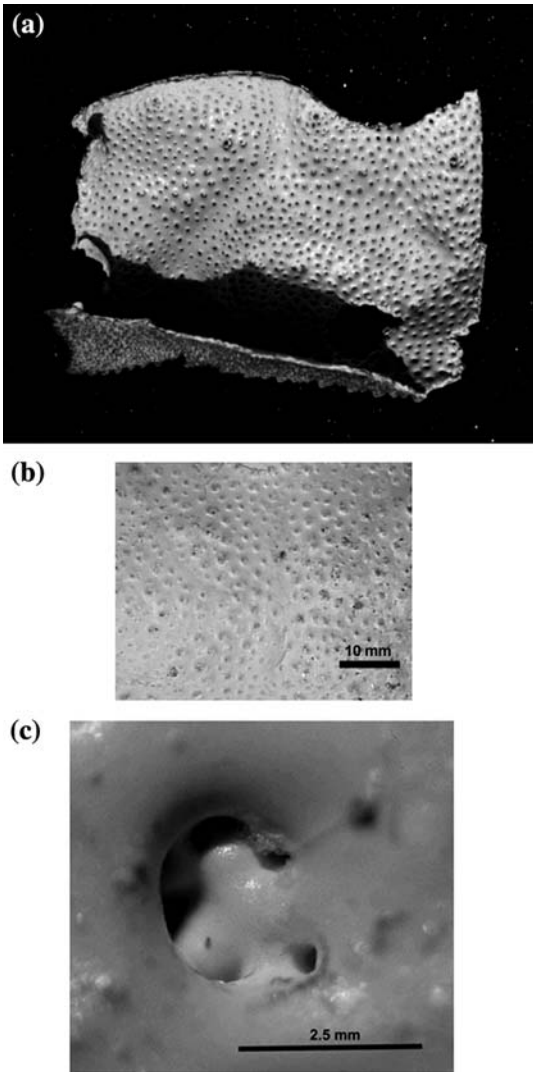

Figure 5. (a) The internal surface of the carapace of the slipper lobster, *Scyllarides latus*, illustrating the well-developed pit system. (b) A close up view of the numerous pits found in the slipper lobster carapace. (c) The complex construction of pits in the carapace of *S. latus* that includes trabeculae formation within the pits. The depth of the pit, along with the trabeculae, allows for the pit rims to be cracked without structural failure of the pit itself.

anhydrase (Fig. 8) demonstrated that carbonic anhydrase was present in the lamellae of the cuticle. This supports the contention that carbonic anhydrase is exported by the epithelial tissue and becomes entombed via the mineralization of the exoskeleton (Horne et al., 2002).

For both spiny and slipper lobsters, tubercle systems are well developed on the outer carapace surface. Histological sections of such tubercles in the spiny lobster (*P. argus*) demonstrated that tubercles are hollow (Fig. 9). Tubercles formed by evaginations of the hypodermis change the direction of the lamella of the cuticle such that they are no longer directed horizontally.

Results of the punch tests

Because fracture of the carapace in one location would likely compromise the integrity of the carapace in other sections, the only comparisons made were for the first puncture location, or the region posterior to the cervical groove of the dorsal carapace (DCG). Data for the second and third puncture locations are reported and discussed in Barshaw et al. (2003). Comparing the three lobster species at this location on the carapace, there was a significant difference in both the force required to puncture the carapace ($F_{(2,\ 0.05)} = 111.615$, $p < 0.0001$) and the thickness of the carapace ($F_{(2,\ 0.05)} = 196.779$; $p < 0.0001$). *Post-hoc*, pair-wise comparisons (analyzed with Bonferoni/Dunn tests), demonstrated that significantly more force (27.777 ± 6.45 SD kg) was required to puncture the carapaces of slipper lobsters than either those of spiny (13.067 ± 3.26 SD kg) or clawed lobsters (6.662 ± 1.6 SD kg) ($p < 0.0001$ for both comparisons, Fig. 10a). Similarly, more force was required to puncture the carapace of spiny lobsters than clawed lobsters ($p < 0.0001$). Concomitant with an increase in force necessary to fracture slipper lobster carapaces, their shells were significantly thicker (1.65 ± 0.18 SD mm) than either those of spiny (0.831 ± 0.16 SD mm) or clawed lobsters (0.679 ± 0.08 SD mm) ($p < 0.0001$ for both, Fig. 10b). Spiny lobster carapaces were significantly thicker than clawed lobster carapaces ($p < 0.0019$). Shell strength and resistance to breakage is related to thickness, increasing as the third power of thickness (Wainwright et al., 1976, p. 256). Thus, very slight increases in thickness, without any additional features such as pitting, should result in substantially greater force needed to break the shell, as demonstrated here by these data. In addition, the complexity of the internal pitting system also contributes to the fracture resistance, and would, therefore, add

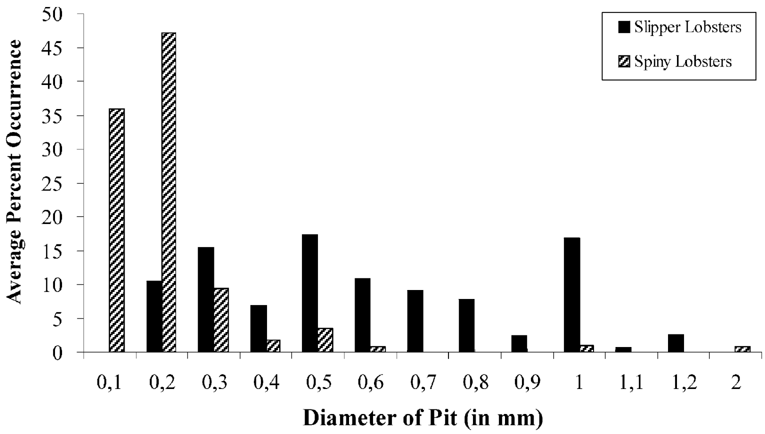

Figure 6. Frequency histograms of pit diameters of spiny lobsters and slipper lobsters. Because clawed lobsters do not have internal pits, they are not included.

to any resistance to breakage due to thickness increases alone. Finally, these data do not represent the actual force needed to break the shells of these species because tests were conducted on non-living specimens. A greater force would be required in living specimens as the animal would no doubt resist the predator and counter stresses would be different from those in the experiment. Nonetheless, the data do provide a relative measure of differences between the species in the three different families and correspond well with the macro- and microscopic observations of differences in the shell's constructional properties.

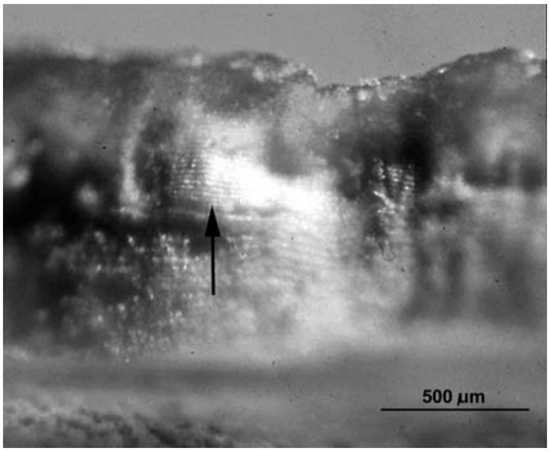

Figure 7. Macroscopic section through the carapace of a slipper lobster, *S. latus*, illustrating the calcified and laminated exoskeleton (arrow).

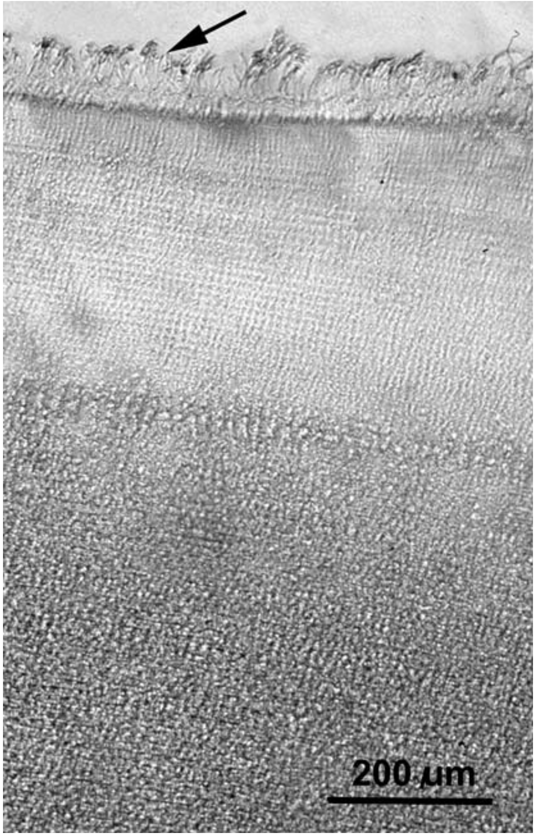

Figure 8. Antibody stained section of a spiny lobster, *P. argus*, carapace showing the layering of the lobster cuticle. Note also the sensory structures distributed on the surface of the carapace (arrow).

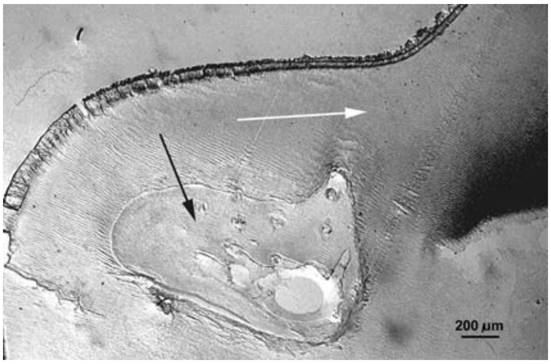

Figure 9. Histological section through the carapace of a spiny lobster, *P. argus*, showing the hollow nature of a tubercle (black arrow) and the laminations (white arrow).

Discussion

How lobster skeletons fit crack-blunting models

The exoskeleton of lobsters provides examples of crack-blunting strategies, layering, and porosity. As arthropods, lobsters have laminated skeletons that calcify by precipitation of calcium carbonate (Simkiss & Wilbur, 1989). They also have a high percentage of fibrous matrix (chitin and protein) – as much as 40% of the total weight – in their exoskeletons (Horne et al., 2002). This high percentage of fibrous matrix mimics the fiber matrix amount of bone, but differs significantly from the matrix fiber composition of many bivalves, where it contributes less than 1% of the weight of the valves (Currey, 1980; Kamat et al., 2000). Despite the high percentage, the fiber orientations are more complex in bone than in arthropods (Currey, 1984). Nonetheless, the high fiber content of crustaceans imparts crack blunting properties often found in such composite materials because the fibers provide many surfaces for crack energy dissipation through plastic flow and delamination (Cook & Gordon, 1964; Hutchinson & Suo, 1992; Curtin, 1999).

Cross-linking in the cuticle (Wainwright et al., 1976) also creates the ability to transfer forces between molecules and, in so doing, increases the surface area the crack must act upon. The repeated

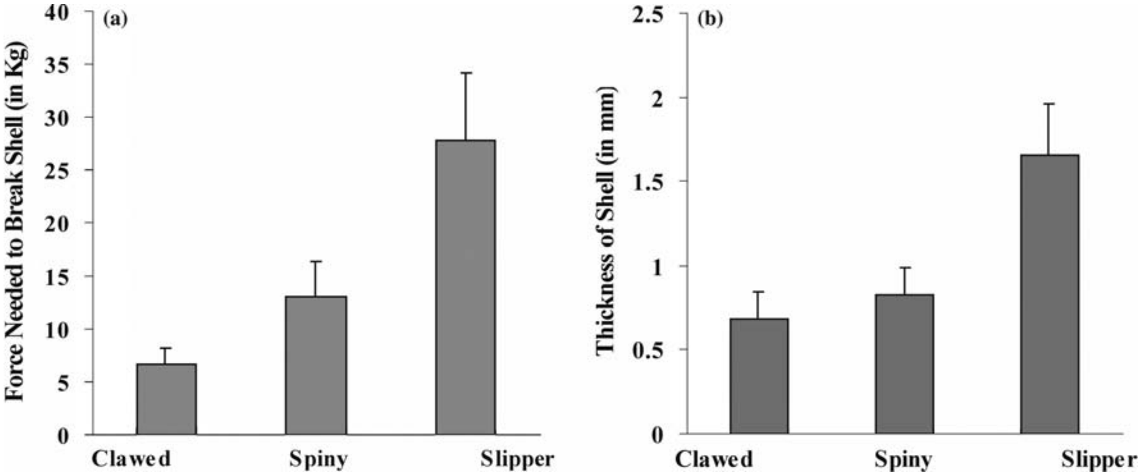

Figure 10. (a) Force (in kg) needed to break a point posterior to the dorsal cervical groove in a representative of each of the three families of lobster. (b) Thickness (in mm) of representative species of each of the three families of lobster (clawed: *H. gammarus*, spiny: *P. elephas*, and slipper: *S. latus*). Numbers in bars represent the mean value; error bars = standard deviation.

layering of skeletal material appears to be found only in mineralized skeletons or those derived from them (i.e., insect exoskeletons). What this suggests for crustaceans, bivalves, echinoderms and corals, is that carbonic anhydrase has been added to each lamellae of the matrix as it is laid down to mineralize the exoskeleton. This raises questions concerning the origin of the insect exoskeleton that is strengthened mainly by cross-linking the cuticular proteins in layers, rather than by calcification (see Brusca & Brusca, 1990 for a discussion of the phylogenetic problems concerning the sister group relationships of the Insecta). Could it be that the non-mineralized insect exoskeleton is the result of the loss of carbonic anhydrase production for export into the cuticle or is it that calcium is less available in a terrestrial setting?

Extending the layering of the exoskeleton by the formation of tubercles and pits

The layering effect of the exoskeleton is enhanced by the formation of significant surface tubercle systems in both spiny and slipper lobsters, but not in clawed lobsters. The tubercles change the direction of the lamella of the exoskeleton and increase the amount of material matrix and crystals that the cracks must act upon (Fig. 11); thus, we see an increase in the force necessary to break spiny and slipper lobster shells compared to clawed lobsters. In addition, these tubercles are hollow and are tied to the development of spine/pit systems in the carapace of slipper and spiny lobsters (Fig. 9), which simply are extensions of the tubercles.

The American clawed lobster, *H. americanus*, lacks an organized internal pit system on the ventral surface of its carapace (Figs. 3a and 3b). The outer surface of the carapace, however, is covered with small shallow pits of sizes ranging between 0.1 and 0.3 mm and shows a limited tubercular pattern seen on the carapaces of spiny and slipper lobsters. Both spiny and slipper lobsters have elaborate pit systems (Figs. 4b and 5b) that correspond to external tubercles and spines. The slipper lobster has the best-developed pits, many consisting of bilayered construction with trabecular struts connecting the walls of the pit (Fig. 5c). Smaller pits occur alongside the larger pits. These pits lead to tubercles on the outer surface of the carapace in slipper lobsters and to tubercles and spines in spiny lobsters. Thus, these tubercles are partially hollow (Fig. 9). As a result, two major systems are operating here in a biomechanical sense: (1) the hollow tubercles may act to mainly blunt vertically-running cracks, and (2) the pit system may act to mainly blunt horizontally-running cracks. The first system – the hollow, tubercles – present three defensive scenarios: (1) to deflect a direct blow and thereby absorb the energy of the attack; (2) to absorb more energy of the blow by presenting more material in line with the possible strike; and (3) to allow the system, using the hollow space, to collapse as a energy absorption system – a lobster crumple zone, so to speak, on a small scale that is only locally destructive. Thus the spiny and slipper lobsters can absorb a number of attacks before the shell is damaged, in contrast to clawed lobsters that have a lessened ability to do so. Overlying this tubercular system is a covering of chitin that also contributes to the energy absorption of a blow.

The second system – the pit or horizontal crack-blunting system – imparts strength via the pit patterns, their constructional principles, and the chitinous membrane adhering to the ventral surface of the carapace underlying these pit sys-

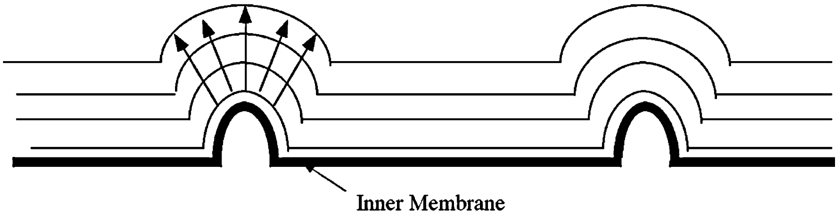

Figure 11. Diagrammatic representation of how the formation of tubercles may act to disperse stresses or blows to prevent fractures. Note how laminations change orientation because the tubercles are formed by evaginations of the tissue forming the exoskeleton.

tems. Cracks generated by the action of predators may run through the carapace and split it open. Each pit larger than 0.6 mm in diameter in the slipper lobster carapace contains one or more trabecular struts extending between the walls of the pit. These struts help prevent the separation of a pit that is cracked through stress. Having a system of pits is a classic way to blunt cracks by: (1) providing no continuity for the crack energy to act upon when it encounters an opening that then expands the crack tip diameter to dissipate the energy, and (2) forcing the cracks to run around the pits and their openings, thereby dissipating energy as the crack has to pass through more material. Having what appears to be bilayered pits in slipper lobster carapaces allows each pit system to better survive a crack without separating and prevents the creation of a fracture that will run to the surface. The more pits, the more the crack is deflected through its run through the carapace. The membrane adhering to the ventral surface of these pits also contributes to the strength of the system by adding a tensile element to the system, which prevents separation of a badly cracked pit system. Examining molt shell remnants of slipper lobsters shows the internal stresses associated with molting that created cracks that ran horizontally but were blunted by the pit system. Despite the stress applied to the shell during molting, only certain predicted weak areas split open, while the rest of the shell remained intact, albeit with stress fractures.

Conclusions

In the examination of clawed, spiny, and slipper lobster carapaces, there is a progressive thickening of the exoskeleton when lobsters of the same size are compared. The thickening arises from the addition of more, but thinner, laminar sheets of chitin and protein, such that slipper lobsters have more thin layers than spiny lobsters, and spiny lobsters have more thin layers than clawed lobsters (Porter, 2004). Clawed lobsters have only two thick layers and it has been suggested that the lack of more thin layers has ramifications for the integrity of the exoskeleton not only from blows, but also from invasive bacteria. For example, in spiny lobsters shell disease spreads laterally and remains confined in the upper cuticular layers, while in clawed lobsters, bacteria are able to invade both laminar layers and reach the membranous layer (Porter, 2004). This thickening appears to coincide with the loss of defensive weapons and modifications to strategies used to ward off predators. Thus, slipper lobsters, which lack defensive weapons such as claws or spinous antennae, have the thickest shells and require the greatest force to crack the carapace. Selective reasons for the development of tubercular patterns may be a reflection of the types of predation each lobster species encounters. The nephropid lobsters (which include *H. americanus* and *H. gammarus*) are all temperate to subarctic in distribution, and they are relatively old and primitive, having diverged little from when they first appeared in the Triassic (Glaessner, 1969; Schram, 1982; Briggs et al., 1993; Tshudy & Babcock, 1997). Both panulirid and scyllarid lobsters are temperate to subtropical in their distribution. While some claim that they both first appeared in the upper Triassic (Förster, 1967), more recent information suggests that palinurids appeared before nephropids in the upper Permian, and scyllarids appeared much later in the middle Jurassic (Briggs et al., 1993). Both palinurids and scyllarids have diverged into many types, with the modern spiny and slipper lobsters appearing in the late Jurassic (George & Main, 1967). Clawed lobsters, evolving at higher latitudes, probably were subjected to more large-mouth fishes (engulfers, swallowers), and may have experienced little pressure to evolve thicker and stronger shells via pitting and tubercles. In contrast, spiny and slipper lobsters living in both temperate and subtropical environments would have been subjected to predation by both large-mouthed and more specialized, shell-breaking predators, and would have clearly benefited from stronger shells to ward off the latter such predators. Extensive layering and the development of tubercular patterns and/or spines are mainly vertical crack-blunting devices that may be the result of adaptation to impact or crushing predators. Studies on gastropod and limpet shell construction vs. latitudinal distribution and predator types encountered (Palmer 1979, Bertness et al. 1981, Lowell 1987, Vermeij 1987) have shown shell thickness differences, as well as differences in tubercle and spine development, with subtropical and tropical species developing thicker, more sculptured shells than temperate species for warding off striking and crushing predators. Vermeij (1977) has argued that molluscan shell morphology that emerged during the Cretaceous is correlated with the increased number of shell breaking predators at the same time. The same trends may also be true for lobsters.

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Biological aspects of *Medorippe lanata* (Linnaeus, 1767) (Brachyura: Dorippidae) from the eastern Ligurian Sea (western Mediterranean)

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Key words: biology, marine crustaceans, trawling, western Mediterranean

Abstract

The aim of the present study is to investigate the demographic structure and to identify some aspects of the biology of an exploited population of *Medorippe lanata* (Brachyura: Dorippidae) in the eastern Ligurian Sea, western Mediterranean. 1364 specimens (639 males and 725 females) of *M. lanata* were collected on a monthly basis from January to December 2001, in a wide area of the eastern Ligurian Sea usually exploited by the Viareggio 'rapido' trawl fleet. *M. lanata* represented an important fraction of the discard, both in weight and in number of individuals. Maximum abundance of this species occurred in late summer-early autumn (up to 3369 ind. km^{-2} and 50.6 kg km^{-2} in August). The overall females:males sex-ratio was 1.13:1, while the monthly sex-ratio did not differ statistically from 1:1 in all months, except in September and October, when females significantly outnumbered males. The sampled population was composed of two cohorts from November to April. Sizes ranged from 10 to 29 mm carapace length (CL) for females and from 9 to 29 mm CL for males. The von Bertalanffy growth curve, computed for both sexes, gave a higher growth rate in males than in females. Recently moulted males and females were observed throughout the year, except in summer, when the highest number of ovigerous females was present. Females with external eggs were collected from March to November, with peaks in August and September. The monthly evolution of the ovarian maturity stages showed no clear temporal trend. At 21 mm CL, 50% of females were ovigerous or showed macroscopically mature ovaries. According to the dimorphism in chelae size, the presence of adult males (post-puberty stage) was observed all year round, from 18 to 29 mm CL, without evident temporal trends.

Introduction

The hairy crab *Medorippe lanata* (Linnaeus, 1767) is distributed in the eastern Atlantic Ocean and in the Mediterranean Sea (Manning & Holthuis, 1981), on sandy and muddy-sandy bottoms from 9 to 952 m (d'Udekem d'Acoz, 1999), but most abundantly between 20 and 100 m depth (Zariquiey Alvarez, 1968; Abelló et al., 1988b). It is a benthic crab distributed on soft bottoms of the Mediterranean continental shelf (Abelló et al., 1988b), belonging to the demersal assemblages currently exploited by trawling (Biagi et al., 2002). *Medorippe lanata* is devoid of commercial interest and is habitually discarded by the Mediterranean trawlers (Carbonell et al., 1997; Fabi & Sartor, 2002).

Classical approaches based on single species evaluation are often found to be inadequate in providing reliable information for a correct management of many fisheries. For this reason, a more holistic approach designed to gather information at the ecosystem level is needed (Caddy & Sharp,

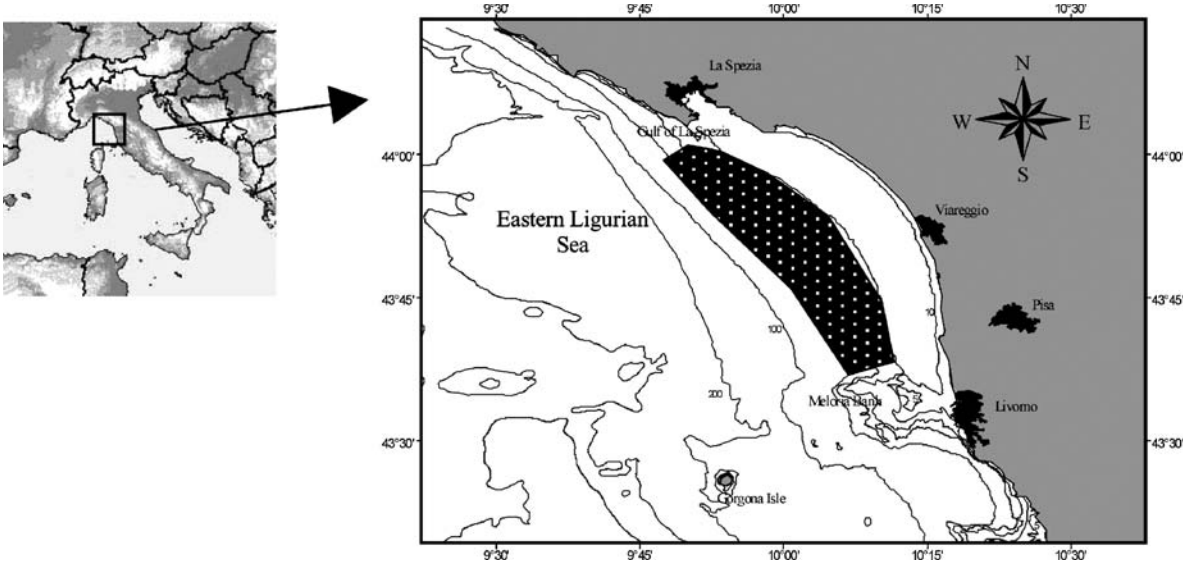

Figure 1. Investigated area, represented by the fishing grounds of the 'rapido' trawl fleet of Viareggio (shaded area).

1986; Brugge & Holden, 1991). In this context, studies designed to increase the knowledge of biology and ecology of species belonging to by-catch and discard have recently been added to the classical investigations focused only on the target species (Alverson et al., 1994; Gislason, 1994; Crowder & Murawski, 1998).

Despite the high occurrence and abundance of *M. lanata* on the continental shelf soft bottoms, only scanty information is available for this species, in particular concerning its reproductive biology and population dynamic. The aim of the present study, performed in the eastern Ligurian Sea (western Mediterranean), was to characterise the demographic structure of an exploited population of *M. lanata* and to identify several peculiar aspects of its life cycle.

Materials and methods

The study area is located in the eastern Ligurian Sea, on fishing grounds generally utilised by the Viareggio 'rapido' trawl fleet. It consists of a wide zone with muddy or sandy-muddy bottoms, comprised between the Meloria Bank to the south and the Gulf of La Spezia to the north (Fig. 1). The area, located between 8 and 12 miles from the coast, extends over a surface of about 800 km^2; the depth range varies between 20 and 60 m. This zone is strongly influenced by the supplies of the rivers Magra (North), Serchio and Arno (South), which carry notable amounts of organic matter and sediments, especially in fall and winter.

Samples of *M. lanata* were collected on a monthly basis during 2001 in the framework of a research project funded by the European Community focused on the 'rapido' trawl fishery characterisation (Fabi & Sartor, 2002). Data collection was performed by scientific personnel on board of a commercial vessel of the fleet of Viareggio, during habitual fishing operations. The 'rapido' trawl is a modified beam trawl with rigid mouth and iron teeth along the lower part, typically used in Italy, above all in northern and central Adriatic Sea (Giovanardi et al., 1998; Pranovi et al., 2001; Fabi & Sartor, 2002). This gear is mainly targeted to exploit flatfishes (*Solea* spp., *Psetta maxima* (Linnaeus, 1758) and *Scophthalmus rhombus* (Linnaeus, 1758)) and scallops (*Pecten jacobaeus* (Linnaeus, 1758) and *Aequipecten opercularis* (Linnaeus, 1758)). At present, in the eastern Ligurian Sea, the 'rapido' trawl is employed by only two vessels of the Viareggio fleet. The fishing boat used for sampling had the following characteristics: overall length of 18.4 m, gross tonnage of 26 and engine power of 206 kW. Two 'rapido' trawls were simultaneously towed at a speed of 9–10 km h^{-1}; each haul lasted about one hour and half. Each gear was equipped with a 4.8 m-long net, with a codend of 39.7 mm mesh size (stretched). Fishing activity was carried out for about 12 h per day, performing a total of 7–8 hauls.

During the period studied, data of 91 commercial hauls were collected. The species composition of each haul was analysed according to the retained and discarded fractions (Alverson et al., 1994); in addition, data on number of individuals and total weight (kg) of the catch of *M. lanata* were collected. Catch data were successively standardised as density and biomass indices (number of individuals km^{-2} and kg km^{-2}, respectively); the area swept was estimated by taking into account the speed and the duration of each haul and the horizontal opening of the gears. These indices were expressed as mean monthly values (with the corresponding standard error, SE).

On each specimen of *M. lanata*, the carapace length (CL) to the next lower 1 mm and sex were recorded. For females, three maturity stages were assigned on the basis of macroscopic analysis of the ovaries, using the scales available for brachyuran crabs (Ryan, 1967; Haefner, 1977; Choy, 1988; Erdman & Blake, 1988), modified as follow:

Stage 1 – Immature or resting gonads: the ovary is small and thin, whitish or translucent;

Stage 2 – Maturing: the ovary begins to develop and swell; it is clearly visible and pale yellow–orange;

Stage 3 – Mature: the gonads are swollen and occupy most of the dorsal part of the carapace cavity; the ovary is dark orange.

In addition, the number of ovigerous females was recorded on a monthly basis.

Maturity of males was assessed by observing the occurrence of heterochelid specimens (right chela clearly bigger than the left one) in the sampled population. According to Mori (1986a), this sexual dimorphism can be referable to different maturity stages: the homochelid situation corresponds to the pre-puberty phase (juveniles and subadults), the heterochelid situation to the post-puberty one (adults). The right chela palm width was measured (to 0.1 mm); a linear regression between size of the chela and CL was computed on Log-transformed data, in order to study the relative growth as a function of the morphological maturity stages.

Finally, the number of recently moulted individuals and the possible presence of specimens carrying parasites among the eggs or in the branchial chambers were investigated.

Sex ratio (f/m + f) was computed monthly and for each 1 mm size class; this analysis was not performed for samples lower than 30 specimens. Statistical significance of the deviations from 1:1 of the sex-ratio was analysed using the Chi-square test.

Monthly size frequency distributions (SFDs) of males and females were calculated using a 2 mm CL interval. The von Bertalanffy absolute growth parameters were estimated by the analysis of the modal progression of SFDs, using the FISAT routine (Gayanilo et al., 1995).

Results

Catch composition

In the studied period, 53.5% of the biomass caught by 'rapido' trawl was represented by commercial species (of which 69.1% fishes, 5.4% crustaceans and 25.5% molluscs) and 46.5% by discarded species (of which 1.8% fishes, 16.5% crustaceans, 63.2% molluscs, 17.9% echinoderms and 0.6% others). The most important discarded species, both in weight and number of individuals, were the gastropods *Turritella communis* Risso, 1826 and *Aporrhais pespelecani* (Linnaeus, 1758) and the crabs *M. lanata, Liocarcinus depurator* (Linnaeus, 1758) and *Goneplax rhomboids* (Linnaeus, 1758). Depending on the season, *M. lanata*, represented 6 to 77% in weight and 4 to 69% in number of the total crustacean discard.

The monthly trend of *M. lanata* density and biomass indices showed a clear temporal pattern, with a marked peak in late summer–early autumn (3369 ± 843.7 ind. km^{-2} and 50.6 ± 12.6 kg km^{-2} in August, 2890 ± 568.1 ind. km^{-2} and 43.5 ± 9.0 kg km^{-2} in September) (Fig. 2). In the subsequent months, the values were considerably lower with the only exception recorded in March, mainly concerning the density index.

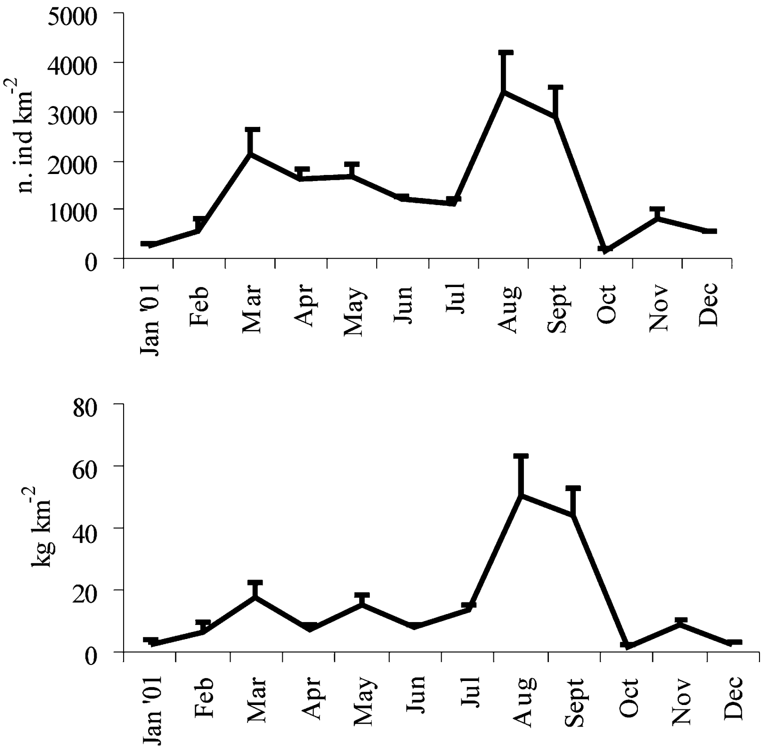

Figure 2. Monthly density and biomass indices of *M. lanata* obtained in the studied period.

Sex ratio

During the monthly sampling at sea, a total of 1364 specimens (639 males and 725 females) were studied. Sizes ranged from 10 to 29 mm CL for females and from 9 to 29 mm CL for males. The overall sex ratio observed (1.13:1) was biased toward females (Chi square Test = 5.42, $p < 0.05$). The monthly sex ratio was not statistically different from 1:1, except in September and October ($p < 0.001$ and $p < 0.05$, respectively), when females significantly outnumbered males. The sex ratio favoured males in the smallest size classes (≤20 mm CL) and females in the largest classes (≥22 mm CL) (Fig. 3).

Demographic structure and growth parameters

The monthly mean sizes of females were higher than those of males ($p < 0.05$, Student's *t*-test) and showed a peak in summer (August and September, with 23.55 ± 0.05 mm CL and 23.64 ± 0.05 mm CL, respectively).

From the analysis of SFDs (Fig. 4), at least two cohorts were singled out and their evolution was followed during the studied period. In January the sampled population was composed of a little amount of small individuals (10–12 mm CL) and of a second demographic component, more abundant, represented by larger specimens (16–26 mm CL); in the following months the first cohort showed a constant increase in size and abundance, becoming dominant in the April and May samples, while the second disappeared from the catch in June. In November, other small

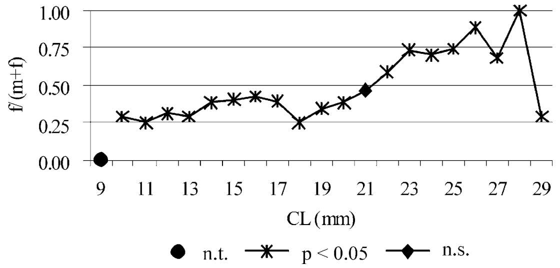

Figure 3. Sex-ratio in relation to carapace length (CL). (n.t. = not testable; n.s. = not significant).

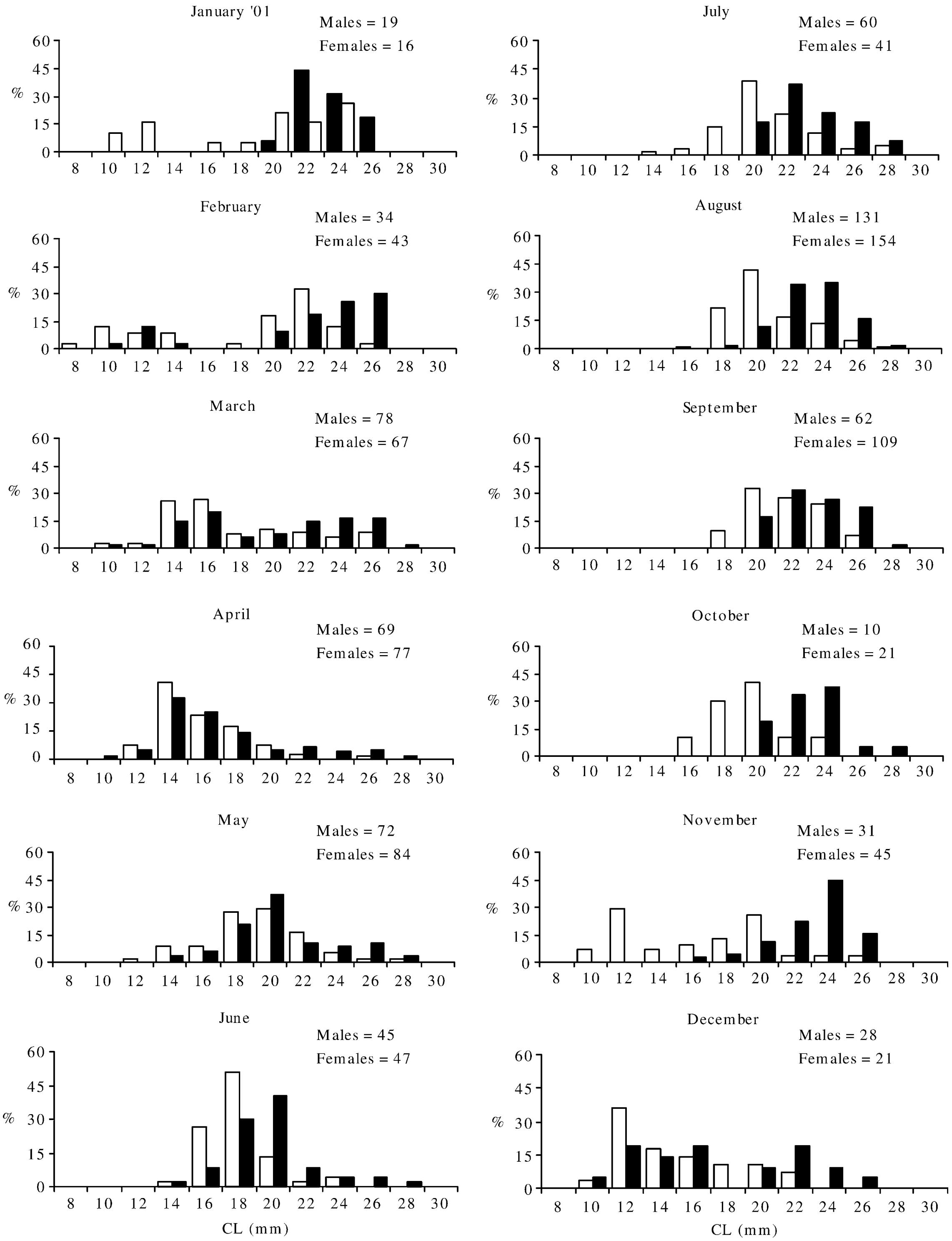

Figure 4. Monthly size frequency distributions for males (white) and females (black) of *M. lanata*.

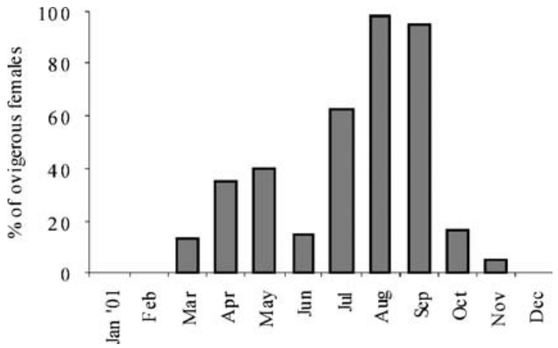

Figure 5. Monthly percentages of ovigerous females related to the total number of females collected each month.

specimens (from 10 to 14 mm CL) appeared in the catch, subsequently constituting the majority of the catch in December.

The estimation of the von Bertalanffy growth parameters gave the following values: $CL_\infty = 33.5$ mm and $K = 1.050$ for females; $CL_\infty = 31.1$ mm and $K = 1.575$ for males.

Maturity aspects

Ovigerous females were observed from 18 to 29 mm CL; from 22 mm CL they represented about 60% of the total females collected. The monthly percentage of ovigerous females in relation to the total number of females showed a clear temporal trend (Fig. 5): females carrying eggs were collected from March to November, with high values recorded from July to September, showing a peak of 98.6% in August. On the other hand, it proved more difficult to single out a clear temporal trend from the monthly evolution of the ovarian maturity stages (Fig. 6). Females with mature ovaries were recorded in almost all months (except June), but they didn't show a temporal pattern. They were observed from 17 to 29 mm CL; at a size of 22 mm CL they represented about 30% of the collected females, and from 28 mm CL they were about 45%.

Taking into account the females with ovaries in stage 3 (mature) plus the ovigerous ones in stages 1 and 2 (immature-resting and maturing), an indication of females that have reached maturity can be obtained. In this way, at 21 mm CL about 50% of the females of our sample was mature and this percentage reached 80% for females greater than 25 mm CL.

Males showed an evident dimorphism in the size of right and left chela during their life span. Two different morphologic patterns were observed during the study: males with both chelae similar in shape and size (homochelid males, juveniles and subadults) and males with right chela typically enlarged and markedly bigger than the left one (heterochelid males, adults). Two different slopes were observed when the regression analysis between CL and right chela palm width was computed, confirming these two different morphological phases in males (Fig. 7). The presence of homochelid (from 12 to 25 mm CL) and heterochelid (from 18 to 29 mm CL) males was observed throughout the year, without

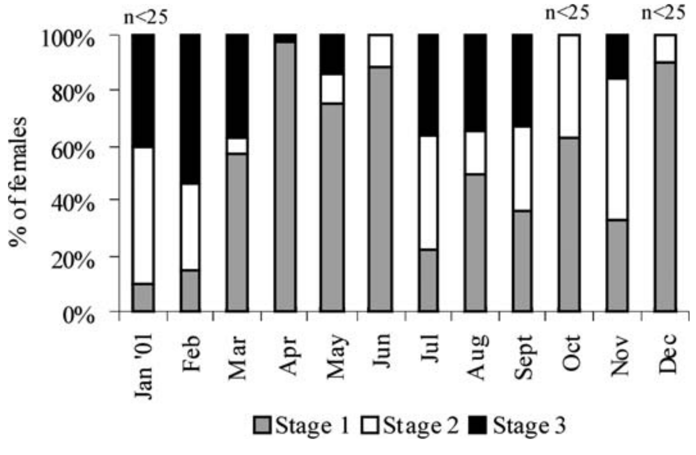

Figure 6. Monthly percentages of females in the three different maturity stages. (n = number of specimens; stage 1: immature or resting; stage 2: maturing; stage 3: mature).

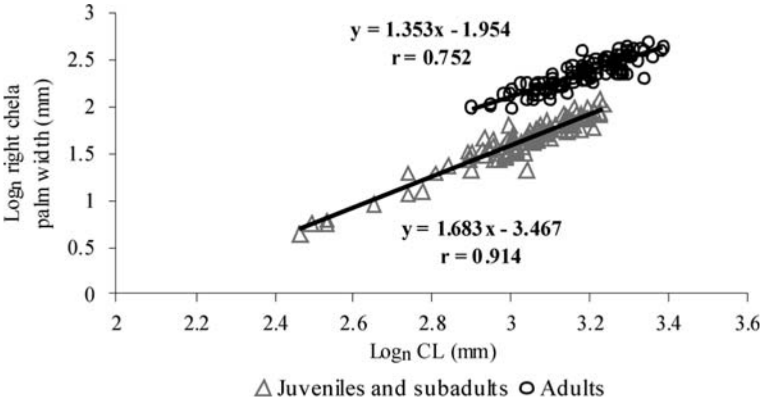

Figure 7. Relationship between carapace length (CL) and right chela palm width in males of *M. lanata*. (Log$_n$ transformed data; *r* = correlation coefficient).

a clear temporal trend. Adult specimens represented from 2.1 to 34.3% of the males sampled each month.

Worthy of note was the finding, in September, of an adult male of 25 mm CL with both chelae enlarged (right and left chela palm width of 11.9 and 11.6 mm, respectively).

Moulting

Recently moulted males and females were observed in most of the sampled months, except from July to October. The percentage of these individuals was in general low, ranging from 6 to 25% of the sampled population, apart from December when a greater proportion (42%) was observed. All females with soft carapace had macroscopically immature ovaries (stage 1) and did not carry external eggs. No evident differences in the occurrence of recently moulted specimens were detected between males and females or in function of the body size.

Presence of parasites

No nemertean predators were found among the eggs carried by ovigerous females, nor were iphitimid polychaete parasites observed in the branchial chamber of both sexes.

Discussion and conclusions

The results of this study highlighted the importance of *M. lanata* in the discarded fraction of the 'rapido' trawl fishery of the eastern Ligurian Sea. In the area studied, abundance and biomass of the species were highest in summer, when the population was mostly constituted of adult specimens. The depth range investigated in the present study (20–60 m) is narrower than that generally reported for this species, mostly found between 20 and 100 m (Abelló et al., 1988b); thus, the temporal peaks in the abundance detected in this study could be explained by the seasonal spatial distribution pattern of the species.

The overall annual reproductive cycle of *M. lanata* described in this study is not markedly different from that recorded by Mori (1986a) in the Gulf of Genoa (western Ligurian Sea). The highest presence of ovigerous females detected in summer is in agreement with the findings of Lo Bianco (1909) in the Gulf of Naples, Pesta (1918) in the Adriatic Sea and Zariquiey Alvarez (1968) in Spain.

In the western Ligurian Sea, small specimens of *M. lanata* were collected from November to May (Mori 1986a), confirming our results that recruitment to the adult population probably occurs during this period. This finding substantially agrees with the high presence of ovigerous females from July to September and indirectly confirms the observations of Lo Bianco (1909) on the presence of pelagic larvae from September to March in the Gulf of Naples.

Males of *M. lanata* are homochelid up to prepuberty, then heterochelid at the post-puberty stage (Mori, 1986a). Hartnoll (1982) explained this sexual dimorphism in terms of the greater role played by adult males in courtship, display and combats. The same morphologic pattern has also been reported for *Corystes cassivelaunus* (Pennant, 1777) (Hartnoll, 1972), *G. rhomboides* (Abellò & Sardà, 1982), *Paromola cuvieri* (Risso, 1816) (Mori, 1986b) and for many brachyuran species (Hartnoll, 1974, 1978).

The von Bertalanffy growth parameters, as well as the analysis of the modal progression of different cohorts during the period of study, identified *M. lanata* as a fast-growing species. However, the growth rate was found to be slower in females than in males, probably due to the energy losses endured by females during the maturing process (Hartnoll, 1982). This result is in agreement with data reported for other brachyurans (Fernández et al., 1991).

In females, the present study revealed an inverse relationship between the maturing process and moulting frequency: recently moulted females showed very low ovarian development, if any. Similar findings have been reported for other brachyuran crustaceans (Mori, 1987; Mori & Zunino, 1987). The temporal shift between moulting and maturation, according to the observations of Abelló (1989) on *L. depurator*, probably is a tool for the reduction of the energy cost of these two processes and for the avoidance of egg loosing. In this context, it is interesting to note that a disjunction between moulting and reproductive cycle of females is reported for many decapods; in general, the minimum percentage of moulting individuals corresponds to the maximum ratio of ovigerous females, and *vice versa* (Gonzalez-Gurriaràn, 1985; Mori & Zunino, 1987; Fernández et al., 1991).

The absence of parasites is at variance with previous studies performed on species of crabs living in similar habitats, such as *L. depurator* (Belloni & Mori, 1985; Mori & Zunino, 1987; Abelló et al., 1988a) and *P. cuvieri* (Mori, 1986b). Such differences could be explained by the different lifestyle and behaviour adopted by *M. lanata*.

The results of this study increase the information on the biology and ecology of *M. lanata*. Further investigations on this species, especially concerning trophic spectrum and post fishing mortality, are therefore advisable in order to acquire a better knowledge of the exploited species assemblage where *M. lanata* is present.

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Review Paper

Reproductive investment in Brachyura

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Key words: Brachyura, reproduction, investment, energy

Abstract

The costs of reproduction in brachyurans are reviewed. In both sexes a number of aspects of reproductive behaviour and physiology can result in higher mortality and/or slower growth. Mortality can be increased by the greater exposure or the reduced mobility of the crabs: these are illustrated by selected examples, but there is a dearth of quantitative data. Slower growth can result from reduced feeding, or the diversion of resources to reproduction. These have been better quantified. The investment by females in egg production is examined in detail. In free living crabs investment per brood in dry weight terms ranges from 3–21%, with a mean of about 11%. This investment is constrained by body morphology. However, in commensal/parasitic crabs the value rises to 60–90%: reasons for this are discussed. Many crabs produce more than one brood per year, and annual investment rises with brood number, though with some trade off. For temperate free living species annual investment ranges from 6–64%, with a mean of 22%. However tropical species produce more broods, and annual investment can reach 250%. In both temperate and tropical regimes there is a higher annual investment by commensal/parasitic species, of up to 470%. Priorities for future research are outlined.

The costs of reproduction

Reproductive activity in crabs can involve behavioural patterns or physiological processes, which will impact on the overall ‘performance’ of the individuals involved. These impacts fall into two primary categories. Firstly those which result in slower growth, or in a reduced ‘scope for growth:’ this may be by either a reduction in energy intake, or a diversion of available energy to reproductive processes. Secondly there may be an increased risk of mortality, either due to an increased availability to predators, or by a reduced ability to escape predators. These various costs will be discussed, and selected examples presented, but there will be no attempt at a comprehensive review. A particular interest will be the degree to which these costs can be quantified.

Costs which result in slower growth, or reduced scope for growth

Slower growth can result from a reduced energy intake due to restrictions on feeding, and limitation on feeding occurs during several reproductive activities.

Reproductive display by males can inhibit feeding – thus male *Uca pugnax* (Smith) do not feed whilst at their breeding burrow, and may engage in 6–8 days of reproductive activity there before moving to the different feeding areas (Christy & Salmon, 1984). In a variety of species males will guard females, normally without feeding, in order to ensure paternity. Such guarding occurs most commonly when mating is possible only between a newly moulted female and a hard male. The male will guard the female from before she moults until she has a hard integument and is no longer receptive to mating. In *Cancer pagurus* Linnaeus the pre-moult pairing lasts a mean 7.6 days, and post-copulatory pairing a mean 8.8 days (Hartnoll & Smith, 1979). Guarding can also occur when females mate when hard, but become capable of mating only for short periods when the operculum of the female genital opening decalcifies and becomes flexible. In the burrowing crab *Corystes cassivelaunus* (Pennant) the opercula decalcify for 12–20 days, and males may carry females from before decalcification until the opercula have re-hardened (Hartnoll, 1968). Since males may mate consecutively with a number of females, the cumulative loss of feeding time may become substantial.

Females may also have feeding restricted during mate guarding, though in those which moult during that period feeding would in any case have been seriously constrained. In females a more general phenomenon is a restriction on feeding during incubation, because incubating females often behave cryptically, reducing the opportunities for feeding. Thus fiddler crabs can feed only on the surface when the sediment is exposed by the tide, but in some species females with eggs are never found on the surface. This is so for *Uca annulipes* (H. Milne Edwards), with an incubation period of about 15 days (Skov & Hartnoll, 2001), for *Uca inversa* (Hoffmann) incubating for 18 days (Skov, 2001), and for the two-week incubation in *Uca pugilator* (Bosc) (Salmon, 1987). On average mature females of *U. annulipes* are ovigerous for 24% of the time, and *U. inversa* for 40% of the time (Skov, 2001), so there is a large reduction in feeding time.

A further limitation on growth in reproducing females is that they cannot moult whilst incubating eggs, or the eggs would be shed with the cast integument and die in the absence of brood care behaviour (see below). Hence ovigerous intermoults will tend to be longer than non-ovigerous ones, limiting the capacity for growth, which occurs only on moulting. In *Pachygrapsus crassipes* Randall the intermoult is 65–70 days for ovigerous instars, but only 40–50 for non-ovigerous ones (Hiatt, 1948). *Hymenosoma orbiculare* Desmarest has a mean of 101 days for non-ovigerous intermoults, but 181 days when ovigerous (Broekhuysen, 1955).

The other basic cause of reduced growth is that energy is diverted to reproductive processes, and is hence not available for somatic growth. Any reproductive behaviour will increase metabolism above the resting rate. Clearly mate searching, display, territorial defence and mate guarding all have energetic costs for males, though none seem to have been quantified in crabs. However, the energetic cost of mate guarding has been demonstrated in amphipods (Robinson & Doyle, 1985) and isopods (Sparkes et al., 1996). In females brooding activity is an important reproductive behaviour. Brooding females have to ensure oxygenation of the tightly packed egg mass, which involves regular standing, abdominal flapping and pleopods beating, especially during the later stages of embryo development. This will increase the energy requirement by the brooding female. Thus in *Cancer setosus* (Molina) the oxygen consumption of females brooding late stage embryos was approximately double that of non-brooding females (Baeza & Fernández, 2002).

However, the major diversion of energy is to the ripening of the gonads and the production of associated reproductive products. There are two problems in determining energy investment in reproductive products by males: firstly the whole contents of the vas deferens are not transferred on copulation, and secondly the frequency of copulation is impossible to determine in the wild. The first problem can be overcome by measuring the ejaculate within the spermathecae of virgin females after copulation (see Rondeau & Sainte-Marie, 2001, for examples and discussion). The ejaculate, as a proportion of the vas deferens content, varies widely both within and between species. It may be <3% in *Chionoecetes opilio* (O. Fabricius) (Rondeau & Sainte-Marie, 2001), or as much as 47% in *Callinectes sapidus* Rathbun (Jivoff, 1997). The frequency of copulation by a male in the wild cannot be determined, though captive studies can find the maximum number of females which can be mated by a male within a breeding season: 10 in *Chionoecetes bairdi* Rathbun (Paul, 1984). Clearly determining the energy invested per year in ejaculate by a male will be very imprecise. In contrast, virtually the entire contents of the ovaries of a female crab are expelled on laying, the egg mass can be quantified, and the number of egg batches produced per year can often be reliably estimated. Hence energy allocation to reproductive products can be accurately evaluated in females, and this allocation is examined in depth in a later section.

Costs which result in increased mortality

Reproductive activities may result directly in intraspecific mortality, either of males during male–male conflicts, or of females due to the attention of competing males. Reproduction may also increase the risk of predation by other species. Activities may increase the visibility or availability of crabs to predators, making them more likely to be observed and attacked. In addition reproduction may limit the mobility of crabs, hindering their ability to escape when attacked.

Fighting between conspecific males is widespread in crabs, but is generally ritualised, and incidences of serious damage are rare (Schöne, 1968): thus the risk to males from combat during reproduction seems small. However, females are also at risk during male–male encounters, especially when (as is the case in many species) they are soft shelled during mating. One such species is the snow crab, *C. opilio*: there is direct evidence that females are accidentally killed by males fighting to mate with attractive females (Sainte-Marie & Hazel, 1992).

There are a number of reproductive situations, which will increase visibility and availability of crabs to predators. During the course of reproduction, males display for a variety of reasons, including the attraction of females, and the defence of territory and exclusion of competing males. This is most obvious with semi-terrestrial crabs such as fiddler crabs, where the display is predominantly visual. Display means that they are, by definition, more visible to potential predators. It also means that feeding is restricted during display, so the total duration of surface activity is extended: even without display they have to spend about twice as long feeding as females, having only the one feeding chela (Valiela et al., 1974). However, studies of *Uca* populations do not indicate a general female bias: indeed direct observational studies of avian predators seem to indicate a preference for females (Christy & Salmon, 1984). Perhaps the large chelae of male fiddlers deter predators, which therefore will favour the less obvious females! Nevertheless, when the risk of predation was experimentally enhanced, males of *Uca beebei* Crane displayed less frequently (Koga et al., 1998), and male fiddler crabs are certainly predated (Ens et al., 1993). Display clearly imposes an added risk for males, even if predators do favour females.

The exposure of females to predation is increased in a variety of situations. In various *Uca* species females wander on the surface 'sampling' a series of displaying males before entering the burrow of one of them to mate, the wandering increases predation risk (Koga et al., 1998): this 'sampling' was reduced when bird presence was enhanced. Many crabs living in exposed situations are highly cryptic (e.g., spider crabs), and are difficult to observe until they move, which they seldom do during the day (Wicksten, 1983). However, the requirements of the embryos mean that incubating females must perform regular bouts of activity to oxygenate the egg mass (see above). In *C. setosus* with late embryos there are bouts of abdomen flapping at least every 4 min or so (Baeza & Fernández, 2002), which will increase visibility to predators. A further factor is the increased vulnerability of females during larval release, which must occur in open water so that the planktonic larvae can disperse. This applies particularly to semi-terrestrial and terrestrial crabs, though it may also be the case for more cryptic marine crabs, which must emerge into the open to allow the larvae to escape the egg mass. Thus fiddler crabs very rarely enter the sea, where there is a very high predation risk: normally at high tide they retreat into and close their burrows before being flooded. However, the larvae must be released into the flooding tide, and although this is almost invariably done at night (e.g., Christy & Salmon, 1984), it will still expose the females to predatory fish and crabs. Gecarcinid land crabs are generally nocturnal and cryptic, and do not approach the sea, except that females must enter the sea to release the larvae. They do so at night, and often in mass events (Bliss et al., 1978), both factors tending to limit the predation risk.

There are several reproductive activities which will limit mobility and hinder escape from predators. Males which are holding or carrying females will be restricted in mobility. This will not be so much a problem for crabs where the pairs are in cryptic habitats (e.g., *Carcinus maenas* (Linnaeus)). However, for species such as *C. cassivelaunus* where the male carries the female on the open sandy bottom (Hartnoll, 1968), it constitutes a risk. In many crabs the egg mass is extremely large, and could well constrain the ability of the female to move rapidly – however, no data are available. However, since berried females do tend to be cryptic, that may limit the scale of this problem.

Can the costs be measured?

A number of demonstrated or putative costs of reproduction have been presented above, where possible with examples. In order to compare these various costs within or between species they need to be quantified. In a number of cases this cannot be done, and this is the case for most of the presumed increases in mortality, though it is clear that additional risks are imposed by a range of reproductive activities. The activities which restrict energy intake can be quantified in a relative way in some instances, and with further work the proportional reduction in energy consumption could be calculated for a number of species. The most objective assessments of the costs of reproduction are in relation to the diversion of energy to reproductive processes.

The factor for which the most widespread data exist is the diversion of energy to the ripening of the ovaries and production of eggs – both in terms of accuracy of assessment, and diversity of taxa studied. The next section of this paper will concentrate on this topic.

Diversion of energy to the ripening of the ovaries and production of eggs

In female crabs effectively the entire contents of the two ovaries are discharged at ovulation to form the egg mass, which is retained attached to the pleopods during incubation. So the investment of a female crab in a brood of eggs could be determined in two ways. One would be to measure the ripe ovaries, the second would be to measure the newly laid egg mass. For egg masses only measurements when newly laid are relevant, since during incubation eggs are lost, and those retained change in size and composition. The measurement of the egg mass is simpler, and has been more widely used. If the number of batches of eggs produced in a year is known (and for many species this is not difficult to estimate), then the annual reproductive investment can be calculated. For comparative purposes the investment is usually expressed as a percentage of the female body.

The units of investment

As discussed above, investment per brood is normally expressed as a proportion or percentage of the female body measure. However, the method used to determine this relationship can have a very large influence upon the values derived: the method is normally selected from wet weight, dry weight, AFDW, and energy content. Wet weight is the simplest, but is inaccurate for small specimens, and even more so for egg masses: the value is very dependent upon the degree of draining or blotting of the material. Dry weight can be determined accurately, though it is more time consuming, and for small egg masses the weights to be measured are very small. The very different ash contents of female body and egg mass distort the relationship. The use of AFDW is still more time consuming, overcomes the problem of ash content, but does not take account of the different energy values for body tissues and eggs. Energy content is the most relevant unit, but to determine it requires equipment which may not be easily available, plus time and technical skill.

The effect of method choice can be examined by looking at specific examples – the spider crabs *Hyas coarctatus* Leach and *Inachus dorsettensis* (Pennant) (Bryant & Hartnoll, 1995). Values are calculated in each case for females of 1 g dry body weight carrying newly laid eggs (Table 1). Values for dry weight and energy are based on comprehensive data. Values for wet weight and AFDW are based on conversion ratios derived from limited data: however, they are adequate to provide comparative values for this analysis. The investment per brood rises from less than 5% based upon wet weight, to 30–40% based upon energy. Since energy is the 'real' currency, the latter values are the true measure of investment in egg production in these species.

Each of these values can be calculated in two ways, though this has been done here only in the

Table 1. The investment per egg mass, calculated as a percentage of the female body, for the spider crabs *Hyas coarctatus* and *Inachus dorsettensis* (based on data in Bryant & Hartnoll, 1995)

	Wet wt. (%)	Dry wt. (%)	AFDW (%)	Energy 1 (%)	Energy 2 (%)
Hyas	4.5	10.2	21.6	42.2	29.7
Inachus	4.4	8.3	17.6	28.6	22.2

For explanation of the two energy terms, see text.

case of energy (Energy 1 and Energy 2 in Table 1). 'Energy 1' has been calculated by the method generally used – for newly laid females the egg mass energy is expressed as a percentage of the female body energy (excluding egg mass). However, in this newly ovigerous condition the female body is at a minimum energy content, before any recovery of the ovaries has occurred. A truer assessment of investment might be to calculate the egg mass energy as a percentage of the total female energy as it was immediately prior to ovulation (i.e., egg mass plus body energy) – 'Energy 2.' This gives a lower value, and the difference between the two calculations increases as the investment ratio rises.

Although there would be good reasons for examining reproductive investment in terms of energy, there are very few examples in the literature. Most published values relate to dry weight, and have been calculated by the first method discussed above. Consequently the comparative analyses of brood investment and annual investment which follow will be in those terms. As a very general rule the dry weight percentage investment can be converted to energy investment by multiplying by 3.5 for free living crabs, and by 2.5 for commensal/parasitic crabs (where the female body has a lower ash content).

Investment per brood

All values considered here are for females with newly laid eggs: the egg mass dry weight is expressed as a percentage of the female body dry weight (excluding the egg mass).

The most comprehensive study of this topic was by Hines (1982), covering 20 species from seven brachyuran families. Variations were found within and between species, but overall the mean value was very close to 10%. Mean values for species ranged from 4.4 to 21% (Table 2). Subsequent studies on other free living crabs (Table 2) have confirmed this tendency: two deep water *Chaceon* spp., 16 and 22% (Hines, 1988); nine species of *Cancer*, 11–19% (Hines, 1991); four assorted species, 3–11% (Hines, 1992); two spider crabs, 8–10% (Bryant & Hartnoll, 1995); *Metapograpsus*, 12% (Sudha & Anilkumar, 1996); two *Sesarma* species, 8 and 21% (Skov, 2001). For non-tropical species there was a mean value of 10.7% ($n = 35$, SD = 4.65), and a range from 3.2 to 21%. Tropical species had a mean of 13.4% ($n = 3$), and a range from 7.6 to 21%. Hines (1982) attributed this constraint upon the investment in an egg batch to the limitations imposed by the rigid cephalothorax of crabs. This allows only restricted space to accommodate the increase in size of the ripening ovary.

All of the studies cited above relate to free living crabs. These have a heavily calcified and inelastic integument enclosing the cephalothorax, and the ovaries are restricted to the cephalothoracic cavity. However, in certain commensal crabs much higher values have been determined (Table 2). Two pinnotherid crabs commensal in bivalves, *Pinnotheres ostreum* Say and *Fabia subquadrata* Dana, had values of 66 and 97%, respectively (Hines, 1992). The cryptochirid *Hapalocarcinus marsupialis*, which inhabits galls in hard corals, had a value of 59% (Kotb & Hartnoll, 2002). Two factors enable these species to escape the constraints imposed on other crabs and to produce such large egg masses, as pointed out by Hines (1992). The protected life style and limited requirement for locomotion enable them to exist with a lightly calcified and flexible integument, which can expand to accommodate the swelling ovaries. For example in *Hapalocarcinus* the ash content averages only 24%, compared to a 42–58% range in free living crabs (Kotb &

Table 2. Investment in egg production by brachyuran crabs

Species	Percentage of investment per brood	Broods per year	Percentage of investment per year	Reference
Free living species				
Cancridae				
Cancer antennarius Stimpson	12.8	1.5	19.2	Hines (1991)
Cancer anthonyi Rathbun	14.7	2	29.4	Hines (1991)
Cancer borealis Stimpson	11.1	1	11.1	Hines (1991)
Cancer gracilis Dana	16.1	1.5	24.2	Hines (1991)
Cancer irroratus Say	10.8	1	10.8	Hines (1991)
Cancer magister Dana	16.8	1	16.8	Hines (1982, 1991)
Cancer oregonensis (Dana)	17.5	1	17.5	Hines (1991)
Cancer pagurus L.	13.4	1	13.4	Hines (1991)
Cancer productus Randall	18.8	1	18.8	Hines (1991)
Portunidae				
Callinectes sapidus Rathbun	17.6	1.5	26.4	Hines (1982)
Ovalipes ocellatus (Herbst)	11.4	1.5	17.1	Hines, 1982
Portunus spinicarpus (Stimpson)	3.2	2	6.4	Hines (1992)
Xanthidae				
Eurypanopeus depressus (Smith)	4.4	2	8	Hines (1982)
Lophopanopeus leucomanus (Lockington)	9.9	2	19.8	Hines (1982)
Menippe nodifrons Stimpson	5.1	2	10.2	Hines, 1992
Neopanope sayi (Smith)	12.2	1.5	18.3	Hines (1992)
Panopeus herbstii H. Milne Edwards	6.1	1	6.1	Hines (1982)
Rhithropanopeus harrisii (Gould)	13.3	3	40.0	Hines (1982)
Geryonidae				
Chaceon fenneri (Manning & Holthuis)	16	1	16	Hines (1988)
Chaceon quinquedens (Smith)	21	1	21	Hines (1982, 1988)
Grapsidae				
Hemigrapsus nudus (Dana)	13	1	13	Hines (1982)
Hemigrapsus oregonensis (Dana)	12.5	2	25	Hines (1982)
Metopograpsus messor (Forskål)	**11.7**	**14**	**163.8**	Sudha & Anilkumar (1996)
Pachygrapsus crassipes Randall	7.8	2	15.5	Hines (1982)
Sesarma ortmanni Crosnier	**21**	**12**	**252**	Skov (2001)
Sesarma guttatum A. Milne Edwards	**7.6**	**10**	**76**	Skov (2001)
Sesarma reticulata (Say)	7.5	2.5	19.8	Hines (1982)
Ocypodidae				
Uca minax (Le Conte)	7	2	14	Hines (1982)
Uca pugnax (Smith)	9.5	2	19	Hines (1982)
Majidae				
Hyas coarctatus Leach	9.6	1	9.6	Bryant & Hartnoll (1995)
Inachus dorsettensis (Pennant)	8.9	3.5	31.2	Bryant & Hartnoll (1995)
Libinia emarginata Leach	4.7	3.5	16.4	Hines, 1982
Loxorhynchus crispatus Stimpson	8.7	3.5	30.6	Hines (1982)
Loxorhynchus grandis Stimpson	5.2	3.5	15.5	Hines (1992)
Mimulus foliatus Stimpson	5.1	6	30.5	Hines (1982)
Pugettia producta (Randall)	8.1	7.5	60.7	Hines, 1982
Pugettia richii Dana	8.1	8	64.4	Hines (1982)

Continued on p. 37

Table 2. (Continued)

Species	Percentage of investment per brood	Broods per year	Percentage of investment per year	Reference
Scyra acutifrons Dana	5.6	7	39.3	Hines (1982)
Commensal species				
Pinnotheridae				
Fabia subquadrata Dana	96.7	1.5	145.1	Hines (1992)
Pinnotheres ostreum Say	66.2	1.5	99.3	Hines (1992)
Cryptochiridae				
Hapalocarcinus marsupialis Stimpson	**59**	**8**	**472**	Kotb & Hartnoll (2002)

Calculated by dry weight, as a percentage of female body weight, excluding the egg mass. Where necessary I have recalculated (and any errors are mine) from data in the cited sources (where the primary data sources may be listed). Tropical species are in bold type.

Hartnoll, 2002). Secondly the developing ovary can extend into the abdomen, which is both flexible and unusually large.

Investment per year

This was similarly investigated by Hines (1982) for a range of 20 species. He found the mean number of broods per year to vary from 1 to 8, and that there was a degree of trade-off between the size of broods and the number of broods per year: the mean investment per year was 21.5%. These results are listed in Table 2, together with those from subsequent studies.

There are 35 results for non-tropical free living species in Table 2. These cover a range of investment from 6 to 64% per year, with a mean of 21.6% (SD 13.2). The level of 'trade-off' between brood size and brood number can be examined by comparing the actual annual investment with the 'expected' investment (Table 3), the latter calculated on the assumption that every brood received the mean investment of 10.7%. For non-tropical species, those producing less than two broods per year exceeded the 'expected' investment, whilst those producing two or more broods did not. The data are sparse for species producing greater numbers

Table 3. Percentage reproductive investment in eggs by free living crabs. 'Expected' investment calculated on the assumption that each brood received the mean investment of 10.7% allocated by non-tropical crabs

Broods per year	*n*	Mean percent per brood	Mean percent per year	
			Actual	Expected
Non-tropical				
1	11	14.0	14.0	10.7
1.5	5	14.0	21.0	16.1
2+	10	8.2	16.7	21.4
3+	5	8.0	25.6	32.1
6+	1	5.1	30.6	64.2
7+	2	6.7	50.0	74.9
8+	1	8.1	64.4	85.6
Tropical				
10+	1	7.6	76	107.0
12+	1	21	252	128.4
14+	1	11.7	164	149.8

of broods, but do tend to confirm Hines' trade–off hypothesis.

There are only three results for tropical species (Table 2). Compared to non-tropical species the number of broods produced per year is higher, and the annual investment also higher, ranging from 76 to 252%. However, there is not a general trade-off, despite the high brood number: in two of the species the actual investment exceeds that expected (Table 3). Perhaps resources are less of a limiting problem for tropical species – certainly they are able to produce successive egg batches rapidly. Thus *Metopograpsus messor* (Forskal) has an incubation period of only 16 days due to the high ambient temperature, and the ovaries mature quickly due to the high metabolic rate, so that it is able to produce successive egg batches in quick succession (Sudha & Anilkumar, 1996). It is to be expected that many other tropical species will show a similar level of reproductive investment. In a study of four Jamaican spider crabs (Hartnoll, 1965) the incubation times ranged from 10 to 14 days, and a new set of eggs was always laid within a few days of the previous ones hatching. This offers the potential to produce over 20 batches in a year, though there are no data on the normal survival period of mature females.

As in the case of investment per brood, annual investment will be expected to be greater in the case of commensal/parasitic crabs. The following data exist for annual investment (broods per year in parentheses).

Pinnotheres ostreum (1.5)	99%	(Hines, 1992)
Fabia subquadrata (1.5)	146%	(Hines, 1992)
Hapalocarcinus marsupialis (8 +)	472+%	(Kotb & Hartnoll, 2002)

The values are indeed high, but especially so in the tropical *Hapalocarcinus*. Again this is due to a short incubation period, and the ability to produce egg batches in quick succession (Kotb & Hartnoll, 2002).

An energy budget for reproduction

One very speculative energy budget is presented below for *C. setosus*, based partly on wet weight and respiratory data in Baeza & Fernandez (2002), and incorporating conversion factors and other data from a variety of other sources. It is an annual budget (Table 4) for a mature female of 120 mm initial CW, assuming that it produces two egg batches, and moults once with a 24% moult increment. Ambient temperature is 14 °C, and the incubation period taken as 40 days.

On this analysis 26% of the assimilated energy is directed to the production of eggs and the respiratory cost of extra incubatory activity: there are probably other reproductive costs as well. If the basic respiration component is excluded, then the reproductive investment rises to over 86% of remaining energy, which might be regarded as that available to provide 'scope for growth'. This indicates a high investment in reproduction.

The only other energy budget which seems to be available is for *C. bairdi* by Paul & Fuji (1989). This study includes data on the energy allocation in the final immature female instar, during which the ovaries mature before the crab moults to the terminal mature instar when the first batch of eggs are very quickly laid. The partitioning of energy is:

Respiration	63%
Somatic growth + exuvia	25%
Ovarian maturation	11.5%

This indicates a lower reproductive investment than the 26% for *C. setosus*, but the two analyses are not strictly comparable. In species with determinate growth such as spider crabs the initial egg batch is often small (e.g., Bryant & Hartnoll, 1995), since in the penultimate instar there is

Table 4. Annual energy budget for mature female *Cancer setosus* with initial CW of 120 mm (see text for further details). Based partly on data from Baeza & Fernandez (2002). Energy values in kJ

Activity	Energy cost	Percentage value	
		Including R	Excluding R
Respiration (R)	6450	70	
Incubatory activity	720	8	26
Egg production	1670	18	60
Somatic growth	370	4	14
Total reproductive	2390	**26**	**86**
Total	9210		

benefit in maximising the growth increment at the terminal moult. Also the value for ovarian maturation is a minimal value for reproductive investment, since it excludes other costs such as incubatory behaviour.

Future research priorities

There is a need to quantify the predation risk of reproductive behaviour – currently there is very little data at all. The effects on growth need to be quantified better, and in terms of a common currency, which should be energy. In that way the relative costs can be compared.

There is a fair data base regarding investment in egg production, but this is predominantly in dry weight terms for free living temperate species producing one or two egg batches per year. It needs development in several ways. Assessments should be in energy. Many more studies are needed on tropical species, and on species leading commensal or parasitic life styles. These should enable reproductive investment to be viewed in relation to the overall energy budget.

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An ultrastructural and histochemical study of the germinal cells contained in hemispermatophores of males of the *Aristaeomorpha foliacea* (Risso, 1827)

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Abstract

The ultrastructure and the oligosaccharide sequence of glycoconjugates of spermatozoa contained in the hemispermatophores of males *Aristaeomorpha foliacea* collected during the main mating and reproductive periods have been investigated. The hemispermatophores from eleven specimens were packed with typical round-shaped spermatozoa (max ø 3.8 ± 0.03 μm), characterised by an electron-lucent nucleus not separated by the nuclear envelope from the thin electron-dense peripheral band of the cytoplasm containing small vesicles and large mitochondria. Hemispermatophores from two out of eight specimens collected in April contained few typical spermatozoa, whereas they were filled with roundish large germinal cells (max ø 5.74 ± 0.16 μm) showing a filamentous chromatin in the nuclear region and the cytoplasm rim rich in large vesicles and myelin-like bodies. These cells were considered as non-mature germ cells, and consequently we defined their hemispermatophores as "immature". The lectin histochemistry showed differences in the glycoconjugate composition between germ cells contained in immature hemispermatophores and spermatozoa. The former had N-linked oligosaccharides containing α-D-Man and internal β-D-GlcNAc (Con A and KOH-sialidase-WGA affinity) as well as terminal NeuAcα2,6Gal/GalNAc and NeuAcα2,3Galβ1, 4-GlcNAc (SNA and MAA binding). Spermatozoa from mature hemispermatophores also displayed O-linked oligosaccharides which terminated with β-D-Gal(1–3)-GalNAc dimer (PNA binding) and αGalNAc (HPA reactivity) in the cytoplasm and with sialic acid linked to β-D-Gal(1–3)-GalNAc (KOH-sialidase-PNA procedure) in the nucleus. The extracellular matrix in immature hemispermatophores displayed both N- and O-linked glycoconjugates, whereas it contained only O-linked oligosaccharides in mature ones. Although the number of specimens examined is low, this study should indicate that the presence of hemispermatophores in the terminal ampullae of male *A. foliacea* and of joined hemipetasmas is not synonymous with maturity.

Introduction

The giant red shrimp *Aristaeomorpha foliacea* (Risso, 1827) represents an important commercial resource and plays an important role in the overall biomass of the muddy bottoms of the Mediterranean Sea. Despite its importance, the studies of spermatozoa of this species are scarce (Medina, 1995a; Desantis et al., 2003).

The males of giant red shrimp *A. foliacea* are considered sexually mature if they present hemispermatophores in the terminal ampullae and joined hemipetasmas, according to the scale of maturity proposed by Sardà & Demestre (1989) for the companion species *Aristeus antennatus* (Risso, 1816). The hemispermatophores of male *A. foliacea* protect the spermatozoa to ensure their survival during mating and their passage into the thelycum of the female.

In the Mediterranean Sea, the highest percentage of mature males has been found between January and July (Mura et al., 1992; Ragonese & Bianchini, 1995; D'Onghia et al., 1998, Belcari et al., 2003). Coupling occurs a few months before ovulation because the highest percentage of females with spermatophores in the thelycum has been observed during March and May (D'Onghia et al., 1998), whereas females with mature ovaries (Levi & Vacchi, 1988; Desantis et al., 2001) occur from May to September with the highest percentage during August.

Glycoconjugates are a fundamental component of eukaryotic cells. Although the biological roles of their oligosaccharide chains remain mostly undefined (Lis & Sharon, 1993; Varki, 1993), the glycoproteins can act as ligands in recognition phenomena (Varki, 1997) and sperm-egg interaction (Geng et al., 1997). Lectins are non-immune glycoproteins of plant or animal origin that bind carbohydrate residues on glycoconjugates. Due to their specific affinities to a particular sugar, lectins are useful probes for the intracellular localization of sugar residues (Ihida et al., 1991; Danguy et al., 1994) and the characterization of distinct cellular populations as well as their morpho-functional changes (Spicer & Schulte, 1992; Danguy et al., 1994).

A. foliacea spermatozoa lack acrosome (Medina, 1995a). The sperm surface oligosaccharides and the cytoplasmic content could be of critical importance in the process of fertilization. Therefore, the aim of this study was to identify the oligosaccharide sequences of glycoconjugates using lectin histochemistry in hemispermatophores of *A. foliacea* during the major mating and reproductive periods. In addition, conventional electron microscope studies were performed to show the ultrastructural characteristics of the germ cells.

Materials and methods

Tissue preparations

The terminal ampullae containing hemispermatophores of 13 considered mature males of *Aristaeomorpha foliacea* with carapace length (CL) >32 mm, captured in spring (April) (n = 8) and summer (July) (n = 5) by commercial bottom-trawl gear in the north-western Ionian Sea (Mediterranean Sea), were removed immediately after capture and processed for either ultrastructural investigations or lectin histochemistry.

For light microscope studies, the terminal ampullae were fixed in Bouin's solution for 24 h at room temperature (RT). Following fixation, the tissues were washed and dehydrated in an ethanol series, cleared in xylene, and embedded in paraffin wax. Sections 4 μm thick were cut and, after de-waxing with xylene and hydration in an ethanol series of descending concentrations, were stained with Mayer's hematoxylin and eosin or by means of the following histochemical methods.

Lectin histochemistry

The lectins used are listed in Table 1. The lectins PNA, RCA_{120}, SBA, HPA, Con A, WGA, GSA-II, UEA-I, LTA were HRP-conjugated and were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). SNA, MAA and GSA I-B_4 were biotinylated lectins and were purchased from Vector Laboratories Inc. (Burlingame, CA, USA).

De-waxed and re-hydrated tissue sections were immersed in 3% H_2O_2 for 10 min to suppress the endogenous peroxidase activity, rinsed in 0.05 M Tris–HCl buffered saline (TBS) pH 7.4 and incubated in lectin solution at appropriate dilutions (Table 1) for 1 h at room temperature (RT). After 3 rinsing in TBS, peroxidase activity was visualized by incubation in a solution containing 0.05% 3,3′-diaminobenzidine (DAB) and 0.003% H_2O_2 in 0.05 M TBS (pH = 7.6) for 10 min at RT before dehydration and mounting. Tissue sections incubated in biotinylated lectins (SNA, MAA and GSA I-B_4) were rinsed three times with 0.05 M phosphate-buffered saline (PBS) and were incubated in streptavidin/peroxidase complex (Vector Lab. Inc.) for 30 min at RT. After washing in PBS, peroxidase was developed in a DAB-H_2O_2 solution as above.

Controls for lectin staining included: (1) substitution of the substrate medium with buffer without lectin; (2) incubation with each lectin in

Table 1. Lectins used, their sugar specificities and inhibitory sugars used in control experiments

Lectin abbreviation	Source of lectin	Concentration (μg/ml)	Sugar specificity	Inhibitory sugar
SNA	*Sambucus nigra*	15	Neu5Acα2,6Gal/GalNAc	NeuAc
MAA	*Maackia amurensis*	10	Neu5acα2,3Galβ1,4GlcNac	NeuAc
PNA	*Arachis hypogea*	20	Terminal Galβ1,3GalNAc	Galactose
RCA_{120}	*Ricinus communis*	20	Terminal Galβ1,4GlcNAc	Galactose
SBA	*Glycine max*	20	Terminal αβGalNAc	GalNAc
HPA	*Helix pomatia*	15	Terminal GalNAc	GalNAc
Con A	*Canavalia ensiformis*	10	Terminal and internal αMan > αGlc	Mannose
GSA I-B_4	*Bandeiraea simplicifolia*	20	Terminal αGal	Galactose
WGA	*Triticum vulgaris*	10	Terminal and internal βGlcNAc > > NeuNAc	GalNAc
GSA II	*Bandeiraea simplicifolia*	20	Terminal D-GlcNAc	GalNAc
UEA I	*Ulex europaeus*	25	Terminal αL-Fuc	Fucose
LTA	*Lotus tetragonolobus*	25	Terminal αL-Fuc	Fucose

Fuc, Fucose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Glc, glucose; GlcNAc, *N*-acetylglucosamine; Man, mannose; NeuAc, *N*-acetyl neuraminic (sialic) acid.

the presence of its hapten sugar (0.2–0.5 M in Tris buffer).

Enzymatic and chemical treatments

Before staining with SNA, MAA, PNA, RCA_{120}, and WGA, some sections were incubated, at 37 °C for 16 h in 0.86 U/mg protein of sialidase (Type V, from *Clostridium perfringens*) (Sigma Chemicals Co., St. Louis, MO, USA) dissolved in 0.1 M sodium acetate buffer, pH 5.5, containing 10 mM $CaCl_2$. Prior to the neuraminidase treatment, a saponification technique was performed to render the enzyme digestion effective, with 0.5% KOH in 70% ethanol for 15 min at RT (Reid et al., 1978). As controls of the enzyme digestion procedure, certain sections were incubated in the enzyme-free buffer solution under the same experimental conditions.

Electron microscopy

Terminal ampullae were fixed in 3% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.2) diluted in sea water for 6 h at 4 °C. After rinsing, tissues were post-fixed in 1% OsO_4 buffered with sodium cacodylate for 2 h at 4 °C, rinsed and dehydrated in an ethanol series. The specimens were then embedded in a mixture of Epon-812/Araldite. Thin sections were cut, contrasted with uranyl acetate and lead citrate, and observed with a Zeiss EM 109.

Results

The hemispermatophores consist of four layers; the first of them surrounds the germ cells, which is enmeshed in an extracellular matrix (Fig. 1a, b). In the hematoxylin-eosin stained specimens, the hemispermatophores from most specimens showed typical spermatozoa, roundish in shape (max ø 3.8 ± 0.03 μm) and characterized by a strongly hematoxylinophil nucleus surrounded by an unstained peripheral band of cytoplasm (Fig. 1a). Only hemispermatophores from two specimens collected in April (CL = 33 and 42 mm) were packed with roundish germinal cells (max ø 5.74 ± 0.16 μm) weakly stained with hematoxylin and among them a few typical spermatozoa were present (Fig. 1b). The larger cells we considered as non-mature spermatozoa and thus we defined their hemispermatophores as immature.

Electron microscopy

Electron microscopy revealed that the mature hemispermatophores were packed with spermatozoa (Fig. 2a) showing a central electron-lucent nuclear region, containing few dense bodies and a

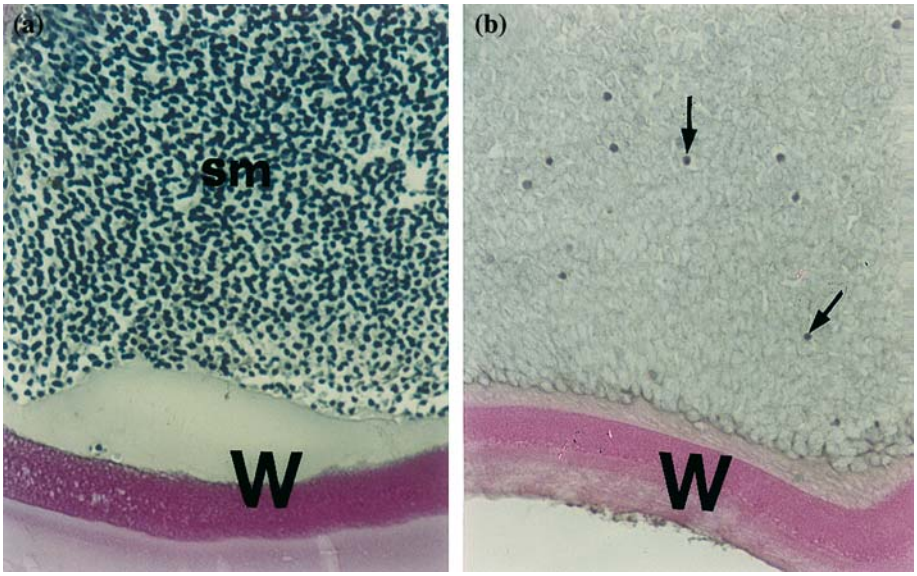

Figure 1. Light micrographs of mature hemispermatophores (a) and immature hemispermatophores (b). sm: sperm masses; W: wall of hemispermatophore; arrow: spermatozoa in immature hemispermatophores packed with immature germ cells. Hematoxylin–Eosin. ×278.

network of wide finely disperse heterochromatin, not separated from the cytoplasm by a nuclear envelope. The nuclear region was surrounded by a peripheral band of electron-dense cytoplasm showing small vesicles and large mitochondria. The immature spring hemispermatophores were filled with germ cells characterized by a nuclear region containing filamentous chromatin surrounded by an electron-dense cytoplasm band rich in large vesicles and myelin-like bodies (Fig. 2b). These hemispermatophores also contained some typical spermatozoa and rare cells characterized by electron-dense cytoplasm including an entirely heterochromatinic nucleus (Fig. 2c) or a nucleus with dense central heterocromatin surrounded by euchromatin (Fig. 2d).

Lectin histochemistry

The lectin binding pattern of hemispermatophore content is summarised in Table 2.

Since the cytoplasm of germ cells consists of a thin perinuclear band, it is very difficult (in light microscopy) to clearly distinguish the cell surface from the cytoplasm. Therefore, where not specified, the material stained outside the nuclear region is indicated as cytoplasm in the following descriptions.

SNA bound the cytoplasm of spermatozoa (Fig. 3a), whereas it linked the cytoplasm and nuclear region of rare germ cells in immature hemispermatophores (Fig. 3b).

MAA marked both the cytoplasm of the germ cells and the extracellular matrix, showing stronger staining of spermatozoa (Fig. 3c) than immature germ cells (Fig. 3d). The intercellular matrix was more marked in immature than in mature hemispermatophores.

PNA reacted weakly with the cytoplasm of spermatozoa as well as the intercellular matrix (Fig. 3e). KOH-sialidase-treatments revealed cryptic binding sites in the nucleus of spermatozoa (Fig. 3f) and in the intercellular matrix (Figs. 3f, g).

HPA stained the cytoplasm of spermatozoa (Fig. 3h), with the intercellular matrix of mature hemispermatophores being less strongly marked than that of immature hemispermatophores (Figs. 3h, i).

Con A marked the cytoplasm and the nucleus of spermatozoa (Fig. 3l), whereas it bound the cytoplasm of germ cells and the extracellular matrix in immature hemispermatophores (Fig. 3m).

The KOH-si-WGA procedure moderately stained the sperm surface and less intensely the whole sperm cell in mature hemispermatophores (Fig. 3n), whereas in immature hemispermato-

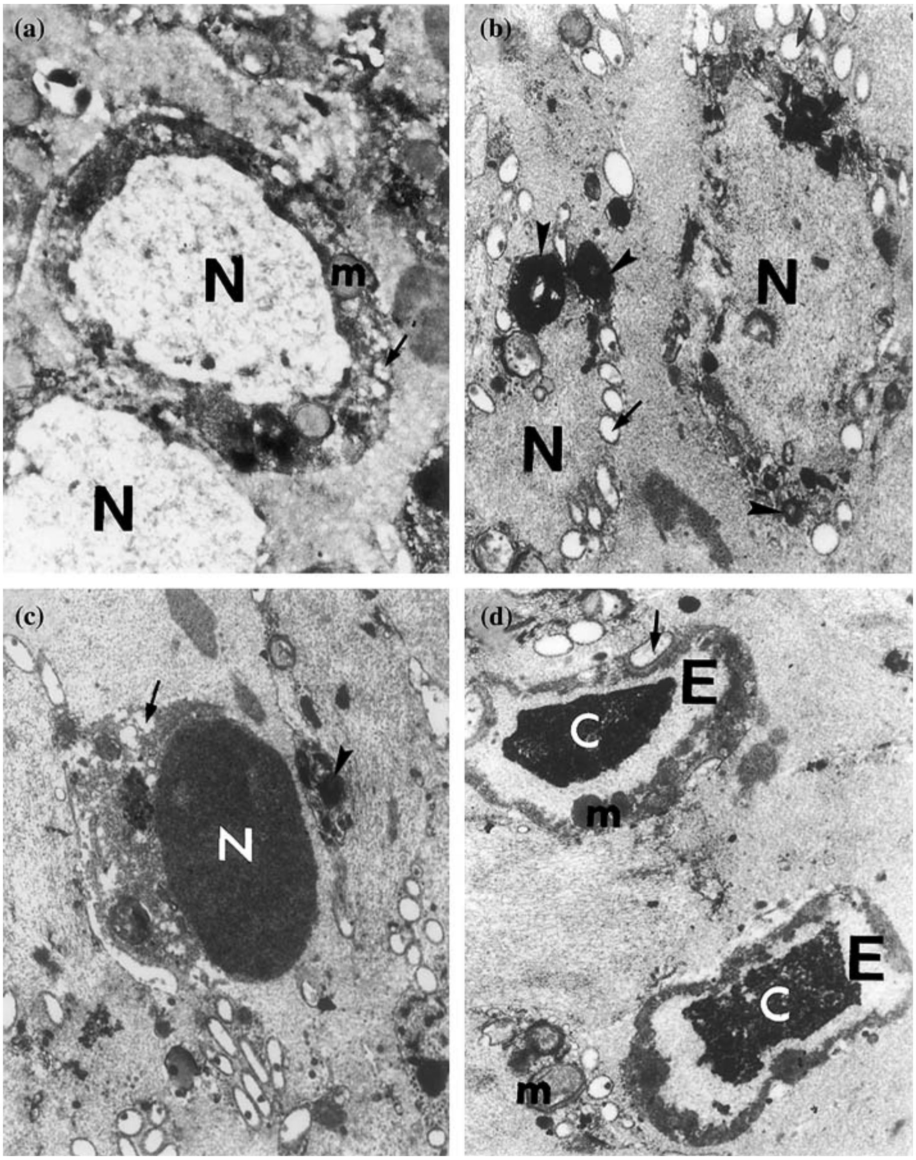

Figure 2. Electron micrographs of typical spermatozoa (a) contained in mature hemispermatophores and germ cells contained in immature hemispermatophores (b, c, d) of *Aristaeomorpha foliacea*. C: central heterochromatin; E: peripheral euchromatin; m: mitochondria; N: nuclear region; arrow: vesicles; arrowhead: myelin-like bodies. Uranyl acetate-lead citrate. (a, b, d) ×13200; c, ×11470.

phores it gave a reaction with the cytoplasm of germ cells and the extracellular matrix.

GSA II reacted with the cytoplasm and the nuclear region of spermatozoa (Fig. 3o), but it did not bind the germ cells in immature spring hemispermatophores. Lectin stained the extracellular matrix more intensely in immature than mature hemispermatophores (Figs. 3o, p).

RCA_{120}, SBA, GSA I-B_4, UEA I, and LTA did not show binding patterns.

Discussion

The general, histological characteristics of all hemispermatophore examined, as described by

Table 2. Summary of the lectins binding to the hemispermatophore content of *Aristaeomorpha foliacea*

Lectin	Mature	Immature
SNA	+ +	–/+ +w*
MAA	+ +/±m	±/+ +m
PNA	+/+m	–/+m
KOH-si-PNA	+w/+ +m	–/+ + +m
RCA$_{120}$	–	–
KOH-si-RCA$_{120}$	–	–
SBA	–	–
HPA	+/+ +m	–/+ + +m
Con A	+ +/+n	+/+ +m
GSA I-B$_4$	–	–
KOH-si-WGA	+ +s/±w	±/+ +m
GSA II	+ +w/±m	+ + +m
UEA I	–	–
LTA	–	–

m, extracellular matrix; n, nucleus; s, cell surface; si, sialidase (neuraminidase); w, whole cell; *, rare positive reaction; –, negative reaction; ±, faintly visible reaction; +, + +, + + +, weak, moderate, intense positive reactions. Where not specified, the reactions concern both the cell surface and the cytoplasm.

Tunesi (1987), consist of four layers with the sperm mass immersed in an extracellular matrix surrounded by the first layer. Our investigations have shown for the first time that hemispermatophores of *A. foliacea* can contain a scarce number of mature spermatozoa while packed with immature germ cells.

According to Medina (1995a), the ultrastructural findings show that spermatozoa of the *A. foliacea* are aflagellate and acrosomal-less cells. They are inconsistent with the dendrobranchiate unistellate sperm plan characterized by a main body housing the nucleus and acrosomal complex formed by a cap region and prolonging spike (Jamieson, 1991). Acrosome-less spermatozoa have also been found in Euphausiacea (Jamieson, 1991), Stenopodidea (Felgenhauer & Abele, 1991), and Sergestoidea (Medina, 1995b). Two of the eight specimens (25%) collected in spring (April) (CL = 33 and 42 mm) had "immature" hemispermatophores. These "immature" hemispermatophores were packed with germ cells showing a peripheral band rich in large vesicles and myelin-like bodies, whereas the "mature" hemispermatophores (collected in April and July) were filled by spermatozoa showing a more electron-dense cytoplasm containing small vesicles and large mitochondria but no myelin-like bodies. The vesicles present in mature spermatozoa are lysosome-like bodies (putative acrosomal-like structures), whereas the myelin-like bodies, occurring only in the large cells of immature hemispermatophores, represent the residual of the nuclear envelope or mitochondria as well as rough endoplasmic reticulum derived from the maturational spermatogenesis events in progress. The occurrence in the immature hemispermatophores of some germ cells with a more or less heterochromatic nucleus may represent a sign of degenerative phenomena such as apoptosis. These findings could seem in contrast with observations by Medina (1995a), who found only mature spermatozoa in hemispermatophores of *A. foliacea*. The specimens used in that study were collected in October, whereas we analysed specimens from two different periods (April and July). In addition, it has to be considered that the reproductive period occurs from May to September (D'Onghia et al., 1998) and that seasonal changes in spermiogenesis have been found (Desantis et al., 2003).

Glycoproteins fall into two main categories according to the attachment of the oligosaccharide to the peptide (Kornfeld & Kornfeld, 1985). The two types include those in which a reducing terminal GalNAc is linked O-glycosidically to a hydroxyl of serine or threonine and those in which a reducing terminal of GlcNAc is bound N-glycosidically to the epsilon amine of asparagine. Among the lectins used in this study, PNA and HPA identified the many O-linked oligosaccharides (Spicer & Schulte, 1992). The other type of glycoproteins contains N-linked oligosaccharides. This type was visualized specifically using Con A (Bernhard & Avrameas, 1971). The results obtained with lectin histochemistry showed immature germinal cells and spermatozoa to have a different glycoconjugate composition. Immature spring germ cells only showed N-linked oligosaccharides which contained terminal and internal α-D-Man and terminal or internal β-D-GlcNAc (shown by Con A and KOH-sialidase-WGA, respectively) as well as NeuAcα2,6Gal/GalNAc and NeuAcα2,3Galβ1,4GlcNAc (reactivity to SNA and MAA, respectively). Spermatozoa from mature hemispermatophores

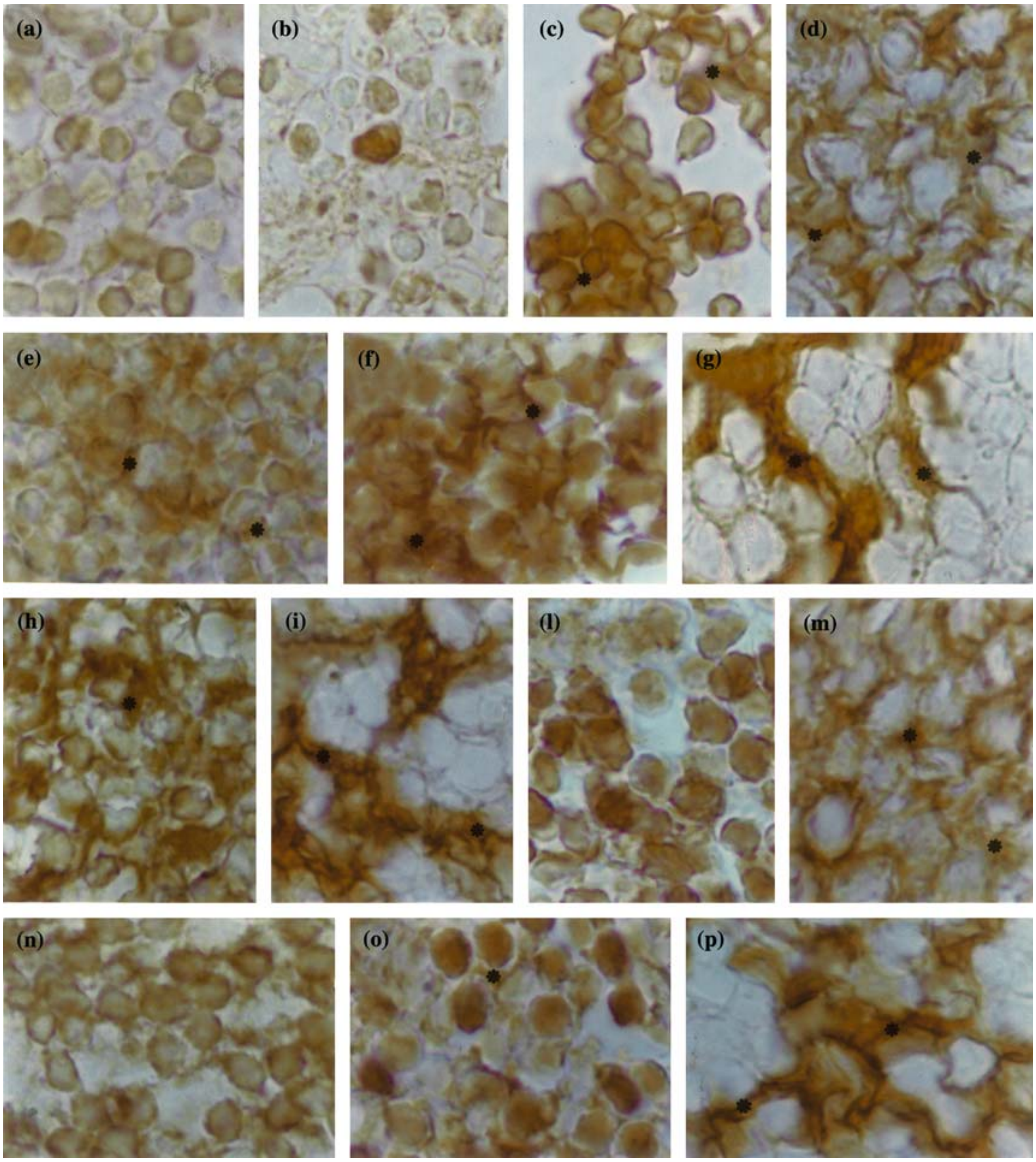

Figure 3. Lectin histochemistry. (a, b), mature and immature hemispermatophores stained with SNA. (c, d), mature and immature hemispermatophores stained with MAA. (e), mature hemispermatophore stained with PNA. (f, g), mature and immature hemispermatophores stained with KOH-sialidase-PNA procedure. (h, i), mature and immature hemispermatophores stained with HPA. (l, m), mature and immature hemispermatophores stained with Con A. (n), mature hemispermatophore stained with KOH-sialidase-WGA procedure. (o, p), mature and immature hemispermatophores stained with GSA II. *: extracellular matrix. ×1390.

displayed a more complex lectin binding pattern, since their cytoplasm contained both N- and O-linked oligosaccharides. The N-linked oligosaccharides also consisted of terminal D-GlcNAc (GSA II binding). The O-linked oligosaccharides terminated with β-D-Gal(1–3)-GalNAc dimer (PNA binding) and/or αGalNAc (HPA) in cytoplasm, whereas they ended with sialic acid linked to β-D-Gal(1–3)-GalNAc (KOH-sialidase-PNA) in the nucleus.

To the best of our knowledge, investigations on carbohydrates of hemispermatophore sperm mass in *A. foliacea* are scarce (Desantis et al., 2003) and few studies have been performed in other Decapoda. In *Macrobrachium rosenbergii* (de Man, 1879) the spermatozoa show a small PAS positive reaction (Yufeng et al., 1997) but they were unstained with Alcian blue procedures (Dougherty et al., 1986). In *Sicyonia ingentis* (Burkenroad, 1949) a PAS positive substance is released on the oocyte surface during the acrosomal reaction (Brown et al., 1976) to facilitate the attachment of sperm to oocyte (Clark et al., 1981). The comparison with the above-cited conventional histochemical data shows that mature hemispermatophore spermatozoa of *A. foliacea* contain sialylglycoconjugates and that the sialic acid residues are much more expressed than in the immature hemispermatophore germ cells.

A. foliacea spermatozoa have a very thin peripheral cytoplasm, therefore it was very difficult to discriminate the cytoplasm from the cell surface at the light microscopy level. The cytoplasmic lectin binding pattern can hide surface glycoproteins or oligosaccharides contained in vesicles. The former could be involved in the interaction with the oocyte and the latter may represent an acrosome-like structure since no acrosomal structure is recognizable at the ultrastructural level. In vertebrate spermatozoa both the cell surface and acrosome are glycoconiugate-rich structures (Arya & Vanha-Perttula, 1984; Burkett et al., 1987; Labate & Desantis, 1995; Ueda et al., 1997).

The extracellular matrix in which the spermatozoa are embedded consists of neutral as well as acidic glycoconjugates. Acidic polysaccharides have been reported in *Albunea symnista* (Linnaeus, 1758) (Subramonian, 1984) and *Panulirus homarus* (Linnaeus, 1758) (Radha & Subramonian, 1985). Neutral glycoproteins and acidic polysaccharides have been found in *M. rosenbergii* (Dougherty et al., 1986; Yufeng et al., 1997). Lectin histochemistry revealed differences in the glycoconjugate composition of the extracellular matrix between immature and mature hemispermatophores. The former displayed much more affinity for the lectin used, evidencing both N- and O-linked glycoconjugates, than mature hemispermatophores, which lack Con A reactivity thus indicating the absence of N-linked oligosaccharides. Mature hemispermatophores contain less sialylglycoconjugates than immature ones. The role of the extracellular matrix in decapods is not well known. It has been suggested that the acid mucopolysaccharide of spermatophores might act as a cementing agent or an antimicrobial agent (Jeanloz, 1970; Sasikala & Subramoniam, 1987) or in the maintenance of spermatozoa during their storage within female spermathecae (Subramoniam, 1991).

In conclusion, this study shows that the hemispermatophores of *A. foliacea* males do not always contain well differentiated spermatozoa and suggests that the presence of hemispermatophores in terminal ampullae and joined hemipetasmas is not synonymous with maturity, the confirmation of which requires further histological as well as experimental investigations.

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Reproduction strategy of the deep-sea hermit crabs *Pagurus alatus* and *Pagurus excavatus* of the Central-Western Mediterranean Sea

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Key words: fecundity, hermit crab, *Pagurus alatus*, *Pagurus excavatus*, reproductive biology, spawning

Abstract

Some aspects of the reproductive biology of two hermit crabs *Pagurus alatus* (Fabricius, 1775) and *P. excavatus* (Herbst, 1791) were studied. Specimens were collected monthly from April 2000 to March 2001 on the continental shelf and the upper-middle slope off the Southwestern coasts of Sardinia, in the central-western Mediterranean Sea. Crab size (minimum and maximum shield length) was 1.7 and 9.5 mm for 1150 females of *P. alatus*; 4.0 and 10.4 mm for 347 females of *P. excavatus*, respectively. Females of *P. alatus* with ripe ovaries were only found in February-April and ovigerous females were observed throughout the year except in March, with the highest incidence in summer. Females of *P. excavatus* with fully developed ovaries were collected during all months of the year reaching a peak from April to June. Ovigerous females of *P. excavatus* occurred throughout the year and the main spawning period occurred in March and April. Fecundity of both species was calculated to assess seasonal variation of reproductive intensity and was positively correlated with the size of the individuals. Monthly fecundity did not reveal significant differences in *P. alatus* ovigerous females, whereas *P. excavatus* exhibited significant differences in monthly fecundity depending on the time of year. Difference in fecundity among *P. alatus* and *P. excavatus* can be related to the size of the ovigerous females and species of hermit crab, but their different reproductive strategies suggest that they are specifically related to different environmental conditions.

Introduction

Hermit crabs *Pagurus alatus* (Fabricius, 1775) and *Pagurus excavatus* (Herbst, 1791) are found in the eastern Atlantic and in the Mediterranean Sea (Ingle, 1985). Of the two species, *P. alatus* has the larger geographical range in the Atlantic, extending from Iceland, Norway, and the North Sea as far as Morocco, at depths between 20 and 2500 m. *P. excavatus* is found throughout the eastern Atlantic from the Bay of Biscay (the northernmost record is N 44°35′05″, W 1°57′30″) to Morocco and Madeira, where it lives at depths of between 54 and 400 m (see Ingle, 1993). In the Mediterranean Sea, *P. alatus* is reported to be commonly found in bathyal bottoms (Pérès & Picard, 1964; Carpine, 1970) and has been found in the entire Mediterranean basin up to a depth of 834 m (Adensamer, 1898). On the contrary, the distribution of *P. excavatus*, which is found on various types of sedimentary bottoms of the continental shelf (Števčić, 1990), is limited to the western part of the Mediterranean basin as far as Cyprus, up to a maximum depth of 450 m (Chintiroglou et al., 1992). Considering the existing systematic uncertainty between *P. alatus* and the congeneric species *P. excavatus*, the determination of the two species has been controversial for years until Ingle (1985) clarified their identity. Thus different studies on *P. alatus* report discordant morphometric, bionomic, or biological indications, which on a more detailed analysis are referable to *P. excavatus* or *vice versa*. As a consequence synonymy has also created misunderstandings in the definition of the larval stages of the two species (Ingle, 1993). In spite of its large distribution, information available on the biology of *P. alatus* is limited to the period in which ovigerous females are present, to the diameter of the eggs (Bouvier, 1940; Zariquiey Álvarez, 1968), and to reproductive patterns (Mura et al., 2001). Data on *P. excavatus* are limited to the description of its relations with different species of symbiotic sea-anemones (Pax & Müller, 1962; Mainardi & Rossi, 1969; Ross, 1979; Doumenc et al., 1985; Chintiroglou et al., 1992), to laboratory observations of their sexual behaviour (Bastock, 1967; Hazlett, 1968), to the period in which ovigerous females are present, to the diameter of the eggs (Zariquiey Álvarez, 1968), as well as to reproductive cycle (Mura & Cau, 2002).

The aim of this paper is to assess some aspects of the reproductive biology (reproductive period, fecundity, and size of ovigerous females) of two populations of *P. alatus* and *P. excavatus* present in neighboring areas off Capo Spartivento (Southern Sardinia).

Materials and methods

The hermit crabs used for this study were obtained in fishing surveys carried out monthly off the South-Western coast of Sardinia (Sardinian Channel) between April 2000 and March 2001. Samples of *Pagurus alatus* were captured on muddy bottoms at depths of between 350 and 450 m along the upper-middle slope, and those of *P. excavatus* at depths of between 130 and 180 m on sandy–muddy bottoms along the continental shelf. An Italian bottom trawl net with a 20 mm stretched mesh cod-end liner, was used. After the specimens were caught, they were preserved at −20 °C and subsequently fixed in a 4% buffered formaldehyde solution. Hermit crabs were removed from their shells, sexed and measured. For each of the females, shield length (SL) was recorded with a caliper to the nearest 0.05 mm and the stage of ovarian maturation was noted based on the size and colour of the ovaries. Three stages were recognized as follows: stage 1, immature or post-spawning condition with thin and translucent ovaries; stage 2, actively developing ovaries, orange in colour, the gonads are swollen and occupy most of the abdomen cavity; stage 3, pre-spawning condition, ripe and fully developed ovaries, dark red in colour with oocytes in advanced vitellogenesis, visible to the naked eye and occupying almost the whole of the abdomen cavity. The pre-spawning condition was checked by observing histological preparations. The ovaries were isolated and included in methylacrilate. Sections of a thickness of 3 μm were stained with the Dominici method (Mazzi, 1977).

Moreover, the presence of ovigerous females was recorded. The eggs were carefully removed from pleopods and counted under a light stereomicroscope. Three stages of egg development were established as reported in Campisi et al. (1998): (1) non-segmented blastoderm or early segmentation of the body; (2) eyes pigmented but abdomen not yet free from the head; (3) well-developed abdomen with little yolk remaining. Fecundity was calculated as the mean number of eggs produced per ovigerous female. Mean monthly number of eggs per brood was compared among ovigerous females for *P. alatus* and *P. excavatus* using the ANOVA test. The comparison of the fecundity among species was carried out with ANCOVA using shield length as the covariate. The relationship between shield length and egg number was expressed by a linear regression after logarithmic transformation. Only the eggs in the first stage of development were used in the fecundity analysis.

Results

A total of 1150 females of *Pagurus alatus* with shield length (SL) between 1.7 and 9.5 mm, and 347 females of *P. excavatus* with SL between 4.0 and 10.4 mm were examined (Fig. 1 and Table 1). Females of *P. alatus* with ripe ovaries (stage 3), starting from SL of 2.0 mm, were only found in April, February and March with monthly frequency of 68.8, 36.8 and 78.2%, respectively. On the other hand, females of *P. excavatus* in ovarian stage 3, starting from SL of 5.0 mm, were found in all the months of the year. Their higher incidence was observed between April and June, with a percentage of occurrence between 42.4 and 50.9% (Fig. 2).

The smallest ovigerous *P. alatus* female of the 765 collected had a SL of 2.1 mm, while the shield

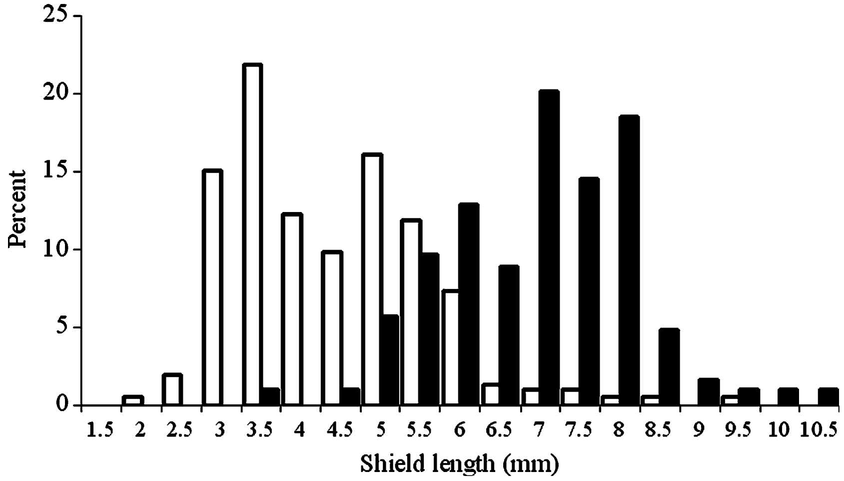

Figure 1. Size-frequency distribution of *Pagurus alatus* (white) and *P. excavatus* (dark) females collected from April 2000 to March 2001.

Table 1. Number of *P. alatus* and *P. excavatus* females examined each month throughout the sampling period (April 2000–March 2001)

	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
P. alatus	121	97	89	93	83	94	80	87	100	95	101	110
P. excavatus	27	30	27	25	35	32	24	30	36	26	31	24

length SL of the smallest *P. excavatus* of the 234 ovigerous females was 5.2 mm (Fig. 3).

Size-frequency distribution of ovigerous females revealed that there were significant differences between the two species during all months throughout the year (K–S = 5.19, $p < 0.01$). On the whole, the mean size of ovigerous females of *P. alatus* proved to be 4.33 ± 1.07 mm SL and that of the females of *P. excavatus* 7.35 ± 1.5 (t-test = 19.12; $p < 0.01$). In both species the mean monthly sizes were not significantly different.

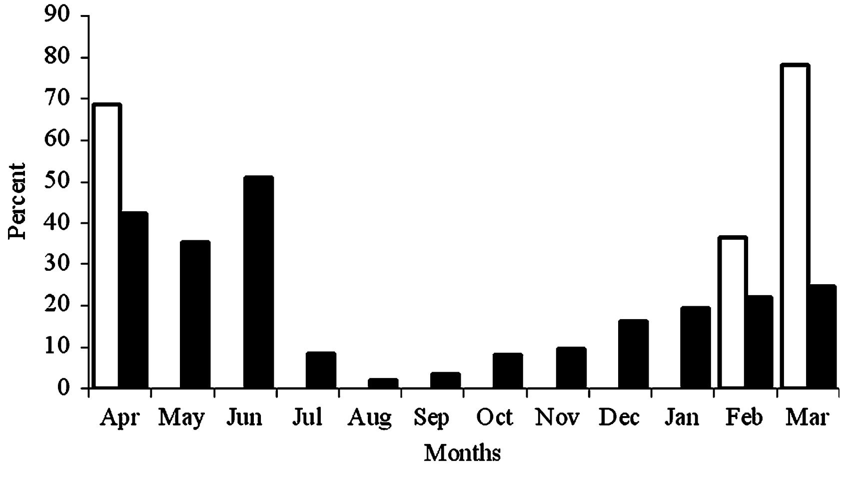

Figure 2. Monthly percentage of females with ripe and fully developed ovaries (stage 3) of *Pagurus alatus* (white) and *P. excavatus* (dark). April 2000–March 2001.

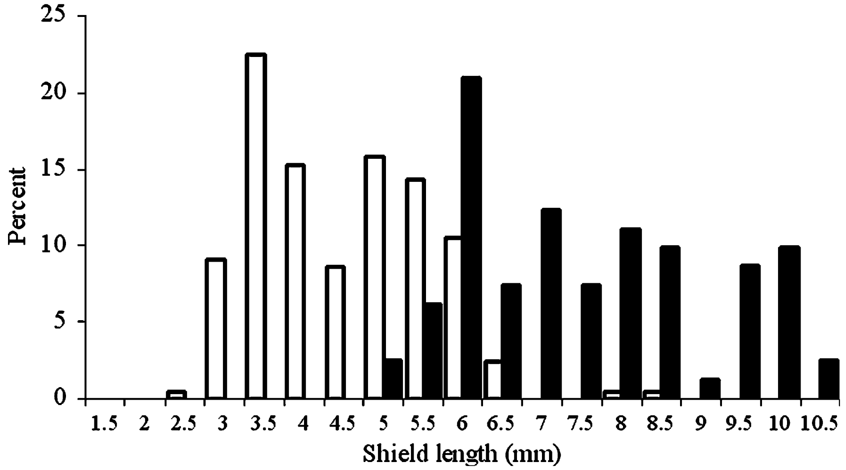

Figure 3. Size-frequency distribution of ovigerous females of *Pagurus alatus* (white) and *P. excavatus* (dark) with eggs in the first stage of development. April 2000–March 2001.

Ovigerous females of *P. alatus* were collected throughout the year except in March with highest frequency in the summer months (Fig. 4). The females of this species showed an annual reproductive cycle characterized by a long egg incubation period and by a marked synchronization of their development in time. The eggs at the first stage of development were observed over ten months from April to January. The eggs at the second stage of development were observed exclusively from November to January, while eggs at the third stage from December to February. All the ovigerous females of this species were found with thin transparent ovaries (stage (1). Post-ovigerous females (characterized by the presence of remains of egg and embryos still attached to the pleopods) were observed from December to March and with highest frequency in February.

Ovigerous females of *P. excavatus* were caught all year round with all egg developmental stages well represented. Their incidence was higher in the winter months and at the beginning of spring. The percentage of ovigerous females reached peak values in March–April and then declined drastically in the following months. Occurrences of ovigerous females lower than 12.3% were recorded from April to October except in August (14.6%). Eggs of *P. excavatus* at the third stage of devel-

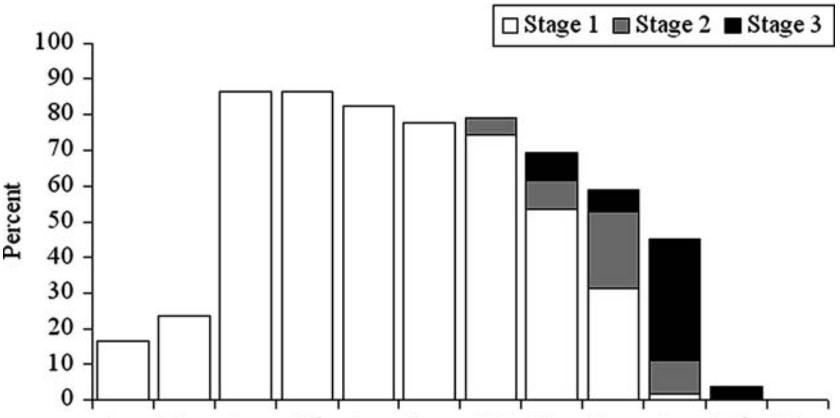

Figure 4. *Pagurus alatus*. Monthly frequencies of the different egg stages in ovigerous females. April 2000-March 2001.

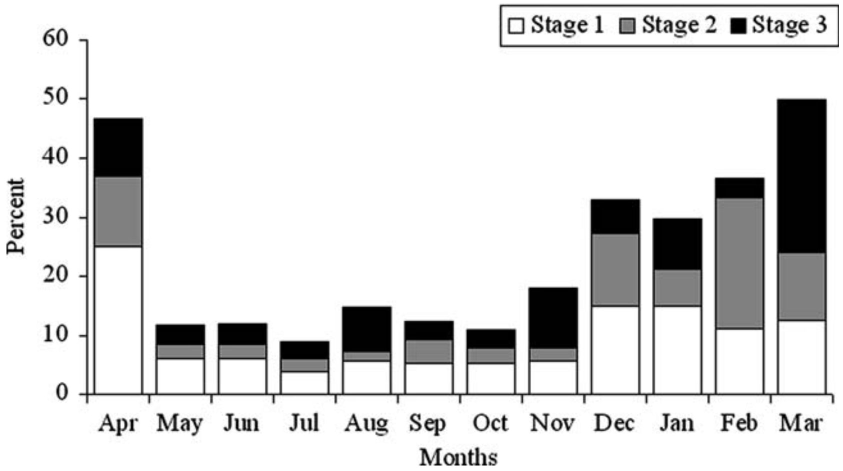

Figure 5. Pagurus excavatus. Monthly frequencies of the different egg stages in ovigerous females. April 2000–March 2001.

opment were observed all year around, with maximum values observed in August, November and March (monthly values 35.3, 20.2, and 46.3% of all ovigerous females, respectively) (Fig. 5). The highest incidence of ripe ovaries in ovigerous females was found in February (47.8%). On the whole, 17.7% of all the ovigerous females examined throughout the sampling period showed fully developed ovaries. It was not possible to determine a clear period in which post-ovigerous females of *P. excavatus* were significantly more frequent.

The mean fecundity of *P. alatus* ovigerous females was 529.84 ± 365.88 ($n = 573$; individual fecundity ranged from 93 to 2,504) while that calculated for *P. excavatus* ovigerous females was 1553.73 ± 558.03 ($n = 138$; individual fecundity ranged from 322 to 4109). The highest number of eggs was observed in a 8.1 mm SL female of *P. alatus* caught in June, and in a 8.9 mm SL female of *P. excavatus* caught in February.

In *P. alatus*, the mean monthly number of eggs was not significantly different throughout the months (mean monthly egg number ranged from 458.6 ± 195.1 in January to 639.1 ± 270.2 in April), while in *P. excavatus* (Fig. 6) the mean monthly number of eggs decreased significantly (ANOVA, $F_{(11,\ 138)} = 13.57$; $p < 0.001$) from February to August to increase later as the season progressed.

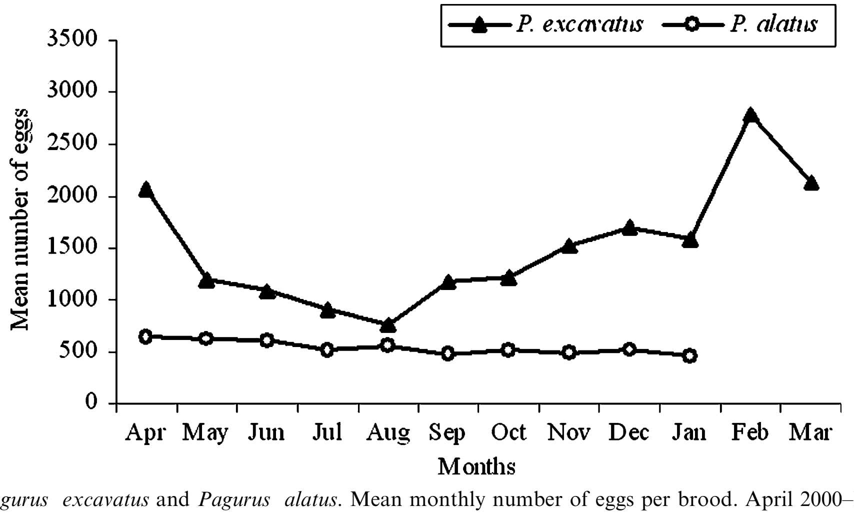

Figure 6. Pagurus excavatus and *Pagurus alatus.* Mean monthly number of eggs per brood. April 2000–March 2001.

The regression parameters of the relationship between carapace shield length and number of eggs in *P. alatus* ovigerous females were the following: log (egg number) $= 3.11 + 2.06$ log (shield length) ($r^2 = 0.74$; *F*-ratio $= 253.77$; $p < 0.01$), while those of the ovigerous females of *P. excavatus* were: log (egg number) $= 1.96 + 1.40$ log (shield length) ($r^2 = 0.78$; *F*-ratio $= 126.2$; $p < 0.01$).

The comparison of fecundity among species, carried out by means of covariance analysis (ANCOVA) using the shield length as the covariate, showed that the difference between the species is caused both by the crab size (source of variation = shield length, $F_{(1,287)} = 415.71; p < 0.001$) and species of hermit crab (source of variation = hermit crab species, $F_{(1,287)} = 17.92; p < 0.001$).

Discussion

Results show that the two hermit-crab species were characterized by very different models of reproductive strategy. In the Sardinian Channel, *Pagurus alatus* is characterized by a long egg incubation period and by a marked synchronization of the egg developmental stages, according to the data provided by Urzelai et al. (1990) in the Bay of Biscay. The observation of ovigerous females with eggs at the first stage of development during a period of ten months, and of females with eggs at the second and third stages of development exclusively in the limited period of four months, and of post-ovigerous females collected mainly in February, confirm some previous observations on the reproductive strategies of this species in the Mediterranean Sea (Zariquiey Álvarez, 1968; Mura et al., 2001). Even the observation that the complete development of the maturity cycle is limited to three months agrees with the proposed seasonal model of reproductive activity (Mura et al., 2001). From the recurring occurrence of ovigerous females with ovaries in the resting state, therefore, it can be ruled out that the females could spawn more than one within the reproductive season per year. Moreover, seasonal migration of some ovigerous females towards deeper areas as the reproductive season advances has also been hypothesized for this species (Urzelai et al., 1990; Mura et al., 2001).

On the other hand, *P. excavatus* females show continuous reproductive activity throughout the year. Ovigerous females of this species (with all three egg developmental stages) have been found in all months even though their monthly frequency indicates the existence of a main reproductive season at the beginning of spring, as seen during a series of preliminary observations that had previously been carried out in the same area (Mura & Cau, 2002). Furthermore, from these early data, which referred to a smaller number of females than that available in this study, it was observed that the period in which *P. excavatus* females with ripe ovaries were more frequent extended from December to March. The present data confirm the existence of a long seasonal maturity cycle, but they indicate that the higher incidence of females with ripe ovaries was found between April and June. Moreover, one fifth of the ovigerous females of *P. excavatus* was observed in pre-spawning condition (e.g. with ripe ovaries) with a high percentage recorded in February. These data suggest that a small number of the ovigerous female have the potential for two consecutive ovipositions.

The ovigerous females of *P. alatus* have smaller mean sizes than those of the congeneric species *P. excavatus*, and also show a mean number of eggs lower than *P. excavatus* at given size. Therefore, the higher incidence of small ovigerous females of *P. alatus*, in relation to the inadequate shell dimension of greatly available gastropods on the continental slope, should be considered (Lancaster, 1990). It has been observed that 52.3% of the *P. alatus* ovigerous females in the area studied live in small shells of the gastropod *Aporrhais serresianus* (Michaud, 1828) (Mura, unpublished data), a characteristic species of Mediterranean bathyal bottoms (Pérès & Picard, 1964). Thus, the fecundity of hermit crabs is related to the availability of local adequate gastropod shells (Elwood et al., 1995; Mantelatto & Garcia, 1999).

The relationship between the shield length of *P. alatus* and *P. excavatus* females and the number of eggs is characterized by a high positive r^2 value. According to Wilber (1989), the number of eggs is positively correlated with the size of ovigerous females, which was also found for *P. alatus* and *P. excavatus*. Ovigerous females of the two species have shown a different mean number of eggs per brood and a significant difference in shield length mean values.

Data referred to the studied area show that: (a) the difference in fecundity among *P. alatus* and *P. excavatus* is caused by the different size of the ovigerous females; (b) the specific size-fecundity is significantly different for the two congeneric species.

While the monthly mean number of eggs per brood in *P. alatus* does not vary during the year, in *P. excavatus* it shows a significant decline starting from February until it reaches a minimum in August and subsequently increases as the season progresses, although the monthly mean size of ovigerous females is not significantly different in the different months of the year. The variable mean monthly number of eggs observed throughout the year shows that *P. excavatus* has low spawning activity in subsequent broodings, except during the main spawning season.

It should be noted that the two congeneric hermit crabs can be probably considered sympatric, given the partial overlapping distributions (Ingle, 1993), and may at times be caught together (Mura, unpublished data) but their different reproductive strategies suggest that they are specifically related to different environmental conditions.

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Diel and seasonal changes in the structure of a Decapod (Crustacea: Decapoda) community of *Cymodocea nodosa* from Southeastern Spain (West Mediterranean Sea)

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Key words: diel changes, decapod, seagrass, Cymodocea, diversity, species richness, Mediterranean Sea

Abstract

The study of a decapod community in a *Cymodocea nodosa* meadow from Southeastern Spain (Western Mediterranean Sea) showed a stable structure, in which the families Hippolytidae, Processidae, Majidae and Portunidae were the most abundant and the species *Hippolyte niezabitowskii* dominated. The animal community was more numerous and diverse during the night, showing the existence of nychthemeral movements, which are essentially related to the trophic behaviour and shelter. In this way, many species increased their abundance as a result of an increasing activity and, also, of an influx of other species and specimens from adjacent sandy bottoms, such as *Processa* spp. (mainly *P. modica*) *Sicyonia carinata, Liocarcinus* spp. (mainly juveniles) and several species of hermit crabs, which were rare or absent during the day. All these changes produced modifications in the dominance curves and in the values of all ecological indices (richness, diversity and evenness). Monthly samples were grouped and ordered (MDS) by the factor "day–night", which showed slight qualitative and quantitative differences (SIMPER, dissimilarity average of the factor day–night = 61.67). On the other hand, no global seasonal differences have been found (one way ANOSIM), but there was a significant level of similarity between winter and spring, while the summer samples were the most different. The differentiation of the summer 1999 can be attributed to a decrease in species abundance and richness, probably due to the dynamics of the decapod populations and the balance with predators (fishes), while that of the summer 2000, to an anomalous event: the massive proliferation of filamentous algae, mainly *Ectocarpus* s.l., which modified the environmental conditions.

Introduction

Three genera of seagrasses can be found in the Western Mediterranean littoral: *Posidonia* (*Posidonia oceanica* (Linnaeus) Delile, 1813), *Cymodocea* (*Cymodocea nodosa* (Ucria) Ascherson, 1869) and *Zostera* (*Zostera marina* Linnaeus, 1753 and *Zostera noltii* Hornemann, 1832), which can form dense and extensive meadows with complex associated communities (Kikuchi & Pérès, 1977).

During the last decades, the animal community of *Posidonia* and *Zostera* beds in general, and to a lesser extent the decapod crustaceans, have been thoroughly studied (see Jacobs & Huisman, 1982; Jacobs et al., 1983; Templado, 1984; Thayer et al., 1984; García Raso, 1990; Curras et al., 1993). On the contrary, little information exists on *Cymodocea* meadows (Sánchez-Jerez et al., 1999), inspite the fact that *C. nodosa* is frequently associated with *P. oceanica*, and represents part of the successional series of the typical seagrass community of the Mediterranean Sea (Molinier & Picard, 1951, 1952; Pérès & Picard, 1964; Hartog, 1977; Hemminga & Duarte, 2000).

Some records of decapods from *C. nodosa* could be found in taxonomic and bionomic papers, but quantitative studies or analyses on seasonal and day–night population changes are scare. In this way, in the Mediterranean, the studies of Ledoyer (1966) and Števčić (1991) could be mentioned. In the former, the macrofauna was analysed throughout a year in different locations, depths, and also during the day and night. In the latter, the decapod fauna of the mixed bottoms (*Zostera* plus *Cymodocea* beds and algae plus *Cymodocea*) was studied monthly around a year using a small beam-trawl.

The main purpose of this study is to obtain information on the quantitative structure of the decapod community and its day-night differences. Also, this information will be useful in a future comparative analysis with other decapod communities of Mediterranean seagrasses, *Zostera* (in preparation) and *Posidonia*, allowing us to acquire a global view.

Materials and methods

The sampling site is located off Genoveses beach, in the Natural Park of Cabo de Gata, Almería, Spain (Fig. 1). In this area, *Cymodocea* beds are distributed as irregular, not very dense patches, in depths between 4 and 15 m.

During the years 1999 and 2000 samples were taken at depths between 10 and 14 m, in different seasons (July 1999, December 1999, March 2000 and June 2000), in the morning (always between 11.00 and 13.00 am) and in the night (in moonless nights, at least 2 h after sunset), using a small Agassiz trawl at a speed of 1 knot (1.85 km/h). This trawl has a dredge frame of 72 cm width and a net mesh of 3 mm (knot to knot). Each haul lasted for 10 min, thus covering an area of 222 m^2. This sampling area is larger than that normally used by other authors in studies on the macrofauna of seagrass communities (1 m^2 or less: Jacobs et al., 1983; Curras et al., 1993; Matheson et al., 1999; 6 m^2: Sánchez-Jerez et al., 1999; 10 m^2: Gore et al., 1981), but it is similar to that used in some fish studies (243 m^2: Mattila et al., 1999). We consider this large area because decapods are medium-large sized and very mobile organisms (unlike other groups of invertebrates such as molluscs), and the employment of such small areas is insufficient (even with replicates) for the study of the decapod community, in which ecological indices are used. Also, two or three replicates were taken, except in June 2000. In the latter sampling period, the material obtained (day and night) was more than twice the normal, as a consequence of an anomalous event, a massive proliferation of filamentous algae, mainly *Ectocarpus* s.l., which modify the environmental conditions. On the other hand, we

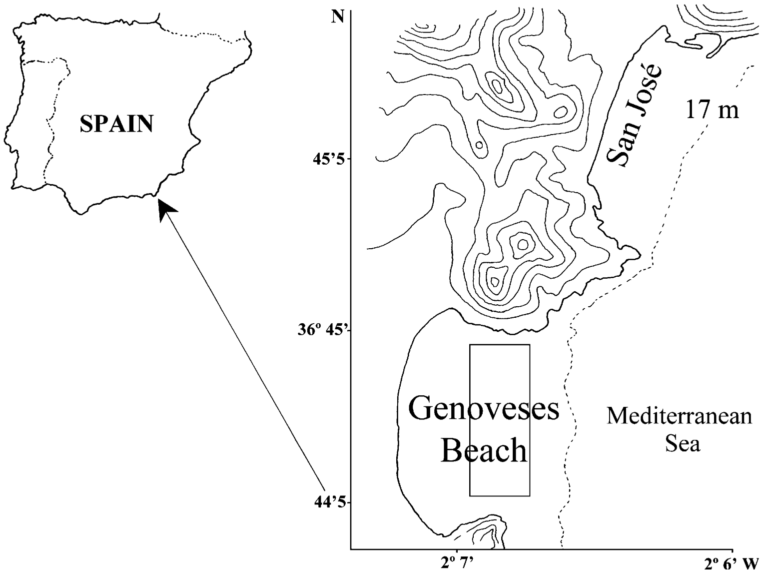

Figure 1. Location of sampling area and the studied meadow (rectangle).

limited the number of samples because the area is included in a protected Natural Park.

In the laboratory, the samples were washed over a column of sieves to separate specimens (smaller mesh size: 1 mm).

For the analysis of the community structure, the PRIMER software (Plymouth Routines In Multivariate Ecological Research, Clarke & Warwick, 1994) was employed using the quantitative data without standardisation and transformation. We assessed possible significant differences between nocturnal and diurnal samples, and between seasons (program ANOSIM); the contribution of each species (program SIMPER); the values of the diversity of Shannon index (Shannon & Weaver, 1963; Krebs, 1989, with e base logarithm), evenness (Pielou, 1969), Margalef's richness, Simpson's diversity (program DIVERSE), and the *k*-dominance curves (Gray & Pearson, 1982, program GEOPLOT). Finally, the Bray–Curtis similarity index was calculated, and aggregation (program CLUSTER) as well as ordination analysis (program MDS) were performed.

Results

The monthly faunistic results (species composition and abundances, as well as the presence of ovigerous females) are presented in the appendix. In Table 1, the total decapod abundance, richness, diversity and evenness values for each sample, as well as the seasonal average values (average of replicates) are given. These data are referred to a surface of 222 m^2. In general, two caridean and two brachyuran families dominated: Hippolytidae, Processidae, Majidae and Portunidae (Table 2). A stable decapod community lived in this biotope, with a total of 48 species caught and an average value of 15 species among seasons (SD = 7.6). However, a significant difference between day and night existed (one way ANOSIM: quantitative, factor day–night, global $R = 0.4$, $p < 0.001$). In total, 34 species were found in day samples, as opposed to 47 species collected in night samples.

The biotope was more rich and diverse during the night (Tables 1, 2, 3 and appendix), because many species increased their abundance, and additional species and families appeared. As a result, 5 to 9 species were caught in a sample during the day (except in June 2000) (average 8.9, SD = 4.4; Tables 1, 3), with *Hippolyte niezabitowskii* d'Udekem d'Acoz, 1996 and *Hippolyte holthuisi* Zariquiey Alvarez, 1953 dominating (Table 2) and having an important contribution to the similarity (SIMPER, similarity average = 52.36; species contribution %: *H. niezabitowskii* 84.66, *H. holthuisi* 7.16). During the night, 17 to 29 species were captured (average 21.22, SD = 4.121; Tables 1, 3) with SIMPER similarity average = 52.76 and principal species contribution %: *H. niezabitowskii* 40.89, *Processa modica* Williamson & Rochanaburanon, 1979 16.35, *Macropodia rostrata* (Linnaeus, 1761) 11.80, *H. holthuisi* 7.94, *Liocarcinus vernalis* (Risso, 1827) 3.92. During the night, nine species accounted for 90.82% of the species contribution.

The most important species, according to the percentages of participation in the dissimilarity of the factor day–night (SIMPER, dissimilarity average = 61.67) were: *H. niezabitowskii* (23.44%), *P. modica* (15.71%), *M. rostrata* (8.63%), *H. holthuisi* (8.08%), *Anapagurus alboranensis* Garcia-Gomez, 1994 (7.01%) and *L. vernalis* (5.85%). All these diel changes produced modifications in the dominance curve (Fig. 2) and in the values of all ecological indices (Tables 1 and 3).

On the other hand, no global seasonal differences have been found (one way ANOSIM: quantitative, factor: summer–winter–spring, global $R = 0.16$, $p < 0.05$), but the pairwise test showed a significant level of similarity between winter (12D and 12N samples) and spring (3D and 3N samples) ($R = 0.45$, $p = 0.02$). The value of the average dissimilarity between these seasons was the lowest, 37.17 (SIMPER).

The aggregation and ordination analyses (Fig. 3a, b) showed that seasonal samples were grouped by the factor day–night; also, the summer samples (June–July) were positioned separately (MDS, stress value: 0.006).

Discussion

The total number of species caught in the biotope of *C. nodosa* (see appendix) is, apparently, similar to that found in the adjacent meadow of *P. oceanica* (rhizome stratum), which has been previously studied (García Raso, 1990). However, total rich-

Table 1. Decapod abundance (*N*), species richness (*S*), Margalef's richness (*d*), Pielou's evenness (*J′*), Shannon's diversity (H′) and Simpson's diversity (1–λ′) of samples

	7D1	7D2	7D3	7N1	7N2	7N 3	12D1	12D2	12D3	12N1	12N2	12N3	3D1	3D2	3N1	3N2	6D	6N
N	33	60	65	285	211	313	263	283	197	433	301	286	158	137	436	415	378	1177
	52.7 (17.2)			269.7 (52.7)			247.7 (45)			340 (80.9)			147.5 (14.8)		425.5 (14.8)			
S	5	9	7	20	20	19	9	9	7	19	17	18	8	6	27	22	20	29
	7 (2.0)			19.7 (0.6)			8.3 (1.2)			18 (1)			7 (1.4)		24.5 (3.5)			
d	1.14	1.95	1.44	3.54	3.55	3.31	1.61	1.42	1.33	2.97	2.98	3.01	1.38	1.02	4.28	3.48	3.2	3.96
	1.51 (0.41)			3.47 (0.14)			1.45 (0.14)			2.99 (0.02)			1.20 (0.25)		3.88 (0.56)			
J′	0.54	0.45	0.54	0.7	0.75	0.74	0.48	0.39	0.42	0.58	0.64	0.63	0.35	0.3	0.7	0.75	0.61	0.71
	0.51 (0.05)			0.73 (0.03)			0.43 (0.05)			0.61 (0.03)			0.33 (0.04)		0.73 (0.04)			
H′	0.87	0.99	1.04	2.14	2.23	2.22	1.11	0.86	0.88	1.72	1.85	1.83	0.73	0.55	2.31	2.33	1.82	2.4
	0.97 (0.09)			2.20 (0.05)			0.95 (0.14)			1.80 (0.07)			0.64 (0.13)		3.32 (0.01)			
1−λ′	0.42	0.41	0.51	0.84	0.86	0.83	0.5	0.4	0.4	0.69	0.74	0.73	0.32	0.26	0.85	0.86	0.72	0.87
	0.45 (0.06)			0.84 (0.02)			0.43 (0.06)			0.72 (0.03)			0.29 (0.04)		0.86 (0.01)			

Seasonal averages, for day and night separately, are given in second line with (SD). In sample notation 7: 13/07/1999; 12: 22/12/1999; 3: 29/03/2000, 6: 22/06/2000; D: day; N: night; 1, 2, 3: replicates.

Table 2. Number of individuals of dominant species caught during the night (NN), the day (ND) and total (NT) with their percentage to all decapods

Dominant species	NN	%	ND	%	NT	%
Hippolyte niezabitowskii	1212	31.42	1095	69.57	2307	42.48
Processa modica	560	14.52	0	0	560	10.31
Hippolyte holthuisi	292	7.57	144	9.15	436	8.03
Macropodia rostrata	299	7.75	46	2.92	345	6.35
Liocarcinus vernalis	200	5.19	12	0.76	212	3.90
Liocarcinus navigator	177	4.59	35	2.22	212	3.90
Hippolyte inermis	112	2.90	87	5.53	199	3.66
Anapagurus alboranensis	167	4.33	5	0.32	172	3.17
Hippolyte garciarasoi	111	2.88	51	3.24	162	2.98
Philocheras monacanthus	94	2.44	29	1.84	123	2.26
Philocheras bispinosus	97	2.51	1	0.06	98	1.80
Processa macrophthalma	96	2.49	1	0.06	97	1.79
Processa edulis	76	1.97	2	0.13	78	1.44
Sicyonia carinata	78	2.02	0	0	78	1.44
Diogenes pugilator	45	1.17	6	0.38	51	0.94
Total of dominant species	3616	93.75	1514	96.19	5130	94.46
Total of all decapods caught	3857		1574		5431	

ness has been underestimated in the study of *Posidonia*, since samples were not collected during the night, when there are additional species coming from adjacent biotopes (Ledoyer, 1966, 1968; Števčić, 1991; López de la Rosa et al., 2002), mainly from sandy bottoms or patches.

Both biotopes, *Cymodocea* and *Posidonia*, share many species in common, because they both provide protection (leaves) and they are neighbouring. However, the dominances are very different. In *Posidonia* beds, there are two well-defined strata (leaves and rhizomes) and, in general, the most abundant decapods are the hermit crabs (*Cestopagurus timidus* (Roux, 1830) and *Calcinus tubularis* (Linnaeus, 1767)), whereas in *Cymodocea*, there is not a comparable rhizome stratum and the Caridea, especially of the family Hippolytidae, dominate. In fact, both biotopes are included within the infralittoral soft bottoms (Pérès & Picard, 1964), but *Posidonia* beds could also be regarded as hard bottoms (Bellan-Santini et al., 1994). Actually, due to its structure and biotic composition, the *Posidonia* meadow represents, a mixed bottom.

In the present study, differences with the studies of Ledoyer (1966) and Števčić (1991), both conducted on *C. nodosa* beds, have been found. In the former, a very small number of decapod specimens and species were caught, which could be the consequence of the different sampling methodology applied. In the latter, the samples were only collected in the morning; this, and probably the different structure of the biotope and the specific characteristics of the geographical location (Adriatic Sea), were the reasons for which decapods, such as *Processa* spp., were not captured, and Hippolytidae were caught only occasionally (few specimens and species).

In our study, the decapod community of *Cymodocea* is dominated during the day by caridean species that live on the leaves' stratum, with *Hippolyte* being the dominant genus, as happens in other seagrass areas (Ledoyer, 1984). This genus and family is perfectly adapted to the seagrass, exhibiting a cryptic coloration and behaviour that reduce vulnerability to certain visually hunting predators (Main, 1987). Other species from the sediment or sandy bottoms have little numerical importance, for example the genus *Philocheras* and some Brachyura, particularly the crabs *Liocarcinus navigator* (Herbst, 1794) and *M. rostrata* (juveniles and medium-sized individuals of the latter species are common in small seagrasses, *Cymodocea* and

Table 3. Average community indices for day and night samples and (SD): Decapod abundance (*N*), species richness (*S*), Margalef's richness (*d*), Pielou's evenness (*J′*), Shannon's diversity (*H′*) and Simpson's diversity (1–λ′)

	Day	Night
N	174.9 (116.4)	428.6 (291.2)
S	8.9 (4.4)	21.22 (4.12)
D	1.61 (0.65)	3.45 (0.45)
J′	0.45 (0.10)	0.69 (0.06)
H′	0.98 (0.36)	2.11 (0.25)
1–λ′	0.44 (0.13)	0.81 (0.07)

Zostera, while large individuals are more abundant in deeper, sandy bottoms).

This species composition is a consequence of the characteristics of the biotope, which include the existence of leaves, accumulation of detritus and sandy patches. However, the above-mentioned species and other resident species are clearly more abundant during night time, as a consequence of an increasing activity. Also, during the night, there is an influx of other species and specimens of adjacent sandy bottoms or patches, which are rare or absent during the day, such as *Processa* spp. (mainly *P. modica*), *Sicyonia carinata* (Brünnich, 1768), *Liocarcinus* spp. (mainly juveniles) and several species of hermit crabs, looking for food resources or shelter. For example, *Processa edulis* (Risso, 1816) is a carnivorous species, and amphipods, decapods and polychaetes are the most abundant prey in *P. oceanica* beds (Chessa et al., 1989).

These diel migrations or movements are normal and frequent in this type of biotopes and in the above-mentioned families and/or genera (Ledoyer, 1966, 1984). Nychthemeral migrations are also known in other groups such as fishes (Bell & Harmelin-Vivien, 1983; Robertson, 1984; Mattila et al., 1999; Guidetti & Bussotti, 2000). For this reason, the diversity and evenness values were higher in night samples; similar results were found by Mattila et al. (1999) in *Z. marina* beds. This decrease in the high dominances during the night makes the curve more depressed.

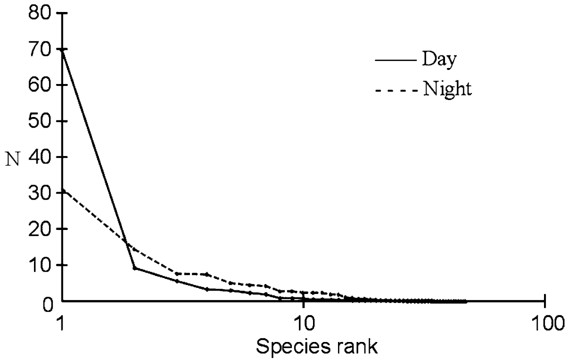

Figure 2. General dominance curves.

As a consequence of these features, the samples are grouped and ordered mainly by the factor "day–night" (Fig. 3a, b). Only the summer samples (June–July) come apart. In the case of July 1999, this differentiation was a consequence of a decrease in species abundances and richness, which was also found in other biotopes during this season (García Raso & Fernández Muñoz, 1987; García Raso, 1990); in June 2000, the most probable reason was an anomalous event. In the summer of this year a massive proliferation of filamentous algae appeared (mainly *Ectocarpus*

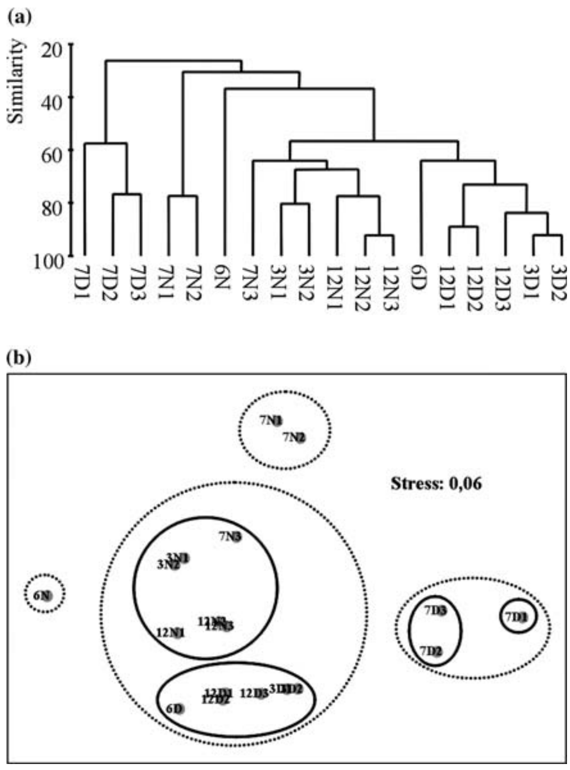

Figure 3. Aggregation (A) and MDS ordination (B) analyses, using quantitative data without standardisation and transformation. 3: March, 6: June, 7: July, 12: December, D: day, N: night. 1, 2, 3: replicates. Stress = 0.06.

s.l., F. Conde, pers. comm.), which produced alterations in the structure of the community: a strong increase in the number of prawns and shrimps, both during the day (mainly *Hippolyte garciarasoi* d'Udekem d'Acoz, 1996, a species very probably associated to seaweed) and the night (particularly *Processa* spp.), as well as a strong general decrease in hermit crabs (and in all Mollusca, C. Salas pers. comm.). Also, together with these changes, many juveniles of the crabs *L. vernalis* and *L. navigator* (= *L. arcuatus*) moved to the seagrass bed during night time, probably looking for food in a protected biotope.

Another factor to be taken into account in relation with these seasonal changes is the effect or balance between the abundance, dynamics and activity of predators (mainly fishes) and the decapod crustacean populations. Actually, crustaceans consist a major food resource for the fishes foraging in seagrass beds, and they play a prominent role in the energy transfer from primary producers to higher trophic levels (Hemminga & Duarte, 2000). In addition, many of fish species use meadows as nursery of juveniles in some periods (Coles et al., 1993; Rooker et al., 1998). In *Posidonia* bottoms from Alicante (SE Spain), Sanchez-Jeréz et al. (1999) found in February an increase of four species of amphipods and gastropods, which was associated with low fish abundance. In the Gulf of Olbia, Guidetti & Bussotti (2000) found the highest density of several necto-benthic fish (mainly labrids and sparids) from late spring to early autumn. In our area and meadow, the fish community associated with *C. nodosa* shows in summer a clear increase in the number of species and individuals, mainly belonging to the families Sparidae (mainly *Boops boops* (Linnaeus, 1758) and *Pagrus pagrus* (Linnaeus, 1758)), Centracanthidae (*Spicara smaris* (Linnaeus, 1758)), Syngnathidae (mainly *Nerophis ophidion* (Linnaeus, 1758)) and Gobiidae (A. Reina, pers. comm.), species, for which the decapods are an important food component.

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Appendix

Appendix A. Decapod species abundance per sample (222 m^2)

Species	Samples																			
	7D1	7D2	7D3	7N1	7N2	7N 3	12D1	12D2	12D3	12N1	12N2	12N3	3D1	3D2	3N1	3N2	6D	6N	Total	%
Hippolyte niezabitowskii d'Udekem d'Acoz, 1996	25*	46*	44	39*	34*	106*	182*	217*	150*	229*	140*	138*	129*	117*	122*	119*	185*	285*	2307	42.48
Hippolyte holthuisi Zariquiey Alvarez, 1953		3*		2*	2*		34*	32*	24*	45*	42*	42*	17*	15*	51*	68*	19*	40*	436	8.03
Hippolyte inermis Leach, 1815	3*		1	7*	2*	12*	20*	18*	12*	17*	8*	11	1*	1	3*		31*	52*	199	3.66
Hippolyte garciarasoi d'Udekem d'Acoz, 1996								1		1	1	3				1*	50*	105*	162	2.98
Hippolyte leptocerus (Heller, 1863)							2											3	5	0.09
Macropodia rostrata (Linnaeus, 1761)	2	4	6	58*	37*	34*	12*	9	3*	26*	17*	17*	2	2	30*	21*	6*	59*	345	6.35
Macropodia czernjawskii (Brandt, 1880)			1	1	2*			1					2				2*	21*	30	0.55
Macropodia longirostris (Fabricius, 1775)							1										3*	2*	6	0.11
Macropodia sp. (juv.) **				3		1	1		1		1								7	0.13
Philocheras monacanthus (Holthuis, 1961)		1*		4*	1	6*	6*	2	4	4*	8*	7*	4*		16*	24*	12*	24*	123	2.27
Philocheras bispinosus (Hailstone, 1835)	1*			1*	1*					18*	10*	14*			40*			13*	98	1.80
Philocheras trispinosus (Hailstone, 1835)			11*	5*		5*										18*		1	40	0.74
Philocheras fasciatus (Risso, 1816)																		1*	1	0.018
Processa acutirostris Nouvel & Holthuis, 1957				3	3	6				2						2	1	3	20	0.37
Processa edulis (Risso, 1816)				1	1	9				7	5	3					2	50	78	1.44
Processa macrophthalma Nouvel & Holthuis, 1957				7	5	3				5	3	3			16	15	1	39	97	1.79
Processa modica Williamson & Rochanaburanon, 1979				17*	21*	52*				53*	44*	31			89*	64*		189*	560	10.31
Sicyonia carinata (Brünnich, 1768)				2	6	14				13	7	2			5	9		20	78	1.44
Liocarcinus maculatus (Risso, 1827)										1									1	0.02
Liocarcinus vernalis (Risso, 1827)				46	25	20				1	4	3			10	10	12	81	212	3.90
Liocarcinus navigator (Herbst, 1794) (= *L. arcuatus*)				2	3	4									1		35	167	212	3.90
Portunus hastatus (Linnaeus, 1767)				1	2	18*									3	14		1	39	0.72
Dardanus arrosor (Herbst, 1796)					1									1	12	15		1	30	0.55
Diogenes pugilator (Roux, 1829)				7	13	3		2	1	4	5	5	2		2	2	1	4*	51	0.94
Anapagurus alboranensis Garcia-Gomez, 1994		1		77*	50*	16*	4								12*	10*		2	172	3.17
Lysmata seticaudata (Risso, 1816)																	1		1	0.02
Thoralus cranchii (Leach, 1817)																	1	3*	4	0.07
Athanas nitescens (Leach, 1814)											1								1	0.02
Palaemon serratus (Pennant, 1777)																	12	1	13	0.24
Palaemon xiphias Risso, 1816	2				1			1											4	0.07
Periclimenes scriptus (Risso, 1822)		2															2*	3*	7	0.13

Continued on p. 68

Appendix A. (Continued)

Species	Samples																		
	7D1	7D2	7D3	7N1	7N2	7N 3	12D1	12D2	12D3	12N1	12N2	12N3	3D1	3D2	3N1	3N2	6D	6N	Total	%
Pontonia flavomaculata Heller, 1864																1			1	0.02
Scyllarus pygmaeus (Bate, 1888)			1	1*	1	1*				4	3	1			1	2			15	0.28
Calcinus tubularis (Linnaeus, 1767)		1													1	2			4	0.07
Anapagurus laevis (Bell, 1845)												1							1	0.02
Anapagurus petiti Dechancé & Forest, 1962		1				1				1*	1				1				5	0.09
Anapagurus sp.			1																1	0.02
Pagurus anachoretus Risso, 1827									2			2			4	9			17	0.31
Pagurus excavatus (Herbst, 1791)											1				2*				3	0.06
Pagurus prideaux Leach, 1815				1								1			4*	5*			11	0.20
Galathea intermedia Lilljeborg, 1851															2		1		3	0.06
Ilia nucleus (Linnaeus, 1758)						1													1	0.02
Achaeus gracilis O. G. Costa, 1839										1					1				2	0.04
Inachus communissimus Rizza, 1839															4*	3*			7	0.13
Pisa carinimana Miers, 1879													1	1	2*	1		5*	10	0.18
Pisa muscosa (Linnaeus, 1758)		1																1	2	0.04
Pisa tetraodon (Pennant, 1777)							1			1		2			1		1		6	0.11
Asthenognathus atlanticus Monod, 1933																		1	1	0.02
Pinnotheres pisum (Linnaeus, 1767)						1									1				2	0.04
Number of decapod individuals caught	33	60	65	285	211	313	263	283	197	433	301	286	158	137	436	415	378	1177	5431	
Average decapod abundance of samples (SD)	301.7 (251.6)																			

*Presence of ovigerous females; ***Macropodia czernjawskii* or *M. longirostris*. In sample notation 7: 13/07/1999; 12: 22/12/1999; 3: 29/03/2000; 6: 22/06/2000; D: day; N: night; 1, 2, 3: replicates

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The use of artificial benthic collectors for assessment of spatial patterns of settlement of megalopae of *Carcinus maenas* (L.) (Brachyura: Portunidae) in the lower Mira Estuary, Portugal

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Key words: megalopae, settlement, artificial collectors, spatial patterns, *Carcinus maenas*, Mira estuary

Abstract

Artificial benthic collectors have been widely used for the assessment of settlement rates of decapod crustaceans. However, to date no consistent works have addressed spatial patterns of settlement in different estuarine habitats, and no specific studies targeted the interaction of artificial surfaces with the surrounding natural substrate. It may be expected that the artificial surface may produce a different thigmotactic response when compared to the natural substrate, which may limit the use of this technique for assessment of natural settlement rates. In this study the settlement rates of megalopae of the estuarine crab *Carcinus maenas* were addressed, specifically deploying artificial benthic collectors in different habitats both intertidal and subtidal in the lower Mira estuary. A number of experiments were performed concerning stratification and temporal fluctuations of settlement. Further, the interaction of collector surface with the surrounding substrate was investigated, by comparing settlement rates in natural and artificial substrates in different habitats. Results have shown significant differences in settlement between different estuarine habitats, both in spatially replicated experiments and in a high-resolution temporal experiment. However, comparison between settlement rates in artificial and natural substrates has shown that there is a strong interference between collectors and surrounding substrate, limiting interpretation of results concerning settlement rates in artificial substrate alone.

Introduction

Most estuarine brachyuran crabs have a pelagic larval phase, which is exported to shelf waters. When the megalopa stage is reached, the animals face the problem of returning to inshore waters and find appropriate habitats to settle (Shanks, 1998). The megalopae penetrate in estuaries and accomplish landwards movement by selective tidal stream transport (Shanks, 1995; Forward et al., 1997; Queiroga, 1998), which is a complex interaction between behaviour (mainly cyclic shifts in vertical position) and physical mechanisms (the tidal cycle and baroclinic water mass movements). Settlement is viewed as the transition process between the pelagic and benthic phases, and occurs as megalopae reach adequate habitats (Rodríguez et al., 1993; Hunt & Scheibling, 1997). According to Rodríguez et al. (1993) the process of settlement in marine invertebrates involves two separate steps: 1) an active behavioural search of adequate substrate, and 2) residence or fixation to the substrate which triggers the juvenile moult. The acquisition of a capacity of thigmotactic response is generally considered as megalopal competency (Goodrich et al., 1989; Jensen, 1991; Rodríguez et al., 1993). Megalopae competent for settling present a remarkable plasticity in advancing or delaying their moult in the face of the presence or absence of given stimuli, such as conspecifics, predators or various structural and chemical characteristics of the substrate (e.g. O'Connor, 1993; Strasser & Felder, 1999). Megalopae thus show a strong adaptation to actively search and choose an adequate habitat for the juvenile phase (Sulkin & van Heukelem, 1986; Wolcott & DeVries, 1994).

Concerning the types of settlement stimuli, visual, tactic and chemical cues have been actually verified or hypothesised (see below). Other factors such as microscale current patterns may modulate megalopal settlement response, as passive sinking is not viewed as a major cause (Hedvall et al., 1998). Instead, it seems that active selection by megalopae is the major factor influencing early juvenile distribution, at least in intertidal crabs (e.g. Paula et al., 2001, 2003). Megalopae of *Callinectes sapidus* Rathbun, 1896 react negatively to predator odour and positively to seagrass environments (Welch et al., 1997). Salinity (Wolcott & DeVries, 1994) and other estuarine waterborne cues (Wolcott & DeVries, 1994; Forward et al., 1997) have also been suggested as guides to recruiting megalopae. Other authors showed that megalopae of several species prefer structurally complex substrates, which provide shelter (e.g. O'Connor, 1993; Eggleston & Armstrong, 1995; Hedvall et al., 1998; Moksnes et al., 1998; Stevens & Kittaka, 1998). These substrates include vegetation (Boström & Bonsdorff, 1997) and shelly gravel (Welch et al., 1997). The presence of conspecifics may enhance settlement in *Uca* spp. (O'Connor, 1993), and several authors referred a number of substrate stimuli capable to accelerate the moult to the first juvenile stage (e.g. O'Connor, 1991; Wolcott & DeVries, 1994; Gebauer et al., 1998; Strasser & Felder, 1999).

Artificial collectors are common tools for studying settlement rates of decapod crustaceans, such as 'hog's hair' filter surfaces (e.g. van Montfrans et al., 1995; Paula et al., 2001) and artificial seaweeds (Phillips, 1972; Phillips & Booth, 1994). These devices intend to simulate the adequate typical settlement grounds (facilitating collection) or to enhance settlement by inducing a positive thigmotactic response as providing refuge. Floating cylindrical artificial collectors have been widely used, covered by a 'hog's hair' filter surface (see van Montfrans et al., 1990 for general methods), especially addressing settlement rates of the portunid blue crab *C. sapidus* (eg Goodrich et al., 1989; van Montfrans et al., 1990; Boylan & Wenner, 1993; Wrona et al., 1995; Hasek & Rabalais, 2001), but also in African mangroves (Paula et al., 2001). The question whether settlement rates thereby obtained actually reflect effective settlement in appropriate areas was not specifically addressed in any of the referred studies. Alternatively, it may be conceived that those settlement rates are a function of megalopal availability in the water mass, being the presence of megalopae on the collectors' surface a temporary event. However we may expect that they do reflect rates of immigration of megalopae from shelf waters into the estuary. Nevertheless megalopal competency to settle may not be achieved by reaching estuarine waters, as suggested by Paula et al. (2001). Unpublished own results indicate that a delay between immigration of megalopae and effective settlement within estuarine benthic habitats does take place in *Carcinus maneas* (Linnaeus, 1758).

In most cases, collection of megalopae of benthic invertebrates in their natural settling grounds is a difficult or impossible task, due to the small size of the animals and their tendency to prefer structured bottoms that provide refuge. Paula et al. (2003) used unfolded 'hog's hair' surfaces to study benthic settlement of brachyuran crabs in a tropical mangrove, obtaining clear temporal and spatial patterns over different mangrove areas. However, collector efficiency in the different areas was not studied. Flores et al. (2002) used nets under boulders for studying settlement rates of the rocky intertidal crabs *Pachygrapsus marmoratus* (Fabricius, 1787) and *Xantho incisus* (Leach, 1814). In this case the natural boulder substrate was used as collecting surface and thus interference with collection method was considered minimal. The possibility of using a natural substrate for collection is however limited to particular environments. Also, the rates of settlement in natural substrates are often low, and less effort is made if suitable surfaces enhancing settlement are used. Settlement intensity in artificial surfaces will not necessarily reflect actual rates in the natural substrate, as its physical nature may in fact decrease or enhance preference by settling megalopae, and thus modulate their thigmotactic response. If megalopae are selective for types of structure or complexity of natural surfaces, then deployment of structured artificial collectors on unstructured bottoms should enhance settlement rates, as widely observed in artificial hard reefs deployed in soft bottoms. It is thus very likely that artificial surfaces hardly reflect natural absolute rates of settlement within an area, and its interference with varying background environment should make spatial comparisons very doubtful.

The main objective of this research was to assess spatial patterns of settlement of megalopae of the crab *C. maenas* in different estuarine habitats, both intertidal and subtidal, at the lower section of the Mira Estuary, south-western Portugal. It was further investigated whether settlement rates on artificial collectors are influenced by the interaction between artificial surfaces and the complexity of the surrounding substrate.

Carcinus maenas is the most common crab in European estuaries, widely distributed in intertidal and subtidal shallow environments. The life cycle of the species comprises an exported planktonic larval phase, which is rapidly flushed out to shelf waters by ebbing currents after release (Queiroga et al., 1994; Queiroga, 1996). Megalopae migrate back to the estuary during spring tides, and transport is accomplished by selective tidal stream transport (Berrill, 1982; Queiroga, 1998; Hedvall et al., 1998; Moksnes et al., 1998). According to Hedvall et al. (1998) and Moksnes et al. (1998) there are indications that opportunistic behaviour is expressed already at settlement, as several different habitats may provide appropriate settling grounds.

Materials and methods

Study area

The Mira Estuary is located in the south-western Portuguese mainland coast (approximately between 37° 40′ N and 8° 45′ W), extending for a length of around 40 km from the mouth to the limit of tidal influence (see Fig. 1). Tides are semi-diurnal, with

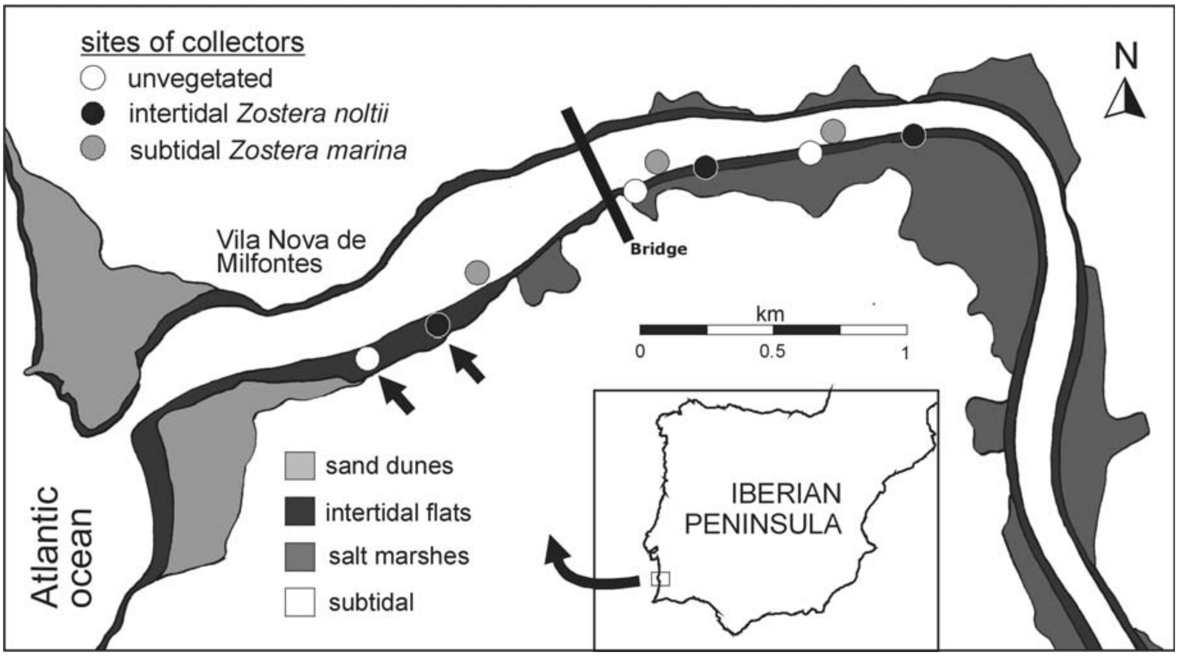

Figure 1. Map of lower Mira Estuary showing sites of collecting of *Carcinus maenas* megalopae. Arrows indicate sites used for temporal series and comparison between natural and artificial substrates.

an amplitude of around 1 m during neap tides and 3 m during spring tides. Tidal penetration varies between around 7.5 km and 2.5 km during respectively average spring and neap tides (Paula, 1998). There is a relatively strong stratification of the water column during the neap tide period, and an almost complete vertical homogenisation during spring tides due to turbulence (Paula, 1998; Blanton & Andrade, 2001).

The lower section of the estuary has a maximum of 400 m width and a maximum depth of between 5 and 10 m. Due to low riverine input, this part of the estuary has a dominant marine character. The intertidal zone is dominated by areas with varying density cover of the seagrass *Zostera noltii* Hornemann, 1832 and bare sandy muddy substrates. Near to *Z. noltii* habitats, the shallow subtidal zone is dominated by dense cover of the seagrass *Zostera marina* Linnaeus, 1752.

Field methods

Stratified spatial settlement

The experiment to study the rates of settlement on different estuarine habitats was designed with a nested approach, using as habitats (treatments) intertidal *Z. noltii*, intertidal bare substrate and subtidal *Z. marina*. For each habitat, three different random areas were selected, each with three randomly deployed replicates in each treatment. The experiment was repeated in two different random starting dates, both during neap-tide 5-d periods (as this was the observed period of maximum settlement, own unpublished data) within the breeding period of *C. maenas*. The measure of settlement was the daily number of settled megalopae in individual collectors. Twenty-seven collectors were deployed each day.

The collectors were made of ‘hog’s hair’ filter, with a dimension of 50×40 cm and 2.5 cm thick, attached to a metal frame and deployed flat on the substrate (see Fig. 2). Preliminary observations have shown that the weight of the metal frame was enough to prevent any movement of the collectors with the currents. Also the coverage of the collector surface by sand, seagrass, algae or debris was found neglectable, and initial intention of using wide mesh boxes to prevent this was abandoned. For the subtidal zone, an additional fine mesh was collocated underneath the ‘hog’s hair’ surface, to avoid loss when lifting collectors for sampling; also, these three replicate collectors were

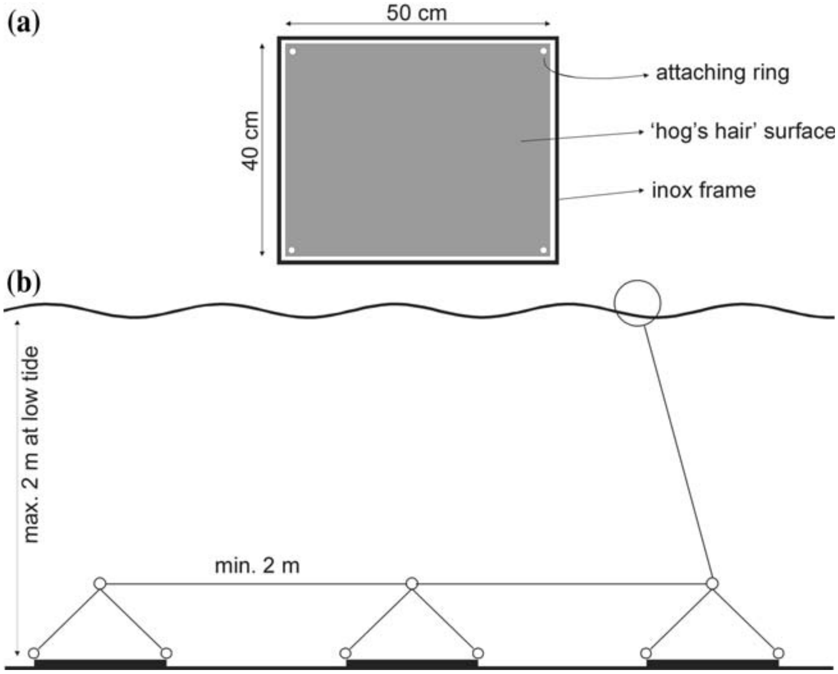

Figure 2. Diagrammatic top view of collector (a) and of scheme of deployment of subtidal series of collectors (b, not to scale).

attached to each other with a 2-m rope between collectors and a terminal buoy. The distance between collectors was set so that when lifting a collector the next one would remain undisturbed at the bottom.

Collectors were changed each day, during the diurnal low tide, thus remaining in the field during two complete tidal cycles. Collectors were collocated in plastic bags and transported rapidly to the laboratory for immediate processing.

Temporal comparison

Previously referred intertidal habitats were then used for a longer temporal comparison, by randomly deploying four replicate collectors in *Z. noltii* meadows and unvegetated substrate. The experiment lasted for 2 months between 22 March and 22 May 2002. General field procedures were similar to those described in the previous experiment, and sampling was carried out on a daily basis at diurnal low tide.

Artificial collectors versus natural substrate

A specific experiment was performed in order to test whether collector efficiency is comparable to rates of settlement in the natural surrounding substrate. In this experiment, two different intertidal substrates were chosen: unvegetated sandy mud and seagrass *Z. noltii* meadow. In both substrates, four random replicate collectors were deployed. In addition, four sampling plots of equivalent size were delimited on natural substrate, not defaunated, in which megalopae were sorted out from the 2-cm upper layer. In this case, bare sand was sieved in the field with a 0.5 mm mesh sieve and seagrass cover was detached from within the plots. All this material was placed in labelled plastic bags and preserved in 4% neutralised formalin until sorting. The experiment was repeated during four dates, coinciding with peak settlement periods.

Laboratory procedure

Megalopae were removed from the collectors' surface by rinsing in abundant freshwater. Detached material, included all debris, were immediately screened under a binocular microscope. Megalopae of *C. maenas* from collectors and sediment samples were preserved in 4% neutralised formalin, and identified using the descriptions by Rice & Ingle (1975) and Ingle (1992).

Data treatment

Balanced analyses could not be performed in all cases because collectors were eventually lost in the stratified experiment. Replicates from different sites were pooled for increased robustness of the analysis, but the spatial scales used (replication within and between sites) incorporated the possible variability at different spatial scales. In addition, Bartlett's, Shapiro-Wilk and Cochran tests (Zar, 1996) indicated heteroscedasticity of data in all analyses and thus non-parametric tests, either the Kruskal–Wallis or the Mann–Whitney procedure, were conducted (Sokal & Rohlf, 1995). In case of significant differences between groups of data, the Dunn's test was used to identify between-group contrasts.

Results

Stratified spatial settlement

On average, 23 megalopae collector^{-1} day^{-1} settled during the first period, while during the second period the mean was 31 (Fig. 3). This difference was found to be significant ($H = 10.985$, $p = 0.0009$), showing temporal instability of megalopal supply. The mean number of megalopae collected at the intertidal unvegetated habitat was higher than in the other vegetated substrates during both sampling periods, with settlement rates being estimated at 39 and 62 megalopae collector^{-1} day^{-1}, respectively. At the intertidal *Zostera noltii* meadow the means were 16 and 10 respectively, while at the subtidal *Z. marina* meadow the means were 8 and 22 respectively. Settlement on the unvegetated areas was significantly higher than on the other areas ($H = 58.6905$, $p < 0.001$), and no statistical differences were found between intertidal and subtidal seagrass meadows. The sampling design used three replicate zones within the lower Mira estuary (see Fig. 1 and Materials and methods section). Comparison of settlement between these areas has shown no statistical differences ($H = 0.4313$, $p = 0.806$).

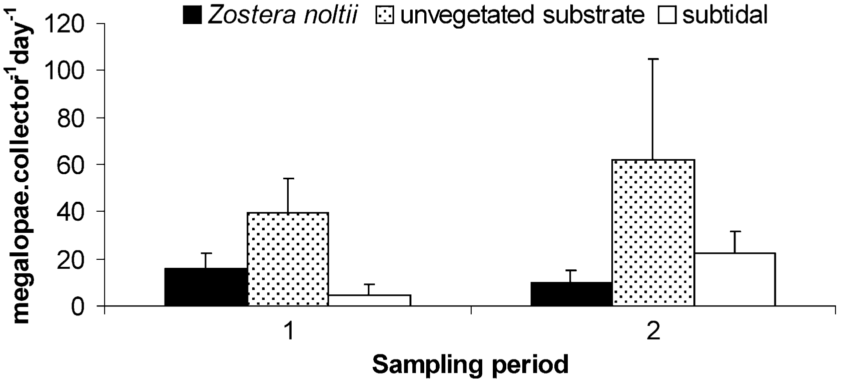

Figure 3. Mean number of collected megalopae of *Carcinus maenas* in the three different bottom types and during both sampling periods for stratified settlement. Error bars = SE.

Temporal comparison

As in the previous study, the collectors deployed on the unvegetated substrate presented higher settlement rates, although significant only during discrete semi-lunar peaks of settlement intensity during neap tides (Fig. 4). Maximum mean daily values were around 126 megalopae collector^{-1} day^{-1} in unvegetated substrate and 25 within the *Z. noltii* meadow. A comparison of the means of both series has revealed a significant difference (Mann–Whitney $U = 1.340$, $p < 0.05$).

Artificial collectors versus natural substrate

Significant differences were found in the unvegetated substrate between artificial and natural substrates ($H = 26.5945$, $p < 0.001$, mean settlement values 36 and 4, respectively), and between artificial collectors at both habitats (Fig. 5). Settlement contrasts between artificial and natural substrates within the *Z. noltii* were not significant (7 and 3 megalopae collector^{-1} day^{-1}, respectively).

Discussion

The present results suggest that *C. maenas* megalopae settle preferentially on collectors deployed in unvegetated (unstructured) substrates, when compared to those deployed in seagrass meadows. These results apparently contradict data on settlement of *C. sapidus* (Olmi et al., 1990). Furthermore, Welch et al. (1997) found that megalopae are attracted by chemical cues of seagrasses, and Forward et al. (1997) have shown that such cues induce a reduction of the duration for moulting in megalopae of *C. sapidus*. Seagrass

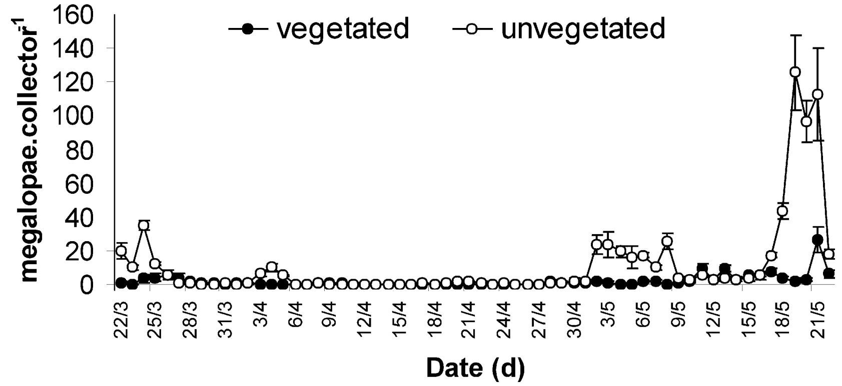

Figure 4. Temporal fluctuation of *Carcinus maenas* megalopal settlement in artificial collectors deployed at intertidal *Zostera noltii* meadow and unvegetated bottom. Error bars = SE.

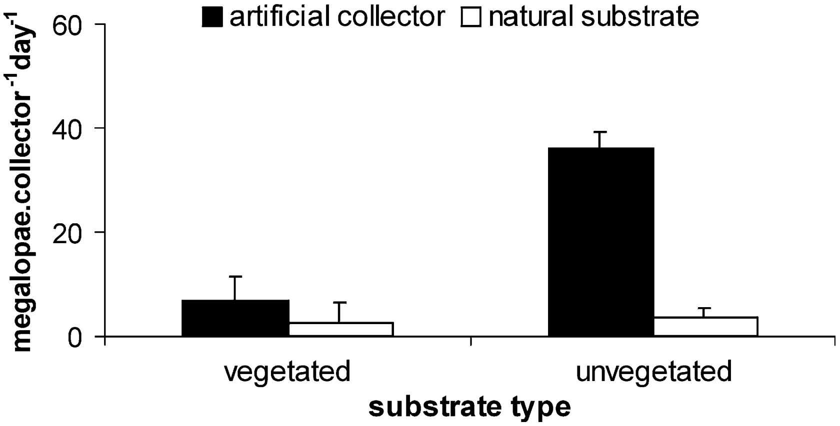

Figure 5. Comparison of settlement of megalopae of *Carcinus maenas* in artificial collectors and natural substrate deployed at intertidal *Zostera noltii* meadow and unvegetated bottom. Error bars = SE.

meadows provide structural and chemical cues which may enhance settlement, such as complexity, food, organic content, lower current speed, higher refuge and stability (Boström & Bonsdorff, 1997). Hedvall et al. (1998) showed that megalopae of *C. maenas* avoid unstructured substrates such as sand. In these types of substrate the predation rates are very high, reaching, according to Moksnes et al. (1998), more than 80% in 2 days. The synthetic fibbers of the collector surface provide shelter to prevent predation and trap organic matter that may further enhance settlement. On the other hand, the collectors also attract juveniles, which are otherwise dispersed over the unvegetated substrate of the tidal flat. Since megalopae may be attracted by conspecifics, the presence of juveniles in the collectors may further enhance settlement of megalopae (O'Connor, 1993).

Previous studies using 'hog's hair' coated collectors (e.g. Goodrich et al., 1989; van Montfrans et al., 1990; Boylan & Wenner, 1993; Wrona et al., 1995; Hasek & Rabalais, 2001) suggest that this type of sampling reflect natural settlement rates and respective temporal intensity patterns. Strictly on a temporal basis it may be true, but when spatial patterns are concerned, the present results make this statement very doubtful. These authors used floating collectors deployed in the water column and not in appropriate settling grounds; whether collected megalopae were competent to recognise appropriate natural settlement cues remains to be assessed.

The validity of the comparison between the rates of settlement obtained in natural and artificial substrates remains also to be clarified. A number of confounding effects may operate, which suggest lines for future investigation. The fact that the natural substrate was not defaunated prior to experiment could have biased the numbers obtained in the third experiment, namely involving post-settlement processes of longer period than the 24-h of artificial collector deployment. However, a few studies describe a rapid moult to first crab instar after settlement (e.g. Paula et al., 2003), especially in the presence of adequate habitat or cues. It is thus most probable that megalopae collected in natural substrate are from settlement during the previous 24 h. Other studies demonstrate that megalopae of *C. maenas* may utilize the artificial substrates temporarily, and in fact that most will show a high rate of emigration even if close to moult (Moksnes & Wennhage, 2001). However this last study used floating collectors, which more likely will collect non-competent megalopae that may exhibit thigmotactic behaviour in the water mass. Further studies are required to elucidate this issue.

In summary, the relative efficiency of artificial collectors clearly depends on the nature of the surrounding habitat. This study strongly suggests that artificial surfaces should be used with caution, at least when deployed in the benthos, and specially when any sort of spatial variability is expected to be studied.

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Feeding habits of *Percnon gibbesi* (H. Milne Edwards, 1853) in the Sicily Strait

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Key words: *Percnon gibbesi*, Grapsidae, alien species, Mediterranean

Abstract

The subtropical grapsid crab *Percnon gibbesi* (H. Milne Edwards, 1853) is one of the most recent alien decapods found in the Mediterranean, where it was discovered at Linosa (Pelagie Islands, Sicily Strait) in summer 1999. At present, the invasion of this species has been recorded in several other insular localities. We studied the feeding habits of the crab in an attempt to understand its success in the Mediterranean. The morphological characteristics of the chela, the feeding adaptations of the gastric mill and the analysis of stomach contents indicate that *P. gibbesi* is a strictly herbivorous species, a characteristic not shared with any other large-sized infralittoral Mediterranean crab.

Introduction

The subtropical grapsid crab *Percnon gibbesi* (H. Milne Edwards, 1853) is one of the most recent alien decapods crustacean found in the Mediterranean. It was discovered in summer 1999, at Linosa (Pelagie Islands, Sicily Strait), where the observation of several individuals in three different localities suggested that the presence of the crab had the characteristics of an invasion (Relini et al., 2000). *P. gibbesi* was almost simultaneously observed at the Balearic Islands: at Ibiza (Müller, 2001) and at Mallorca, Menorca and Formentera (Garcia & Reviriego, 2000). Shortly after it was also found at Lampedusa (E. Azzurro, pers. obs.), Malta (M. Camilleri pers. com.), Pantelleria and along the coast of the Sicily (Pipitone et al., 2001).

Investigations are in progress in an attempt to understand which biological characteristics can explain its success in invading the Mediterranean (Puccio et al., 2003): this note presents a preliminary study of feeding habits of this crab, through the analysis of stomach contents.

Materials and methods

Sampling was carried out at Linosa and Lampedusa (Pelagie Islands), two small islands located in the centre of the Sicily Strait, from August to November 2000 and from July to August 2002. Crabs were randomly caught at the uppermost sublittoral levels, where they live on a rocky substrate abounding in holes and fissures. Nineteen specimens were fixed in 70% alcohol and brought to the laboratory, where the carapace length (CL) was recorded.

The stomach of each crab was removed and opened; its content was put in a Petri dish, cleaned from traces of crab tissues, weighed (SCW) and finally analysed; algal and animal items were separated and each remain was identified to the lowest possible taxonomic level. Some of the most interesting stomach contents were photographed using an image analyser system (Leica DC software). In order to ascertain the alimentary adaptation of *P. gibbesi*, the pieces of the gastric mill were extracted; zygocardiac and urocardiac ossicles were observed under a dissecting stereomicroscope and compared with the morphological categories described by Warner (1977). They were then photographed using the same image analyser system as above.

Results and discussion

Table 1 shows, for the 10 female (F) and 9 male (M) eviscerated specimens, the carapace length range, the stomach content weight range and its composition in algal, animal and abiotic fraction expressed by the frequency of occurrence of each food item.

Analysis of stomach contents

Algae were the most common food item (71.6%); considering the ingested biomass, algae reached 99%. Non-algal items included single foraminiferans, very small skeletal fragments of bryozoans and hydrozoans, very small copepods (three cases), gastropods (two cases) and decapods (one case); considering their very scarce biomass, the animal food seems not to be chosen but occasionally ingested.

Following the classification proposed by Steneck & Watling (1982), four functional groups were identified in the algal portion of stomach contents (see Table 1): filamentous algae, calcareous algae and corticated macrophytes were represented by almost equal parts in biomass, while foliose algae by a smaller amount.

Inside functional groups such as calcareous algae and corticated macrophytes, articulated Corallinaceae and Sphacelariaceae, respectively, (Fig. 1) represented the main fractions. The higher variety of filamentous algae is likely due to their tendency to create thick felts, from which the crab easily gets a polyspecific meal.

The relationship between the individual size of a crab and the amount of food ingested is shown in Figure 2. The linear trend of the CL–SCW relationship, with $r = 0.847$, indicates that most of the sampled specimens had a full-stomach, suggesting the great food availability for *P. gibbesi* in its new habitat and, at the same time, its great fitness in feeding.

Morphofunctional characteristics related to feeding

As regards feeding adaptation of chelae, a distinction must to be drawn between algivorous crabs and those, which eat vascular plants. Mediterranean algivorous grapsids are characterized by the possession of spoon-tipped chelae which scrape algae from rocks and deliver to the mouth a mix of algae, rocky fragments and a greater or lesser proportion of detritus, sponges, hydroids and bryozoans; in vascular plant eaters, having a much tougher material to cope with, chela tips tend to be sharp since they are used to tear off pieces of vegetation rather than simply to scrape (Warner, 1977). The chelae of *P. gibbesi* present an external straight cutting edge, therefore, resembling to those of vascular plant eaters; as a result, they are fitted out to break up tough vegetal food, too.

According to the morphological categories indicated by Warner (1977), the zygocardiac and urocardiac ossicles of *P. gibbesi* gastric mill present intermediate characteristics between algivore and vascular plant-eater type; therefore, in any case, they conform to grazer and not predator features (see Figure 3).

Living in the upper infralittoral levels, *P. gibbesi* probably hardly experiences shortage of food, thanks to the local abundance of algal covering and to the scarcity of competitors. In fact, in these levels only few other crab species occur, such as some majids and xanthids, most of which are not strictly herbivorous or they are small-sized (Zariquiey Alvarez, 1968). Nevertheless, *P. gibbesi* is increasing its population inside the geographical area of *Pachygrapsus marmoratus* (Fabricius), another grapsid that presents an algal input in its diet (Cannicci et al., 2002). However, the latter species occupies the intertidal belt, while *P. gibbesi* has not been observed in the emerged shore yet. Furthermore, *P. marmoratus* is considered not an algivorous but an omnivorous-not opportunistic species; it can select its food, getting an equal intake of algal and animal items, irrespectively to the community composition of the substrate (Cannicci et al., 2002). Considering size, the infralittoral distribution and partially the feeding habits, *P. gibbesi* could also find a competitor in *Maja crispata* (Risso, 1827). Nevertheless, the latter feeds

Table 1. Percnon gibbesi: analysis of stomach contents

		Frequency of occurrence		
Sex and numbers		F (10 ind.)	M (9 ind.)	TOT.
CL range (mm)		16–34	18.5–32	
Stomach content weight SCW (g) range		0.04–0.24	0–0.34	2.76
Calcareous algae	*Jania rubens*	4	6	10
	Amphiroa sp.	1	1	2
	Haliptilon sp.	1	1	2
	Hydrolithon farinosum	0	2	2
	n.i. articulated Corallinaceae	1	2	3
	n.i. not articulated Corallinaceae	4	3	7
Filamentous algae	*Ceramium flaccidum*	1	0	1
	Ceramium echionotum	1	0	1
	Ceramium sp.	1	0	1
	Polysiphonia atlantica	0	1	1
	Polysiphonia elongata	0	1	1
	Polysiphonia sp.	2	5	7
	Herposiphonia secunda	1	3	4
	n.i. Ceramiales	0	1	1
	Audouinella sp.	0	1	1
	Ulothrix sp.	1	2	3
	Chaetomorpha linum	2	1	3
	Cladophora sp.	1	0	1
	Bryopsis sp.	1	0	1
Corticated macrophytes	*Gelidium spinosum*	1	1	2
	Gelidium sp.	0	1	1
	n.i. Gelidiales	0	1	1
	Gigartina acicularis	2	0	2
	Stypocaulon scoparium	0	3	3
	Sphacelaria cirrosa	0	1	1
	n.i. Sphacelariaceae	6	7	13
Foliose algae	n.i. foliose Rhodophyta	1	0	1
	Dictyota linearis	1	0	1
	Dictyota sp.	0	1	1
Foraminifera	*Elphidium crispum*	5	5	10
	Planorbulina sp.	1	2	3
Bryozoa	*Sertella* sp.	4	3	7
Cnidaria	Chitinous filaments Hydroidea	3	0	3
Gastropoda	n.i. Gastropoda	1	1	2
Crustacea	Copepoda	2	1	3
	Decapoda	0	1	1
	Eggs	2	0	2
Other	Indistinct material	2	0	2
	Sand grains	6	4	10

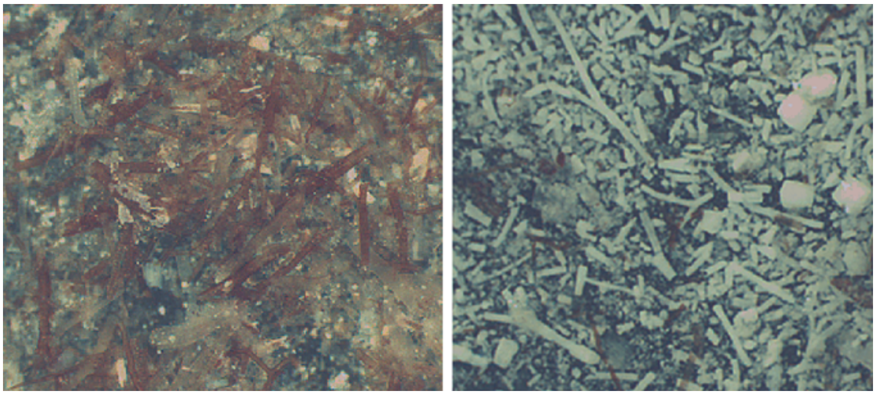

Figure 1. Two different types of meals in *P. gibbesi*: one with a prevalence of Sphacelariaceae (left); the other based on Corallinaceae (right).

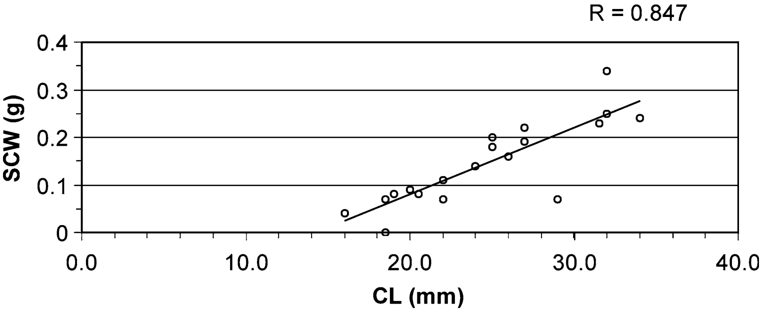

Figure 2. Relationship between individual size, CL (mm) and stomach contents weight, SCW (g).

mainly on animal organisms, including Echinoidea, Polyplacophora, Crustacea Decapoda, Bivalvia, Ophiuroidea, fishes, Gastropoda, Polychaeta, Porifera, Bryozoa, Hydroidea, Foraminifera and Amphipoda (Števčić, 1985).

Conclusions

The morphology of chelae, the feeding adaptations of the gastric mill and the results of the analysis of stomach contents indicate that *P. gibbesi* is a strictly herbivorous species, able to take both soft and tough algal meals. This characteristic, confirmed by the finding of a very coriaceous algal fraction in its stomach, may represent another reason of its success, since this crab apparently exploits food that other herbivorous decapods can not.

The alimentary preferences of *P. gibbesi* suggest that further studies on the ecological position of this new infralittoral herbivore should be carried out. Given its clearly algivorous feeding habits, it would be interesting to evaluate the possibility of competition with the sea urchins *Paracentrotus lividus* (Lamarck) and *Arbacia lixula* (Linnaeus), two of the most important Mediterranean grazers that are able to limit algal abundance in the upper infralittoral levels (Verlaque & Nedelec, 1983; Verlaque, 1984; Benedetti-Cecchi et al., 1998).

The feeding competitiveness, in addition to the exceptionally large megalopa that produces a robust first crab (Paula & Hartnoll, 1989; Hartnoll, 1992) and the high fecundity (Puccio et al., In Press) can explain – at least in part – the rapid spreading of this invader.

Figure 3. Gastric mill pieces: zygocardiac ossicle (left) and urocardiac ossicle (right).

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The decapod crustacean community of the Guadalquivir Estuary (SW Spain): seasonal and inter-year changes in community structure

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Key words: decapod crustacean, community structure, Guadalquivir Estuary

Abstract

Monthly samples were taken from May 1997 to March 2002 at three sampling sites within the last 32 km of the Guadalquivir Estuary. Twenty-four decapod crustacean species were recorded of which *Crangon crangon* (Linnaeus), *Melicertus kerathurus* (Forskal), and *Palaemon* spp. represented 99% of all collected individuals. These three dominant species showed a similar seasonal density pattern even though peaks in *M. kerathurus* were lower. Their densities were positively correlated ($p < 0.01$) with water temperature and salinity, but negatively with turbidity. The highest correlation corresponded to temperature in *Palaemon* spp. and to salinity in *C. crangon* and *M. kerathurus*. Therefore, the total estuarine decapod density also showed a regular seasonal pattern having the lowest figures in late autumn and winter and the highest in spring and summer. In addition, it was positively correlated with water temperature and salinity, but negatively with turbidity. Density decreased upstream, mainly due to the higher density of *C. crangon* and *M. kerathurus* in more saline waters. Non-metric multi-dimensional scaling ordination of samples also indicated a regular seasonal change in the community, even though inter-year differences between dry and rainy winters were especially great. The first ordination axis was significantly correlated with environmental variables, while the second axis seemed to split samples up following seasonal community changes in species' composition and dominance.

Introduction

Estuaries are highly productive ecosystems that provide permanent habitats for estuarine species as well as play an important role as breeding and adult-feeding grounds for marine species (Sorbe, 1980; Marchand, 1981; Kuipers & Dapper, 1984). As the physicochemical conditions of these ecosystems are highly variable, environmental factors are among the main forces that structure these communities. In particular, water temperature and salinity are among the most significant control factors in temperate estuaries. Both variables follow a similar seasonal pattern in most temperate European estuaries having maximum values in summer and minimum in winter (Cattrijsse et al., 1994; Azeiteiro & Marques, 1999). As a result, it is very difficult to determine the separate individual contribution of each factor to the observed seasonal community changes. Furthermore, due to the fluctuating estuarine environmental conditions from one year to another, a long-term time series of biological data is necessary in order to have a good understanding of temporal changes within these communities (Atrill et al., 1999; Jackson & Jones, 1999).

Decapod crustaceans are known to play a critical role in metabolizing and controlling the flow of energy in estuarine ecosystems. Even though the decapod species are conspicuous inhabitants of European temperate estuaries and have a great economic impact on them, few comprehensive studies that include the evaluation of abundance and biomass (Cattrijsse et al., 1994) have been done on the decapod community structure in these estuaries.

The Guadalquivir Estuary is a temperate well-mixed estuarine system, which has a gradual salinity gradient (Vannéy, 1970). Nevertheless, the freshwater input into the estuary is controlled at a dam located 110 km upstream. For this reason the seasonal variation of estuarine salinity in dry years, when water temperature and salinity maximums do not coincide, is very different from the expected natural patterns. This 'peculiarity' makes it possible to distinguish the effects of these two factors by comparing seasonal community changes in rainy years with those occurring in dry years. The estuary's aquatic macrofauna shows considerable seasonal and annual changes in which decapod crustaceans represent anywhere from 4 to 11% of its biomass (Drake et al., 2002). This study, which is part of a long-term effort to study the Guadalquivir Estuary aquatic macrofauna, focuses on temporal changes of the estuarine decapod crustacean community during five annual cycles.

Materials and methods

Study area

The Guadalquivir Estuary (SW Spain: 36°47′–37°25′ N, 4°58′–7°00′ W) is a non-stratified system in which a gradual longitudinal change in water salinity occurs (Vannéy, 1970). The tidal influence reaches inland up to approximately 110 km from the river mouth (maximum amplitude < 3.5 m). The location of the estuarine mixing zone changes, both seasonally and annually, from high tide to low, but sea water flow penetrates inland, on average, up to about 32 km upstream. The sampling sites, Tarfía (Ta), La Esparraguera (Es) and Bonanza (Bo), were located at 32, 20 and 8 km inland, respectively, from the river mouth (Fig. 1).

Sampling protocols

Monthly, at each new phase of the moon, samplings were conducted from May 1997 to March 2002 from an anchored boat equipped with on-board fishing gear and a 10-m net with an opening area of 7×3 m and a 1 mm mesh. At each sampling, four samples were taken during a 24-h period at each site. Each sample consisted of an approximately 120-min passive haul. Samples were taken in synchronization with tidal phases within the first 2 h of diurnal and nocturnal high and low tides at depths generally ranging from 3 to 6 m. The total catch was discharged into a calibrated container where its volume was estimated. A maximum of 13 l of the collected material, or less in smaller catches, was preserved in 10% formaldehyde. During sampling, water current velocity was measured with a digital flow-meter placed near the nets. Water temperature and salinity were measured at the beginning of each sampling. In the laboratory, individual decapod crustaceans were sorted out and separated from the other aquatic macrofauna, classified by species, counted and weighed.

Data analysis

Community structure was analyzed based on the total number of different species and total density or number of individuals per 100 m^3. Constancy (*C*) of each species was estimated as the percentage of months in which a species was collected at each sampling site. Due to elevated estuary hydrodynamism whose horizontal tidal displacement was roughly the equivalent of the distance between sites, and to the mobility of the studied species, monthly samples from the three stations were combined to get a single monthly estimate in order to analyze the temporal pattern of the community. In the three most abundant species, temporal changes in density, size and weight of individual samples were also analyzed.

A multivariate approach to the analysis of temporal (seasonal and inter-annual) differences in the community structure was followed using the PRIMER (Plymouth Routines in Multivariate Ecological Research) computer software pack. Following the recommendation of Clarke & Warwick (1994), rare species were removed from the data matrix prior to analysis. Such species were those present in less than 10% of the months at all three sampling sites (Table 1). Multivariate data analysis was carried out by non-metric multidimensional scaling (MDS) ordination with the Bray-Curtis similarity measurement calculated

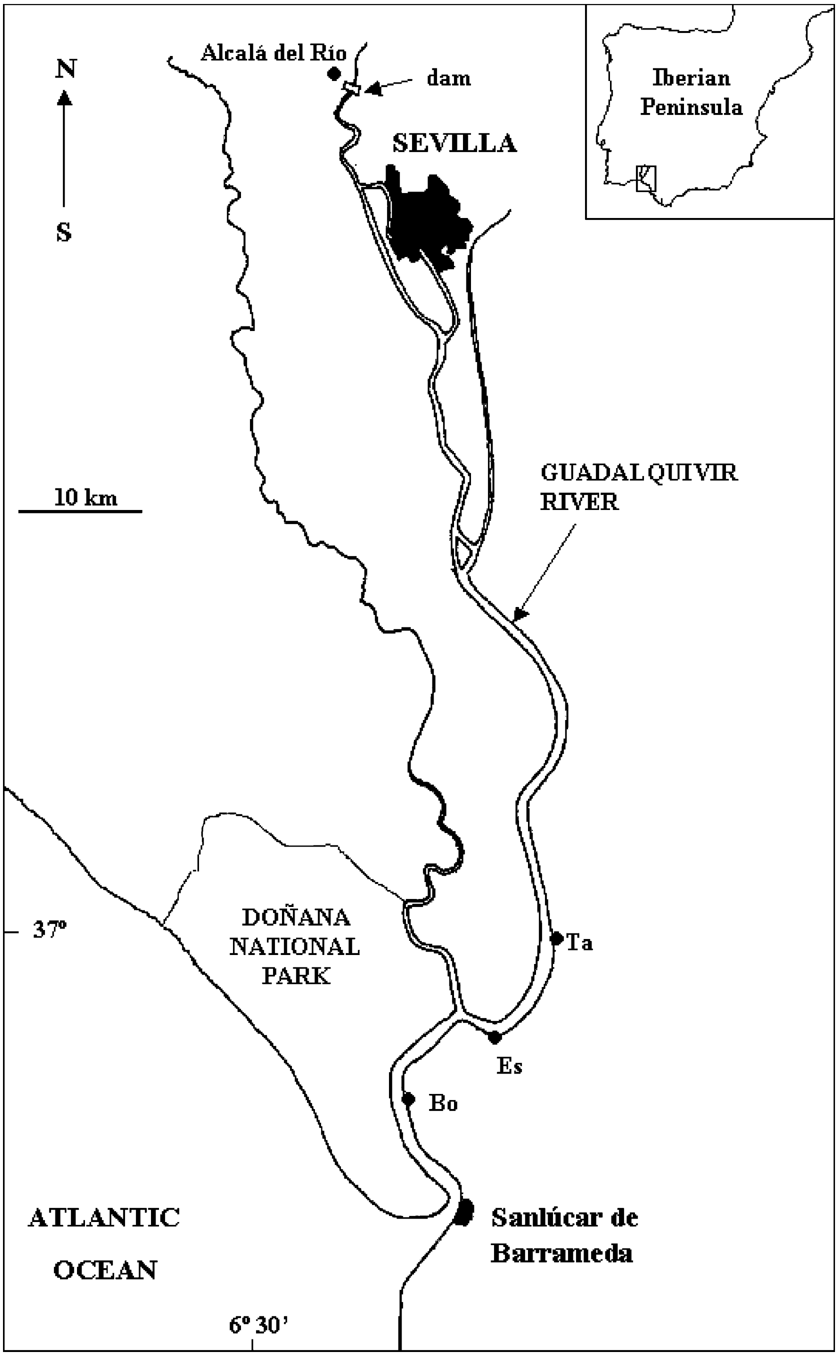

Figure 1. Map of the Guadalquivir Estuary (Southwest Spain) showing the location of the three sampling sites: (Ta) Tarfia, (Es) Esparraguera, (Bo) Bonanza.

on root–root transformed abundance data (Clarke, 1993). This method has proved to be a useful tool in the detection of possible community structure changes (Clarke & Warwick, 1994). The Spearman rank coefficient was used to estimate the relation between the two first axes of the MDS ordination and environmental variable outcomes and that between densities of the most abundant

Table 1. Average density for decapod crustacean species (families) collected at the Guadalquivir Estuary and constancy (*C*) of each species at the different sampling sites

Species	Density	Constancy		
	No Ind./10^5 m^3	Ta	Es	Bo
*Crangon crangon** (Crangonidae)	10,489.18	66.7	88.3	93.3
Palaemon spp.* (Palaemonidae)	4345.81	100.0	100.0	98.3
*Melicertus kerathurus** (Penaeidae)	693.04	18.3	33.3	55.0
*Palaemon serratus** (Palaemonidae)	89.60	23.3	33.3	51.7
*Palaemonetes varians** (Palaemonidae)	64.98	96.7	90.0	43.3
Processa sp.* (Processidae)	22.98	0.0	0.0	10.0
*Liocarcinus vernalis** (Portunidae)	20.41	8.3	8.3	40.0
Lysmata sp. (Hyppolytidae)	6.59	0.0	0.0	3.3
*Pachygrapsus marmoratus** (Grapsidae)	6.30	1.7	5.0	13.3
*Carcinus maenas** (Portunidae)	5.73	0.0	1.7	16.6
*Palaemon adspersus** (Palaemonidae)	3.31	5.0	15.0	26.7
*Ogyrides rarispina** (Ogyridae)	3.02	0.0	0.0	10.0
Upogebia tipica (Callanassidae)	1.15	0.0	5.0	5.0
Eriocheir sinensis (Varunidae)	0.65	0.0	1.7	5.0
Nepinnotheres pinnotheres (Pinnotheridae)	0.40	1.7	0.0	8.3
Jaxea nocturna (Laomediidae)	0.38	0.0	0.0	1.7
Athanas nitescens (Alpheidae)	0.21	0.0	0.0	6.7
Axius stirhynchus (Axiidae)	0.16	0.0	0.0	1.7
Rhithropanopeus harrisii (Panopeidae)	0.05	3.3	1.7	0.0
Macropodia rostrata (Majidae)	0.03	0.0	0.0	3.3
Pisidia longicornis (Porcellanidae)	0.02	0.0	0.0	1.1
Sicyonia carinata (Sicyoniidae)	0.02	0.0	1.7	0.0
Atyaephyra desmarestii (Atyidae)	0.01	5.0	3.3	0.0
Hippolyte inermis (Hyppolytidae)	0.01	0.0	0.0	1.7

Ta, Tarfía; Es, La Esparraguera; Bo, Bonanza.
*Species included in the multivariate analyses.

species and environmental variables. The relative importance of temporal changes in the community structure was estimated by calculating the average similarity of samples: (a) within each year (monthly samples), for seasonal changes; and (b) among years (average annual samples), for inter-annual variations.

Results

Abiotic parameters

On each sampling date, water temperature and turbidity were quite homogenous throughout the estuary, while salinity showed a clear horizontal gradient throughout the sampling sites: mean salinity at Ta, Es and Bo was 5.0, 10.4 and 20.6 psu, respectively. From December 1997 to January 1998, on one hand, and from December 2000 to January 2001, on another, the river flow increased considerably due to heavy rainfall pushing fresh inland water to the outer limits of the estuary and causing the saline gradient to temporarily disappear (Fig. 2).

During the 5-year study period, water temperature displayed a very regular seasonal pattern having minimum values in winter and maximum in summer. A less-regular seasonal pattern was also observed in turbidity, which showed minimum values in summer from June to August and maximum in winter from November to February.

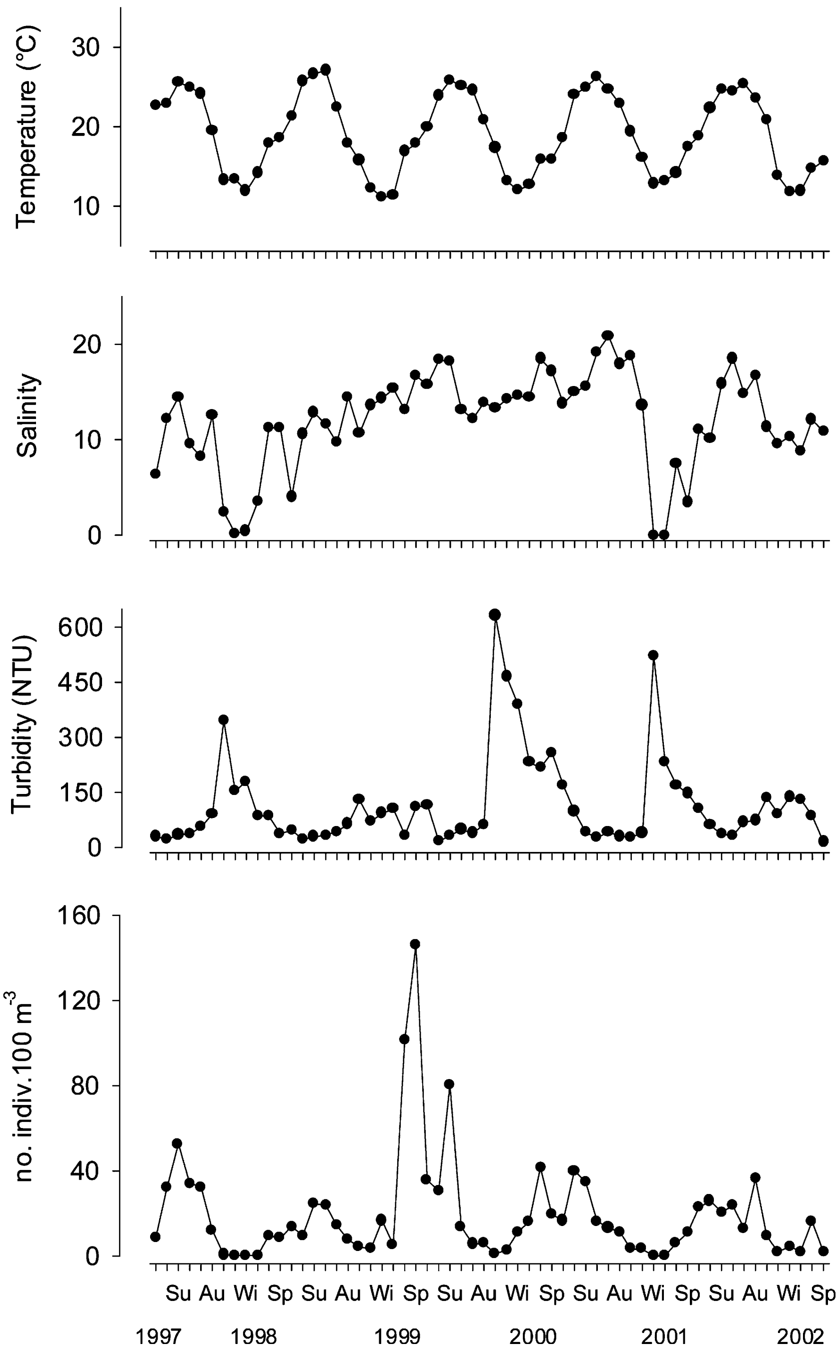

Figure 2. Water temperature, salinity, turbidity and total estuarine decapod density at the Guadalquivir Estuary from May 1997 to March 2002. For each variable, the mean values of the three sampling sites are shown. Su: summer; Au: autumn; Wi: winter, Sp: spring.

During the first and the fourth annual cycles, salinity exhibited a seasonal pattern, which was attributable to rainy winters. Seasonal changes in salinity were not observed during the other three annual cycles, which turned out to be very dry (Fig. 2).

Species composition and densities

Twenty-four decapod crustacean species representing 19 families were recorded in the Guadalquivir estuary during the 5-year study period (see Table 1). The community was strongly dominated by *Crangon crangon* (Linnaeus, 1758), *Melicertus kerathurus* (Forskal, 1775), and *Palaemon* spp., which were the three species that comprised 99% of all collected individuals. *C. crangon* and *M. kerathurus* are marine species that use the estuary as nursery as well as, in the case of *C. crangon*, adult feeding grounds. On the other hand, *Palaemon* spp. are estuarine species that complete their life cycle within the estuary. From the other species, 13 were collected sporadically ($C < 9\%$) and considered as rare species, all being marine except *Rhithropanopeus harrisii* (Gould, 1841) and *Atyaephyra demarestii* (Millet, 1831). As a result, they were not included in the multivariate analysis of the community.

Spatially, the community density decreased upstream, due to the higher density of marine species (mainly *C. crangon* and *M. kerathurus*) in more saline waters (Fig. 3). Temperately, the total estuarine decapod density showed a regular

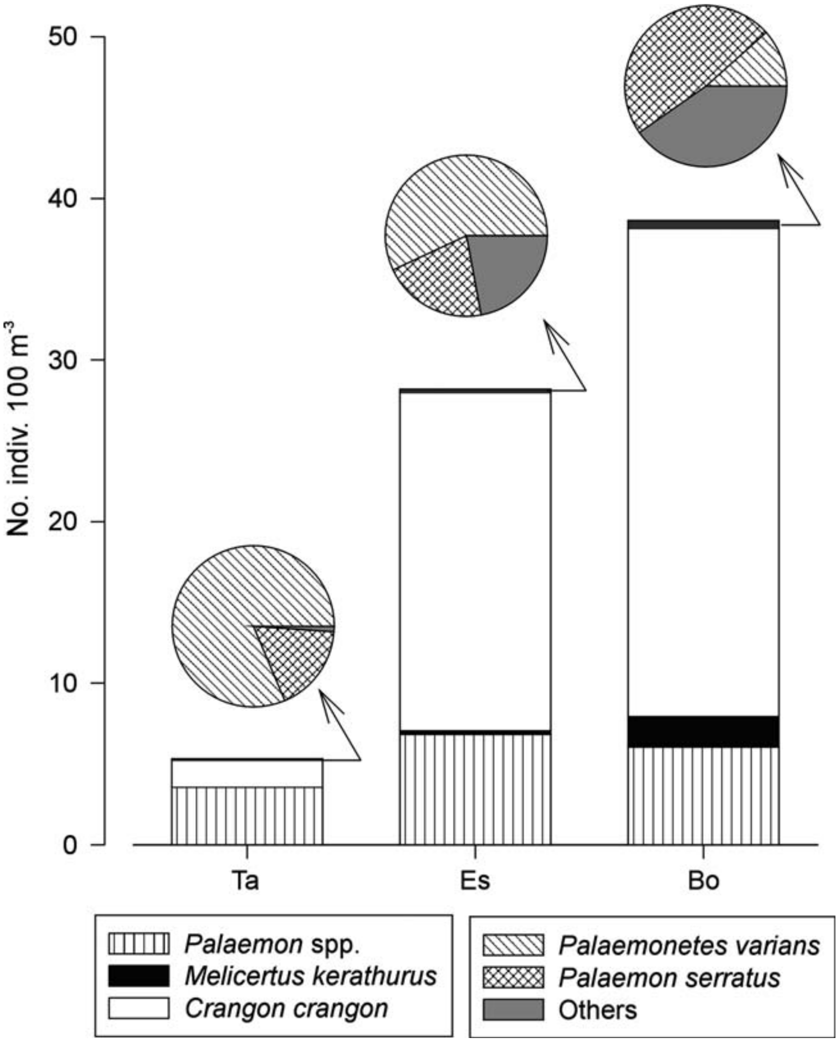

Figure 3. Decapod species' composition and abundance at the three sampling sites. For each sampling site the mean values of abundance of each species during the study period are shown. (Ta) Tarfia, (Es) Esparraguera, (Bo) Bonanza.

seasonal pattern indicating minimum values in late autumn and winter and maximum in spring and summer (Fig. 2). Water temperature and salinity showed significant ($p < 0.01$) positive correlation with total density. Turbidity, on the other hand, was negatively correlated with total density (Table 2).

Such a seasonal pattern in total density was just a result of the similar seasonal pattern seen in the three dominant species. Nevertheless, *Palaemon* spp. and *C. crangon* were present almost all year round, while peak duration of *M. kerathurus* was always shorter (5–6 months) (Fig. 4). Densities for the three species were positively correlated ($p < 0.01$) with the water temperature and salinity, and negatively ($p < 0.01$, excepting *M. kerathurus* $p < 0.05$) with turbidity. The highest correlations corresponded to temperature in *Palaemon* spp. and salinity in *C. crangon* and *M. kerathurus* (Table 2).

A continuous arrival of *C. crangon* juveniles occurred each year from late winter to early summer. The smallest *Palaemon* spp. individuals appeared mainly in spring although, in some years, new individuals seemed to arrive in successive brood waves from spring to early autumn. Due to its lower density, such arrival pattern was not clearly observed in the smallest *M. kerathurus* individuals (Fig. 3). Nevertheless, peaks in density of the three species usually coincided with the arrival of smaller individuals. Furthermore, there was a significant ($p < 0.01$) negative correlation between the mean weight of individuals and the natural logarithm of densities (correlation coefficients: −0.85, −0.50 and −0.46 for *C. crangon*, *Palaemon* spp., and *M. kerathurus*, respectively).

Temporal changes in community structure

Non-metric Multi-Dimensional Scaling ordination of samples indicated a regular seasonal change in the community (Fig. 5). Samples corresponding to warmer months were located on the right side of the plot, while samples from colder months were situated on the left. Such a temporal segregation of samples was especially remarkable for the years with rainy winters (Fig. 5b, e). Furthermore, spring and autumn samples were joined to the upper and lower middle parts of the plot, respectively. As a result, the first ordination axis was significantly ($p < 0.01$) correlated with environmental variables, especially with salinity and water

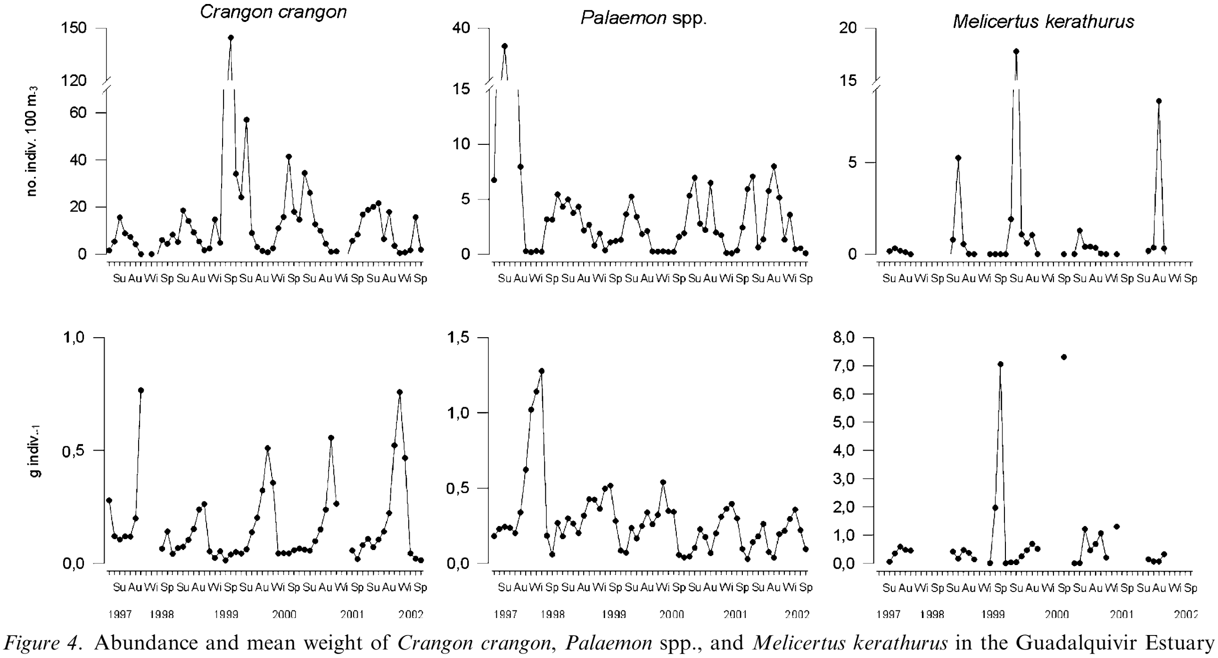

Figure 4. Abundance and mean weight of *Crangon crangon*, *Palaemon* spp., and *Melicertus kerathurus* in the Guadalquivir Estuary from May 1997 to March 2001. For each variable, the mean figures of the three sampling sites are showed. Su: summer; Au: autumn; Wi: winter; Sp: spring.

Table 2. Spearman rank coefficients (*p*: level of significance) between environmental variables and decapod densities (ln-transformed); and between environmental variables and first and second axis co-ordinates corresponding to each sample in the non-metric Multi-Dimensional Scaling ordination plot

	Salinity	Temperature	Turbidity
Total density	0.68**	0.58**	−0.47**
Crangon crangon	0.70**	0.41**	−0.35**
Palaemon s.p.p.	0.29*	0.72**	−0.58**
Melicertus kerathurus	0.44**	0.61**	−0.25*
MDS axis I	0.76**	0.70**	−0.49**
MDS axis II	0.08 NS	−0.08 NS	−0.05 NS

$^{**}p < 0.01$; $^{*}p < 0.05$; NS: $p > 0.05$.

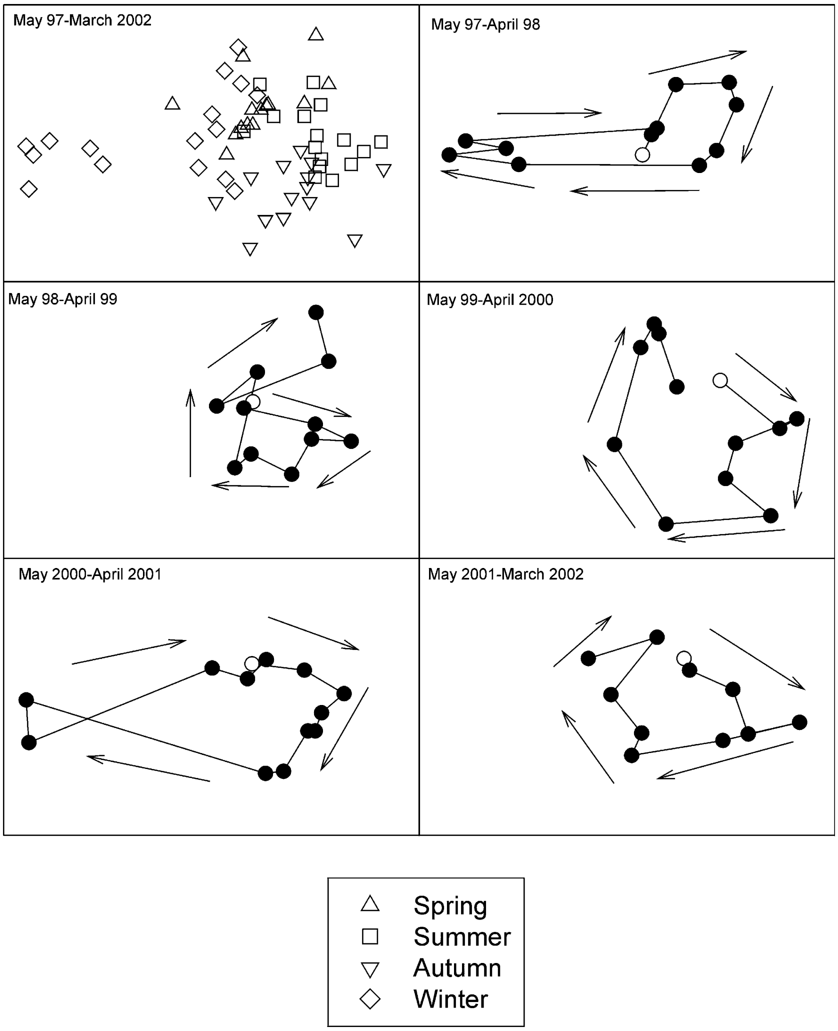

Figure 5. Non-metric Multi-Dimensional Scaling (MDS) ordination based on the root–root transformed abundance data of selected decapod species at the Guadalquivir Estuary. (a) All samples taken from May 1997 to March 2002; (b) first annual cycle; (c) second annual cycle; (d) third annual cycle; (e) fourth annual cycle. Empty symbols correspond to the first sample of each annual cycle, while arrows indicate temporal sequence of samples.

temperature (Table 2), while the second one seemed to split up samples following seasonal community changes in species' composition and species densities.

When similarity in averages of samples was used to quantify community changes at different temporal levels, the conclusion was reached that monthly differences were greater (a lower similarity) in years with rainy winters (Bray-Curtis similarity: 0.62). Besides, the inter-annual difference in yearly averages was always lower (Bray-Curtis similarity: 0.81) than monthly differences within each year, even in those years in which the weather was more stable (Bray-Curtis similarity: 0.66–0.70).

Discussion

The high specific richness found in the Guadalquivir Estuary is a result of the strong marine component of the outer sampling site (Bonanza), which is 8 km from the river mouth and where 21 species were reported. In the inner site (Tarfia), which has the lowest average salinity figures, only 11 species were collected. On one hand, the number of species collected and, on the other, the abundance and biomass data are similar to that found in other temperate estuaries (Atrill & Thomas, 1996; Szedlmayer & Able, 1996; Able et al., 2002). However, due to the fact that this sampling method mainly collects from the water column, benthonic and inter-tidal decapod species have been especially undercounted, such as brachyuran crabs *Uca tangeri* (Eydoux, 1835), *Carcinus maenas* (Linnaeus, 1758), *Pachygrapsus marmoratus* (J. C. Fabricius, 1787), *Eriocheir sinensis* H. Milne Edwards, 1853 and *R. harrisii*, which also have important populations in the Guadalquivir Estuary.

The studied community showed considerable temporal (both seasonal and inter-yearly) changes, with salinity and temperature being highly correlated with the total decapod crustacean density. Although with some exceptions (Atrill et al., 1999), both environmental variables have long been evoked as being among the most significant factors affecting estuarine species densities (Cartaxana, 1994; Szedlmayer & Able, 1996). In the Guadalquivir Estuary, water temperature showed a clear seasonal trend in every year while salinity did so only during the first and fourth annual cycle. Therefore, it can be said that salinity could possibly be responsible of the inter-year community changes observed during this study. Nevertheless, due to the strong dominance of the most abundant species, which represented 99% of individuals, this general community correlation probably showed the relations of dominant species' densities with both environmental variables. As these species made different uses of the Guadalquivir Estuary, such relations will be discussed separately in the following paragraphs.

The brown *C. crangon* shrimp is a marine species that uses the estuary as both a nursery and an adult-feeding ground as previously reported in other estuarine areas (Cattrijsse et al., 1997). Although its density showed a clear seasonal trend in each year of the 5-year period, new recruits started to arrive sooner in dry winters because of higher winter salinity, and they also left the estuary earlier. As a result, shrimp density and water temperature displayed a coincident seasonal pattern during rainy years, while their minimum and maximum values did not match in dry years. Following such behavior, and even though water temperature may have an important influence on the timing of the reproductive activity of this species (mainly outside the estuary), estuarine salinity seems to control the arrival of *C. crangon* juveniles to the estuary. Furthermore, during warmer periods, there were temporal declines in shrimp density that often coincided with decreases in salinity. Inter-annual differences at the start of settlement of new generations to nursery areas have been previously observed in the German and Dutch Wadden Sea, where settlement of new groups started later than usual after cold winters (Beukema, 1992; Temming & Damm, 2002). Nevertheless, bearing in mind that winter temperatures in the Guadalquivir Estuary correspond to spring and autumn temperatures in the Wadden Sea, such a different role of temperature in both geographical areas was expectable. Besides, *C. crangon* density also declined earlier than usual in summers following dry winters. This could be due to juvenile migration to the sea after reaching a given size and/or to the earlier-than-usual arrival (Baldó & Drake, unpublished data) of its principal predators, the fish *Argyrosomus regius* (Asso,

1801) and *Dicentrarchus punctatus* (Bloch, 1792) (Baldó & Drake, 2002), after a dry winter.

The *Palaemon* spp. group consists of two species, *Palaemon longirostris* H. (Milne Edwards, 1837), on one hand, and *Palaemon macrodactylus* (Rathbun, 1902), on the other, which is an introduced species (Cuesta et al., 2004). *P. macrodactylus* individuals are well defined and clearly different from *P. longirostris*, from a molecular-data point of view, even though they are difficult to distinguish from each other in juvenile stages. For this reason, and taking into account the similar ecological roles and life cycles of both species, in this study we have considered them as a unique group called *Palaemon* spp. These species are permanent residents of the Guadalquivir Estuary, where they complete their life cycles. The *Palaemon* spp. density displayed a clear seasonal trend, which coincided with that of water temperature in each year of the 5-year period. During dry winters and despite the high salinity, *Palaemon* spp. densities also declined. Therefore, the significant correlation between white shrimp density and estuarine salinity seems to merely be a result of the observed correlation between water temperature and salinity in rainy winters.

The prawn *M. kerathurus* is a marine species that only uses the Guadalquivir Estuary as a nursery ground. In the studied area (Gulf of Cádiz), this species shows a clear seasonal reproductive pattern, with a high percentage of mature females from May to September (Rodríguez, 1985). As a result, the significant correlation observed between the estuarine density and water temperature found there was mainly due to peaks in the arrival of young individuals in summer. Although salinity seems to play a less important role in the temporal pattern of *M. kerathurus* arrival to the estuary, its real role is hidden by the fact that the reproductive period coincides with the warm season in which salinity is high. In fact, when the spacial salinity component, or estuarine gradient, is considered, the degree of penetration of this species into the estuary was closely related with the salinity gradient. This explains its distribution throughout the sampling points (see Constancy values, Table 1).

While seasonal community changes in the first axis of the MDS plot seem to be correlated with changes in estuarine environmental conditions, those in the second axis were related to changes in species' composition and dominances. A seasonal pattern showing well-defined temporal changes in the species' composition has been previously found in aquatic communities in temperate estuaries (Potter et al., 1997; Maes et al., 1998; Thiel & Potter, 2001). It is clear that in temperate latitudes with four different well-defined seasons, the associated changes in the light-time/night-time pattern and water temperature play an important role in the timing of the reproductive activities of aquatic species and, as a result, in the seasonal succession of these species in estuarine communities. Furthermore, the greater inter-monthly than inter-yearly changes observed in the studied community is not at all surprising due to the strong seasonal effect on temporal aquatic community changes in temperate estuaries, which show both reproductive activities in it and seasonal migrations to and from nearby marine areas.

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Preliminary estimation of the factors influencing the distribution of the midlittoral crab *Portumnus lysianassa* (Herbst, 1796)

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Key words: distribution, Mediterranean Sea, midlittoral, *Portumnus*

Abstract

In order to acquire information on the factors influencing the distribution of the Mediterranean endemic species *Portumnus lysianassa* (Herbst, 1796), which lives in the soft substratum midlittoral zone, 83 stations were selected along almost the entire Greek coast. In each sampling station, dredging was carried out, just above the rise of the surf zone, and sediment samples were taken with a core sampler. Two faunal samples were also taken by means of a quadrat from the lower midlittoral zone. The preliminary results of this study show that: *P. lysianassa* prefers to burrow in the rise of the surf zone; wherever the sediment becomes very coarse or very fine the crab populations decline; there is a correlation between the population abundance of the midlittoral bivalve *Donacilla cornea* (Poli, 1795) and *P. lysianassa*, and, moreover, this crab species is related to the composition of the midlittoral assemblage; also, *P. lysianassa* abundance is negatively correlated with the organic matter content of the sediment; in very exposed areas, *D. cornea* can be replaced or co-exist with the infralittoral species *Donax trunculus* (Linnaeus, 1758), and, in these cases, *P. lysianassa* can be replaced by the closely related infralittoral species *Portumnus latipes* (Pennant, 1777); when low temperatures or intensive wave action, as well as disturbance caused by swimmers in summer prevail, the population of this crab descends in the upper infralittoral zone.

Introduction

A review of the relevant literature, shows that the ecology and biology of the Mediterranean endemic species *Portumnus lysianassa* (Herbst, 1796), previously known as *Portumnus pestai* (Forest, 1967; Sakai, 1999) are almost unknown. The only existing information is that it is found in sediments of fine to coarse sand (d'Udekem d'Acoz, 1994), at depths from the midlittoral zone (Števčić, 1990) to 6 m (Forest, 1967). This is a common species that seems to have a limited distribution in a well-defined zone, located in the north-central Mediterranean (d'Udekem d'Acoz, 1994).

The aim of this study is to give information on the factors influencing the distribution of *P. lysianassa*.

Material and methods

A considerable number of stations (83), covering a wide range of midlittoral sediments, were selected along almost the entire Greek coast (Fig. 1). Sampling was carried out in autumn 2001 and spring/summer 2002. Very coarse or muddy sediments were not sampled, as they are inhabited by assemblages that differ from the typical midlittoral sandy sediment assemblage.

In each sampling station, 2–4 samples were collected just above the rise of the surf zone, with a specially designed triangular dredge of 40 cm opening, on which a 0.5 cm mesh-size net was attached. The distance of each hauling was 5 m, thus, each sample covered a sampling area of 2 m^2, along the midlittoral zone.

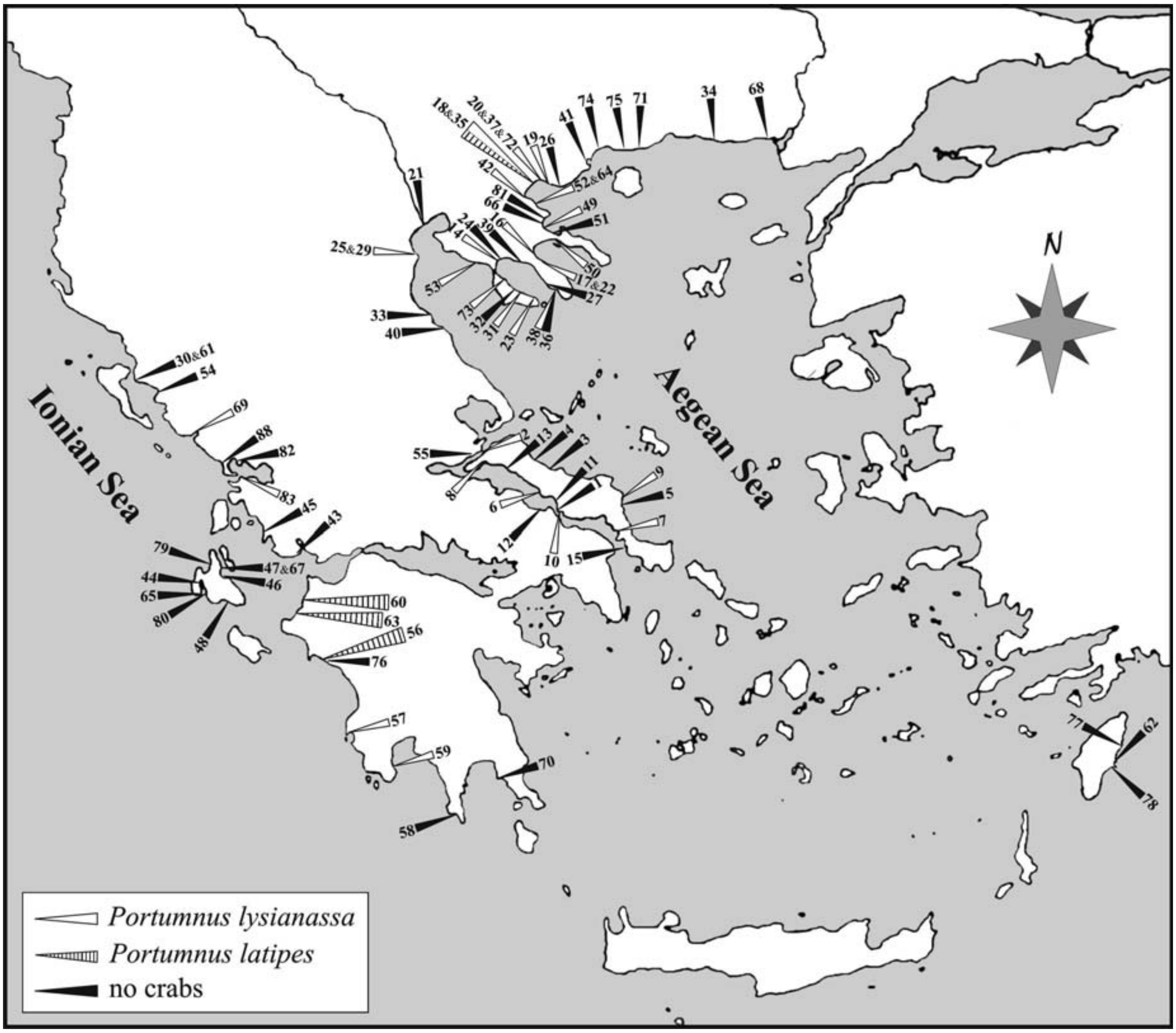

Figure 1. Map of Greece indicating the sampling stations.

In all sampling stations, sediment samples were also taken by means of a core sampler (3 cm diameter) down to 10 cm depth. Sediment particle size analysis and estimation of organic matter content were carried out according to the methods described by Buchanan (1984).

Additionally, two faunal samples were taken with a quadrat (20×20 cm) from the lower midlittoral zone, in order to determine *Donacilla cornea* (Poli, 1795) population abundance.

Correlation analysis, using the Spearman correlation coefficient, was performed to determine significant relationships among the studied variables.

Results

The values of the sediment median diameter (Md), quartile deviation (QD) and organic matter content, as well as the abundances of *P. lysianassa* and *D. cornea* for all sampling stations are given in the Appendix A.

The faunal composition of all sampling stations, in relation to the sediment Md and QD values is given in Fig. 2. All stations are distinguished in the faunal categories given in the legend. Most of the stations that did not yield crabs and *D. cornea* (27 out of 28), either had Md values higher than 1950 μm and QD values higher than 1 (poorly sorted sediments) or had Md values lower than 200 μm and QD values lower than 0.7 (moderately well–very well sorted sediments). In most stations where only the bivalve *D. cornea* was found (16 out of 22), the Md values ranged from 250 to 1650 μm (medium–very coarse sand), and the QD values ranged from 0.35 to 1 (well–moderately sorted sediments).

In all the other stations where crabs were found (33 in total), Md values ranged from 177 to 1790 μm (fine–very coarse sand), and QD values ranged from 0.31 to 1.94 (very well–poorly sorted sediments). According to the results of the Spearman correlation analysis, given in Table 1, the correlation of *P. lysianassa* abundance with the Md and Qd values is not statistically significant.

The distribution of the sampling stations in which *P. lysianassa* was not found, in relation to the organic matter content and the sediment Md values, is given in Fig. 3a. In most of the stations where neither crabs nor *D. cornea* were found, the sediments either had organic matter content values higher than 1.5% or high Md values (> 1700 μm). On the contrary, the sediments of most sampling stations where *D. cornea* or *Portumnus latipes* (Pennant, 1777) were found had organic matter content values lower than 1.5% and Md values from 250 to 1700 μm. Spearman correlation analysis showed that there is a statistically significant negative correlation between *D. cornea* abundance and the organic matter content of the sediment (Table 1).

The distribution of *P. lysianassa* abundances, in the sampling stations where this species was found, in relation to the organic matter content and the Md values of sediment is given in Fig. 3b. *P. lysianassa* abundances in the above stations ranged from 1 to more than 10 individuals per 2 m^2. In most sediments, the organic matter content values ranged from 0.3 to 1.8%, and Md values from 250 to 1800 μm. However, the highest abundances were recorded in 3 stations with sediment organic matter content 0.4–1% and Md 308–871 μm (medium-coarse sand). As it is shown in Table 1, the organic matter content of the sediment is negatively correlated with *P. lysianassa* abundance and this correlation is statistically significant.

The triangular graph of Fig. 4a illustrates the distribution of sampling stations where

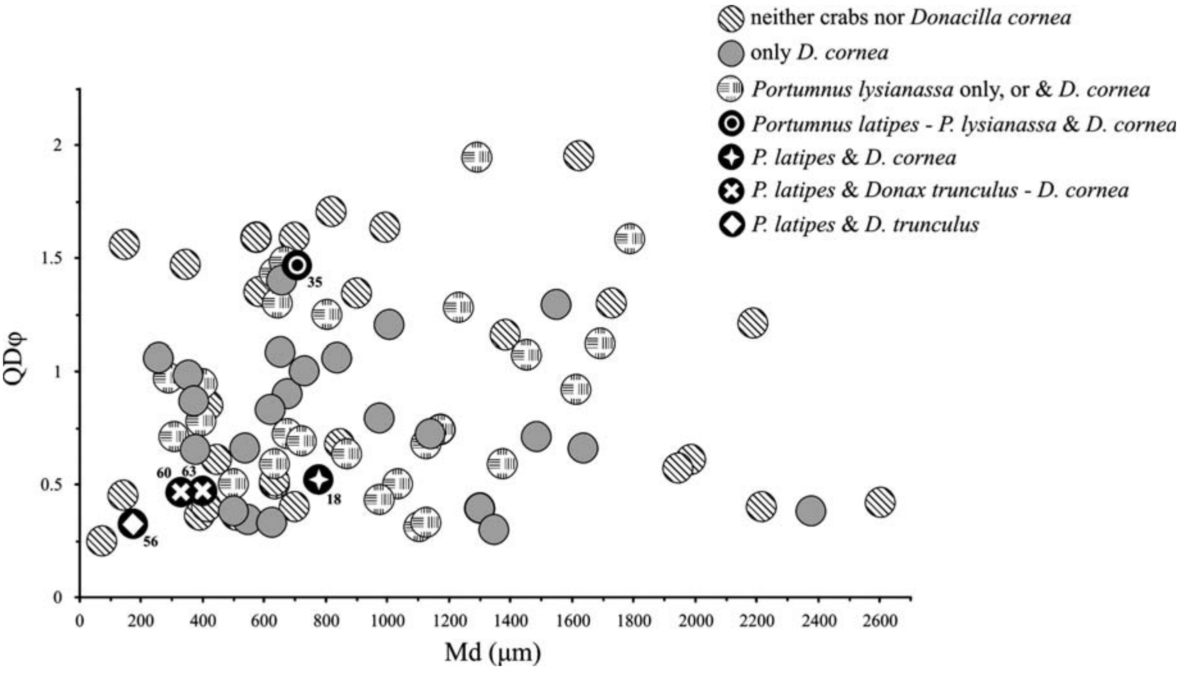

Figure 2. Faunal composition of all sampling stations, in relation to the median diameter (Md) and quartile deviation (QD) values of the sediment.

Table 1. Results of Spearman correlation analysis between the studied variables

	Md	QD	Organic matter content	*P. lysianassa* abundance	*D. cornea* abundance
Md	–	0.1194	−0.1484	0.0681	−0.0472
		0.2824	0.1816	0.5400	0.6707
QD		–	**0.2600**	−0.0026	0.0440
			0.0193	0.9812	0.6920
Organic matter content			–	**−0.3813**	**−0.3263**
				0.0006	**0.0033**
P. lysianassa abundance				–	**0.3829**
					0.0006
D. cornea abundance					–

Statistically significant correlations are marked in bold.

P. lysianassa was not found, in relation to sediment particle size. It is obvious that in the stations where only *D. cornea* was found, the intermediate fraction constituted the higher percentage of the sediment. The sediments of the stations where neither crabs nor *D. cornea* were recorded had a wide distribution among the three sediment fractions. In three of the stations where *P. latipes* was collected, the fine fraction participated with more than 70% in the sediment composition.

The distribution of *P. lysianassa* abundances in the sampling stations where this species was found, in relation to sediment particle size is given in Fig. 4b. The three stations with the highest abundances of *P. lysianassa* had sediments with a high participation of the intermediate and fine fractions together.

The distribution of *P. lysianassa* abundances in the sampling stations where it was found, in relation to the abundances of *D. cornea* in the corresponding stations and the Md values of these station sediments, is given in Fig. 5. This figure shows that: (a) the low abundances of *P. lysianassa* (the first three classes in the scale given in the legend) appeared mostly in stations (68%) where *D. cornea* abundance was lower than 9 ind./400 cm^2, while in three stations (12%), *D. cornea* abundances had intermediate values (15–40 ind./400 cm^2); only in two stations (8%) *D. cornea* abundances were high (100 and 150 ind./400 cm^2, respectively). On the other hand, the three highest abundance values of *P. lysianassa* appeared in the stations (12%) where *D. cornea* abundance is intermediate, ranging from 31 to 46 ind./400 cm^2; (b) the gradation of *D. cornea* abundances in the sampling stations seems to be discontinued, since all the abundance values recorded were either lower than 46 ind./400 cm^2 or higher than 100 ind./400 cm^2; (c) a discontinuity in the abundance gradation also appeared in *P. lysianassa*, since two abundance classes (6–7 and 8–9 ind./2 m^2) were absent. Thus, a positive correlation between the abundance of *P. lysianassa* and the corresponding one of the bivalve *D. cornea* seems to emerge and this correlation is statistically significant (Table 1). However, this correlation has to be investigated in detail. It is remarkable that a preliminary analysis of *P. lysianassa* stomach content showed that it feeds on the bivalve *D. cornea*, various Crustacea and organic detritus. On this basis, it might be assumed that the abundance range of this crab may be related to the composition of the midlittoral assemblage. This seems to be supported by the fact that one of the three highest *P. lysianassa* abundance values was recorded in station 72. In the midlittoral assemblage of this station, *D. cornea* had an intermediate abundance and coexisted with the mysid species *Gastrosaccus sanctus* (Van Beneden, 1861), which occurred in a very high abundance.

Discussion

The results of this study show that *P. lysianassa* is a true component of the midlittoral sandy sediments assemblage. When sediments become very coarse or very fine and the assemblage tends to disorganize, the population abundance of *P. lysianassa* declines. This species prefers to

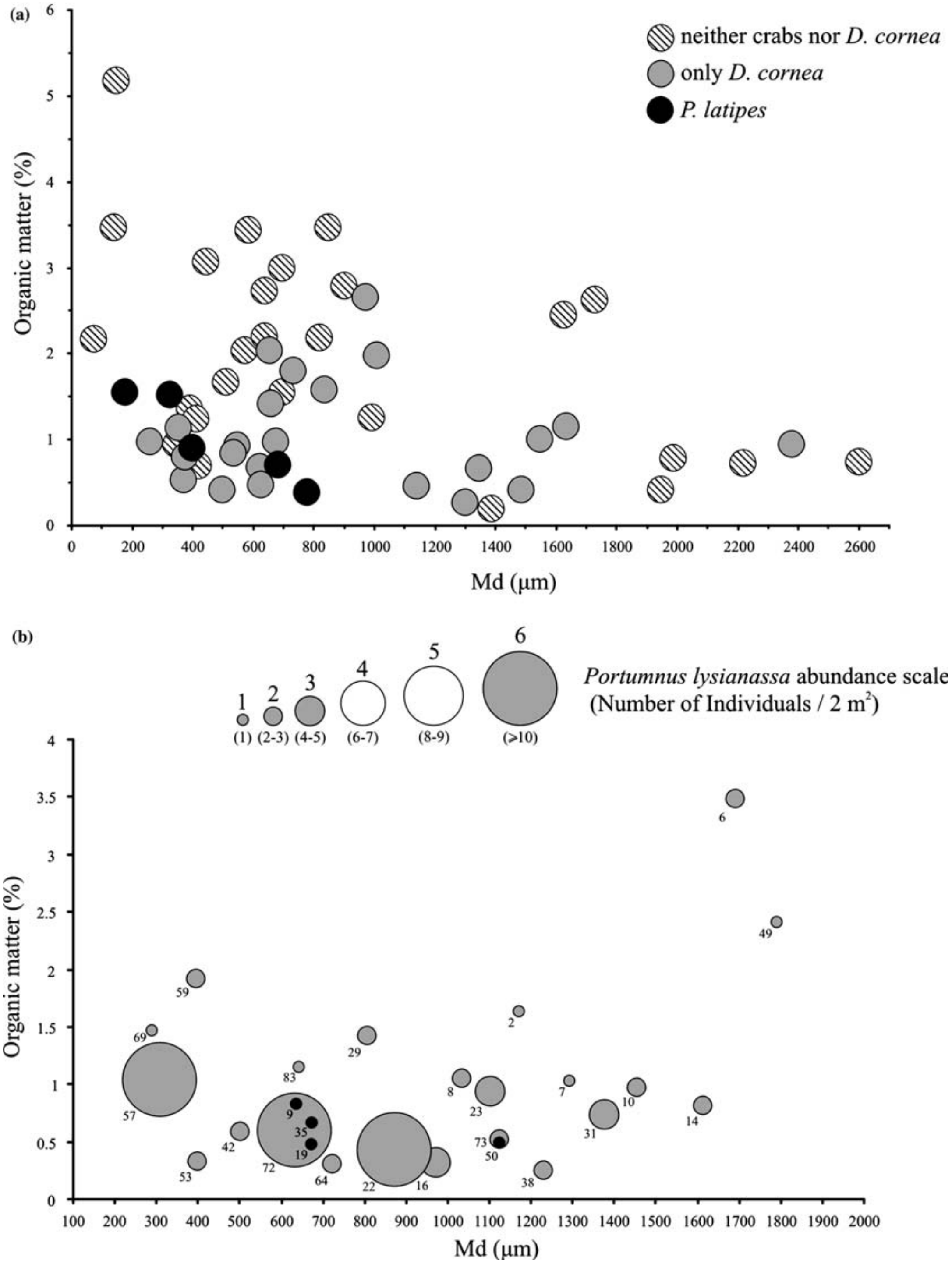

Figure 3. Distribution of: (a), the sampling stations in which *P. lysianassa* was not found; (b), *P. lysianassa* abundances in the sampling stations where this species was found, in relation to the organic matter content and the median diameter (Md) values of the sediment.

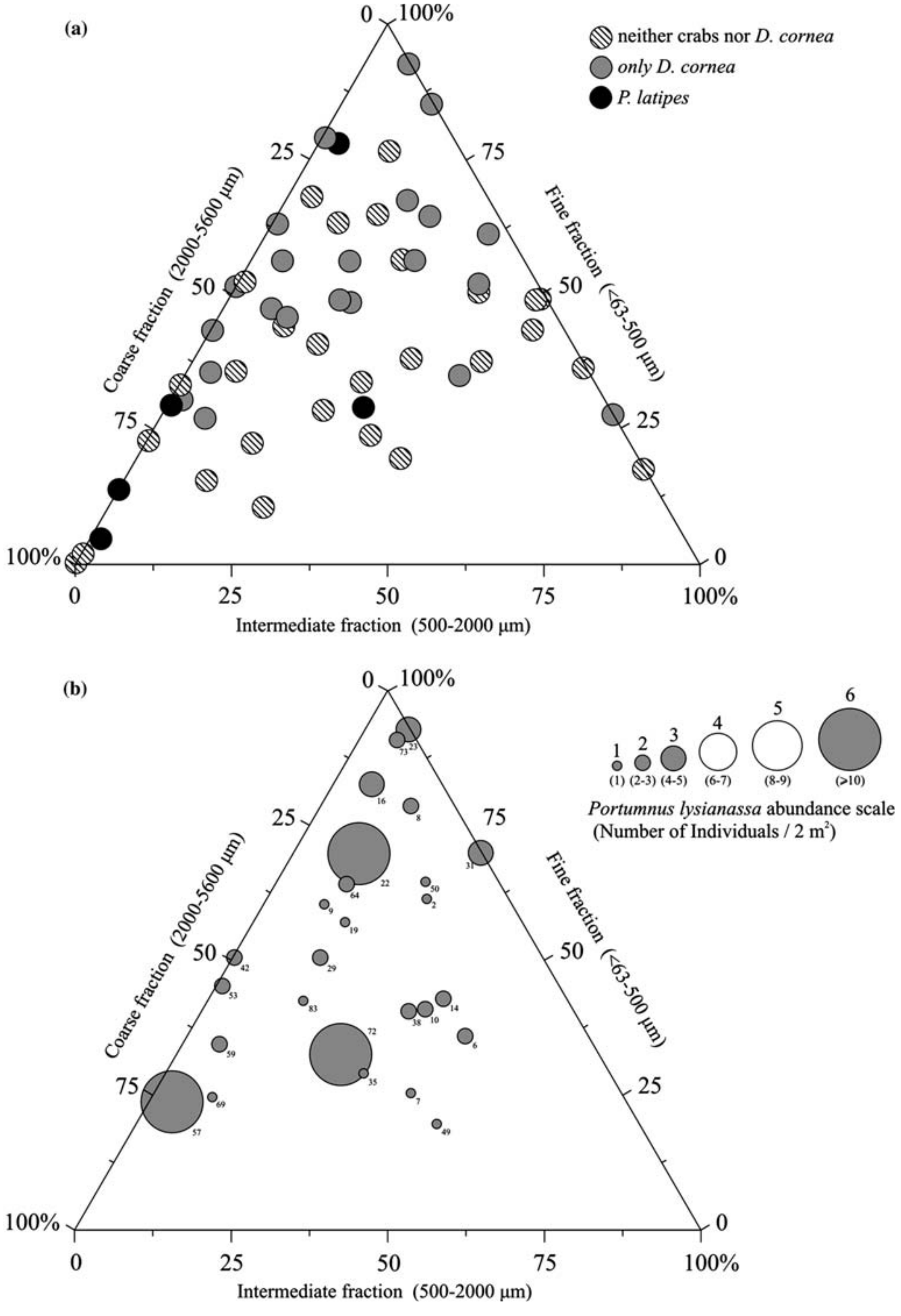

Figure 4. Distribution of: (a), the sampling stations where *P. lysianassa* was not found; (b), *P. lysianassa* abundances in the sampling stations where this species was found, in relation to sediment particle size.

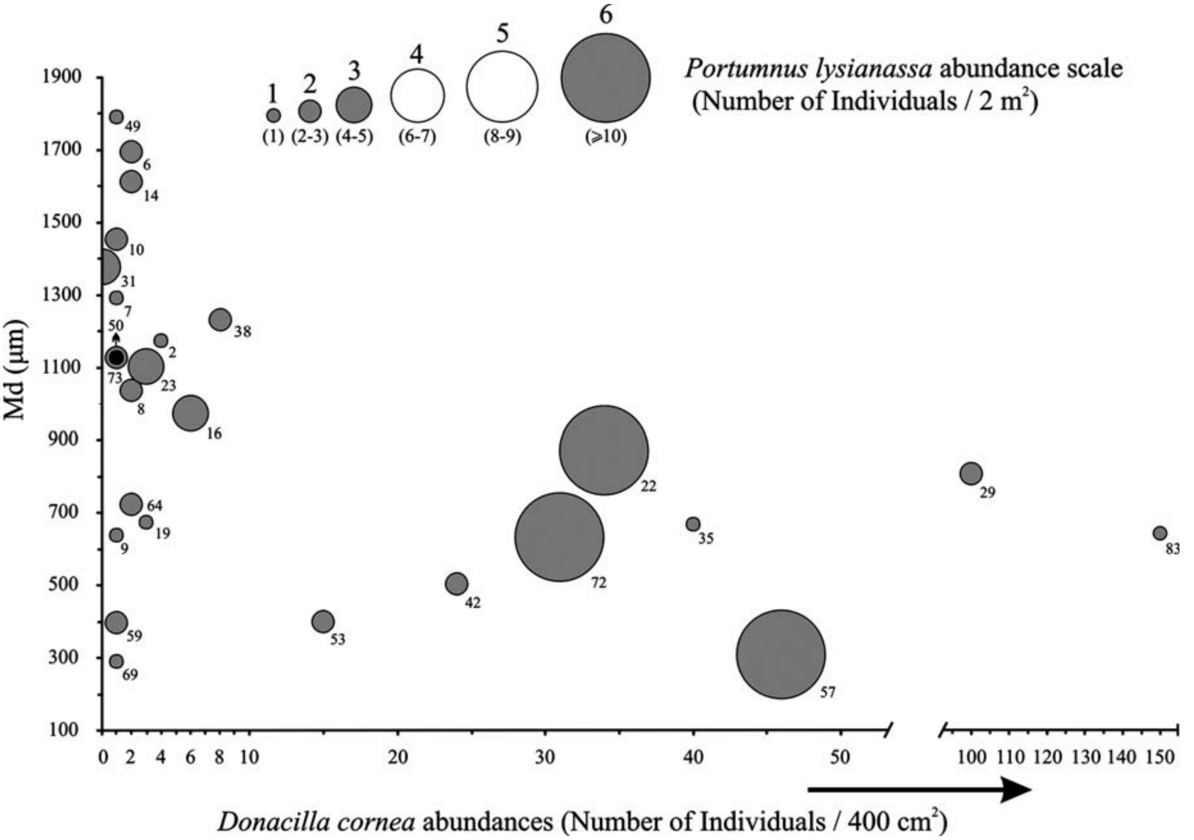

Figure 5. Distribution of *P. lysianassa* abundances in the sampling stations where it was found, in relation to the abundances of *D. cornea* in the corresponding stations and the median diameter (Md) values of the sediment.

burrow in the rise of the surf zone, where the usual disturbance of the sediment by the wave action is more intense. At this shore level, it seems to be facilitated in capturing its food, which mainly consists of organisms and organic detritus derived mainly from the midlittoral assemblage. It should be mentioned that in the west coast of Peloponnisos and specifically in station 56, the infralittoral crab *P. latipes* and the bivalve *Donax trunculus* (Linnaeus, 1758) were found instead of the crab *P. lysianassa* and the bivalve *D. cornea*, respectively. In other two stations of the same area (60 and 63), the crab *P. latipes* and the bivalves *D. trunculus* and *D. cornea* were found (station 60: 6 *D. trunculus* and 5 *D. cornea* individuals; station 63: 22 *D. trunculus* and 1 *D. cornea*). These two stations should be considered as very exposed, due to their long fetch, even though their sediments (during the sampling period) had Md and QD values, which do not justify high exposure. In these cases we could accept that *P. lysianassa* and *D. cornea* are absent because of the prevailing adverse conditions caused by the frequent intensive wave action. In such a case, *P. lysianassa* and D. cornea can be replaced by the relative infralittoral species *P. latipes* and *D. trunculus*, respectively. In station 18 (Fig. 1), where repetitive samplings were carried out, there is a permanent *D. cornea* population of high abundance, but *P. lysianassa* is replaced by *P. latipes* (only once, an individual of *P. lysianassa* was found). This station could be considered as exposed (long fetch, coarse sand moderately sorted). In this particular case, we could assume that the hydrodynamism intensity is such that, on one hand allows the maintenance of a *D. cornea* population, while, on the other hand, severely limits the presence of *P. lysianassa*. This view seems to be supported by the fact that populations of

P. lysianassa were observed to descend in the upper infralittoral zone, when intensive wave action (e.g. storms), or disturbance caused by swimmers in summer prevail.

A different case of long-term descent of *P. lysianassa* population in the upper infralittoral zone was observed in station 72 (Fig. 1). In this station a *P. lysianassa* population of high abundance was recorded in October 2001, while at the end of January of the next year its abundance in the midlittoral zone was null. Sampling in the upper infralittoral zone, at depths of approximately 0.5–1 m, revealed a *P. lysianassa* population of high abundance. This long-term descent of the population should be attributed to the fact that for several days before January sampling, very low temperatures were recorded.

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Appendix

Appendix A. Sediment parameters and abundances of *Portumnus lysianassa* and *Donacilla cornea* in the sampling stations

Station number	Date day/mth/year	Sediment			Abundance	
		Organic matter (%)	Md (μm)	QDφ	*P. lysianassa* (ind./2 m^2)	*D. cornea* (ind./400 cm^2)
1	10/05/02	2.04	574	1.59	–	–
2	08/05/02	1.63	1173	0.74	1	4
3	09/05/02	2.79	901	1.34	–	–
4	09/05/02	2.63	1729	1.30	–	–
5	10/05/02	0.69	420	0.85	–	–
6	08/05/02	3.48	1693	1.12	2	2
7	10/05/02	1.02	1292	1.94	1	1
8	08/05/02	1.05	1035	0.50	3	2
9	10/05/02	0.82	637	0.59	1	1
10	10/05/02	0.97	1454	1.07	2	1
11	09/05/02	5.17	148	1.56	–	–
12	09/05/02	2.18	818	1.70	–	–
13	09/05/02	–	2189	1.21	–	–
14	15/05/02	0.81	1613	0.92	2	2
15	10/05/02	1.26	993	1.63	–	–
16	21/04/02	0.32	973	0.43	5	6
17	21/04/02	0.27	824	0.75	10	34
18	14/04/02	0.38	779	0.52	–	75
19	14/04/02	0.47	674	0.72	1	3
20	14/04/02	0.35	497	0.51	10	31
21	19/09/01	0.94	344	1.47	–	–

Continued on p. 105

Appendix A. (Continued)

Station number	Date day/mth/year	Sediment			Abundance	
		Organic matter (%)	Md (μm)	QDφ	*P. lysianassa* (ind./2 m^2)	*D. cornea* (ind./400 cm^2)
22	09/09/01	0.43	871	0.63	10	34
23	18/10/01	0.94	1102	0.31	4	3
24	09/09/01	1.00	1548	1.29	–	1
25	19/10/01	1.14	354	0.98	2	100
26	08/11/01	0.46	1141	0.72	–	1
27	17/02/02	0.72	2219	0.40	–	–
28	06/05/02	0.94	2378	0.38	–	8
29	19/09/01	1.42	807	1.25	2	100
30	26/10/01	0.98	674	0.90	–	114
31	18/10/01	0.73	1376	0.59	4	–
32	18/10/01	0.19	1385	1.16	–	–
33	15/11/01	0.68	620	0.83	–	1
34	28/10/01	0.93	547	0.34	–	58
35	20/10/01	0.66	669	1.48	1	40
36	17/02/02	0.97	259	1.06	–	1
37	27/01/02	0.49	382	0.49	10	31
38	17/02/02	0.25	1231	1.28	2	8
39	09/09/01	0.53	371	0.87	–	1
40	15/11/01	0.84	536	0.66	–	11
41	06/11/01	0.42	1485	0.71	–	24
42	20/10/01	0.59	503	0.50	3	24
43	04/05/02	2.16	74	0.25	–	–
44	02/05/02	3.46	141	0.45	–	–
45	05/05/02	0.78	1986	0.61	–	–
46	04/05/02	0.73	2603	0.42	–	–
47	03/05/02	1.15	1636	0.66	–	36
48	02/05/02	2.45	1625	1.95	–	–
49	09/03/02	2.41	1790	1.58	1	1
50	09/03/02	0.47	1125	0.68	1	1
51	09/03/02	0.42	1945	0.57	–	–
52	09/03/02	0.27	1301	0.39	2	2
53	15/03/02	0.33	398	0.94	2	15
54	27/10/01	1.97	1007	1.20	–	26
55	19/09/01	1.55	697	1.59	–	–
56	24/09/01	1.55	177	0.32	–	–
57	25/09/01	1.04	308	0.71	10	46
58	27/09/01	1.66	511	0.36	–	–
59	26/09/01	1.91	395	0.78	2	1
60	22/09/01	1.52	325	0.47	–	5
61	27/10/01	2.04	655	1.08	–	114
62	26/12/01	3.47	847	0.68	–	–
63	22/09/01	0.90	398	0.47	–	1
64	09/03/02	0.30	722	0.69	2	2

Continued on p. 106

Appendix A. (Continued)

Station number	Date day/mth/year	Sediment			Abundance	
		Organic matter (%)	Md (μm)	QDφ	*P. lysianassa* (ind./2 m^2)	*D. cornea* (ind./400 cm^2)
65	02/05/02	3.43	586	1.35	–	–
66	09/03/02	0.67	1347	0.30	–	1
67	03/05/02	1.58	835	1.06	–	36
68	27/10/01	1.42	660	1.40	–	60
69	27/10/01	1.46	289	0.97	1	1
70	28/09/01	3.07	444	0.61	–	–
71	06/11/01	0.47	624	0.33	–	4
72	20/10/01	0.60	633	1.43	10	31
73	18/10/01	0.52	1125	0.33	3	1
74	08/11/01	0.79	376	0.65	–	300
75	08/11/01	0.41	500	0.38	–	6
76	24/09/01	1.36	390	0.36	–	–
77	26/12/01	1.24	412	0.40	–	–
78	26/12/01	2.72	637	0.50	–	–
79	03/05/02	2.65	973	0.79	–	18
80	02/05/02	2.20	637	0.51	–	–
81	09/03/02	2.99	697	0.40	–	–
82	06/05/02	1.80	732	1.00	–	100
83	04/05/02	1.15	642	1.30	1	150

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Chromatic patterns of the hermit crab *Calcinus tubularis* related to the occupied shell

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Key words: morphs, chromatism, shell preference, camouflage

Abstract

Crustacea are known to develop different chromatic patterns due to many factors. Regarding decapods, chromatism was mainly studied in crabs, while very little is known about chromatic patterns in hermit crabs. *Calcinus tubularis* is a typical infralittoral rocky bottom hermit crab, studied for different aspects of its biology except chromatic variations. This paper aims at describing the different colour morphologies of *C. tubularis*, discussing hypothesis of why they develop, and testing if in nature the crab prefers a shell with a chromatic pattern similar to that of its body. One hundred and forty crabs were observed and filmed in the laboratory. They were subdivided into two groups, according to their chromatic pattern: 1) light and 2) dark crabs; the shells they occupied were also subdivided into the two groups of 1) light and 2) dark shells on the basis of the epibionts encrusting them. Observations of 129 crabs suggest that the colour depends neither on depth nor on size, intermoult period, diet, reproductive period but it might be connected to genetic factors and might help crab to camouflage. Camouflage is suggested by the fact that 79.3% of the total examined specimens occupy shells with a chromatic pattern resembling that of the crabs themselves. This phenomenon is significantly more recurrent in females than in males and could help the crabs to be cryptic, first with the occupied shell and secondly with the habitat (rocks encrusted by photophylous algae).

Introduction

Crustacea are known to develop different chromatic patterns due to different factors (Pessani & Premoli, 1992; Guarino et al., 1993; Wolfrath, 1993; Abelló et al., 1997; Merilaita & Jormalainen, 1997; Wolf, 1998).

Regarding decapods, chromatism has been studied recently in particular in the crab *Carcinus maenas* (Linnaeus, 1758) (McGaw et al., 1992; McGaw & Naylor, 1992; Abelló et al., 1997; Reid et al., 1997; Wolf, 1998; Styrishave et al., 2000). According to Wolf (1998), colour pattern changes in *C. maenas* are connected with the crab size, and therefore with its age. Reid et al. (1997) ascribe the two morphs to photodenaturation of pigments over a long intermoult period, to different toleration of salinity fluctuations, and to aerial exposure. Pessani & Premoli (1992) observed red and green specimens of the hermit crab *Cestopagurus timidus* (P. Roux, 1830) and considered the two forms as a consequence of genetic polymorphism.

Calcinus tubularis (Linnaeus, 1767) is a typical infralittoral rocky bottom hermit crab, partially studied for its biology, shell choice, and geographical distribution (Amoroux, 1974; Zibrowius, 1978; Gherardi, 1990; Manjon Cabeza & Garcia Raso, 1995; Pessani et al., 2000b). In different populations of Ligurian Sea, Damiano et al. (1998) observed that 10% of *C. tubularis* specimens live in empty tubes of vermetids, while the remaining 90% occupy shells covered by epibionts, mainly red algae and secondly bryozoans and tubes of serpulids. In agreement with Stachowitsch

(1980), the presence of epibionts is considered as an antipredatory adaptation to strengthen the structure of low quality shells against crushing predators. In the Mediterranean Sea main predators of hermit crabs in infralittoral rocky bottom are *Octopus vulgaris* Cuvier, 1797, which pulls the crabs out of their shells using the tip of one of its arms (Tirelli et al., 2000), and different fish species belonging to *Diplodus* genus, which crush shells: these are all species that hunt by sight. Nothing is known up to now about chromatic variations of *C. tubularis*, but we observed specimens of *C. tubularis* showing two different body colour patterns. They often occupied shells covered with epibionts, thus giving the shell a hue similar to that of the crab body.

Aims of this study were: 1) to describe the different colour patterns of *C. tubularis* observed; 2) to find a relation between colour patterns of the crab and those of the occupied shell; 3) to discuss hypotheses about its development.

Materials and methods

Sampling

The samplings were performed in two sites of the Ligurian Sea (Villefranche sur Mer, France and Punta Sestri, Italy), both placed on infralittoral rocky bottom rich in photophylous algae; they were characterised by micro-hollows among sparse rocks (∅ about 50 cm), covered mostly by green and red algae (mainly *Corallina elongata* Ellis & Solander, 1786) and partially (approximately 30% of the surface) by concretions of polychaetes and bryozoans. *Calcinus tubularis* specimens were collected: a) in June 2001, at Villefranche sur Mer at 7–8 m depth and 10–15 m off the coast (29 crabs), b) in August 2001, at Punta Sestri at 12–13 m depth and about 50 m off the coast (65 crabs), and c) in October 2002, at the same site (46 crabs).

Observations

In the laboratory specimens were individually kept in glass bowls (∅ 5 cm), filled with 75 cm^3 of sea water (salinity 35‰), which was renewed once a week, and fed with pieces of *Mytilus* sp. mantle twice a week. The bowls were placed into a thermostatic room ($T = 19 \pm 1$ °C) with a photoperiod according to the season.

All the specimens were observed, filmed, and subdivided into two groups, according to their chromatic pattern: light and dark crabs (from here on named as LC and DC). In order to film the two different morphs, each single specimen was overturned putting its shell aperture towards the camera: during its attempt to recover the right position, the crab offered a complete sight of carapace to the observer. The shell was also filmed.

The crabs were counted, sexed, measured, and divided into four categories on the basis of their carapace length (CL): a) CL $\leq$ 4 mm, b) 4 < CL $\leq$ 6 mm, c) 6 < CL $\leq$ 8 mm, and d) CL > 8 mm.

We investigated relationships between the two morph types and 1) depth (i.e. distance from the coast); 2) sex; 3) age, using size (CL) as its estimate, and 4) intermoult period (as observed in the laboratory).

The shells occupied were identified at generic level, and subdivided into two groups (light = LS and dark = DS).

For the statistical analysis G, χ^2, and Student's *t* tests were used.

Results

Description of the chromatic patterns of crabs and shells

LC individuals show a light blue spot on the shield and a light blue–grey carapace, with sparse little red spots. Claws have the same colour pattern with the carapace but the red spots are closer than those of the carapace; the top of the fingers is white. Pereiopods are grey with longitudinal orange stripes, dactyls are white with rare red spots. LC specimens have an overall orange–red aspect.

DC individuals show an electric blue carapace with red stripes on each side; the shield has a very dark blue–violet spot. Chelipeds have the same blue of the carapace with very close red spots. Fingers are darker blue than the rest of the body and partially covered with red spots; their top is white. Pereiopods are electric blue with longitudinal orange stripes; dactyls resemble those of LC. DC specimens have an overall dark red hue. This morph type corresponds to the basic chromatic pattern already known, as described by Fenizia (1933).

About 80% of the 140 crabs observed occupied *Cerithium vulgatum* (Brugière, 1792) shells; the others lived in *Cantharus* sp. ($n=7$), *Columbella* sp. ($n=6$), *Trunculariopsis* sp. ($n=4$), *Buccinulum* sp. ($n=3$), *Mitrella* sp. ($n=3$), *Hinia* sp. ($n=1$), *Hexaplex* sp. ($n=1$), *Bittium* ($n=1$), and empty tubes of vermetids ($n=2$).

The occupied shell is described as DS when widely covered by red algae (≥50% of the shell surface encrusted), and as LS when either presenting no encrustation or covered by polychaetes and bryozoans, or when slightly covered by red algae (< 50% of their surface encrusted).

Crab chromatic patterns

Data on crab and shell colour obtained from our videotapes are shown in Table 1.

Ninety out of 140 (64.3%) crabs were DC; 111 (79.3%) specimens occupied shells with a chromatic pattern resembling that of the crab (χ^2 test: $\chi^2=51.24$, df = 1, $p<0.001$).

The 94 crabs collected during the reproductive season in the two sites (Villefranche sur Mer and Punta Sestri) (Table 1) were analysed together because the two areas were characterised by the same habitat and showed no statistical difference in the number of L and D females (G test: G = 0.744, df = 1, p = ns), as well as of L and D males (G test: G = 3.511, df = 1, p = ns). Therefore the crabs were considered altogether as a single sample: 32 (= 34%) LC and 62 (66.0%) DC.

In the laboratory, we took into account the intermoult period of a few crabs and could ascertain that their chromatic patterns do not depend on it (mean intermoult period: LC = 80.6 days, n = 19; DC = 103.7 days, n = 41; t-Student test: $t=1.57$, df = 58, p = ns).

We could not determine the sex of 11 crabs out of the 140 specimens observed; therefore, the subsequent analyses were performed on 129 specimens. Table 2 shows *C. tubularis* specimens subdivided according to sex, morph, and occupied shell.

Table 3 shows the number and percentage of females and males according to the morph. Sex ratio (number of males/total number of crabs) did not differ significantly between DC and LC (respectively, DC = 0.38; LC = 0.32; G test: G = 0.433, df = 1, p = ns).

Chromatic patterns did not depend on size (G test: G = 0.378, df = 3, p = ns) (Fig. 1). The number of LC and DC for each shell hue per sex is shown in Fig. 2. Females inhabited shells with a chromatic pattern close to that of their body significantly more often than males (72 females against 31 males) (χ^2 test: $\chi^2=16.78$, df = 1, $p<0.001$).

Discussion

According to Hedgecock et al. (1982), in crustaceans nearly 95% of the about 230 genes that show visible effects concern differences in colour and colour pattern. As a consequence, colour seems to be the effect of the action of numerous genes.

Nevertheless, some other factors but genes may play an important role in the presence of different chromatic patterns, such as: 1) distance from the open sea, 2) age, 3) intermoult period, 4) photodenaturation of pigment and diet, 5) reproductive season, and 6) camouflage.

A relation between colour and distance from the open sea has been found in *C. maenas* (Abelló

Table 1. Number of crabs of the two morphs of light (LC) and dark (DC) crabs occupying light (LS) and dark (DS) shells, according to the reproductive season

	Reproductive season				Non reproductive season	
	June 2001		August 2001		October 2002	
	DS	LS	DS	LS	DS	LS
DC	18	5	30	9	22	6
LC	1	5	4	22	5	13

Table 2. Number and percentage of the analysed crabs according to sex; morph: light (LC) and dark (DC) crabs; and occupied shell: light (LS) and dark (DS) shells

		LC		DC		Total
		f	m	f	m	
Cerithium vulgatum	LS	21	8	3	8	40
	DS	1	4	40	18	63
Columbella sp.	LS	1		1		2
	DS	1		2		3
Cantharus sp.	LS	2		4	1	7
Truncularіopsis sp.	LS		1	1	1	3
	DS			1		1
Buccinulum sp.	LS				1	1
	DS			1	1	2
Mitrella sp.	LS	1	1			2
Bittium sp.	LS	1				1
Hinia sp.	DS				1	1
Hexaplex sp.	DS				1	1
Tubes of vermetids	LS	2				2
Total		30	14	53	32	129

Table 3. Number (*n*) and percentage (%) of the analysed crabs according to sex and morph: light (LC) and dark (DC) crabs

	Females		Males		Total
	n	%	*n*	%	*n*
LC	30	68.2	14	31.8	44
DC	53	62.4	32	37.6	85
Total	83	64.3	46	35.7	129

et al., 1997), while a relation between different life phases and bathymetry has been seen in *Diogenes pugilator* (P. Roux, 1829) (Pessani et al., 2000a). Our data do not support this theory in this species, because the distance from the coast is not enough to cause a different distribution of the two morphs. As regards depth, in the two sampling sites *C. tubularis* was found only at depths equal or greater than the bathymetric limit reported for this species (7 m; Ingle, 1993). Consequently, it is not possible to know if, and in case how, a minor depth could influence the chromatic pattern.

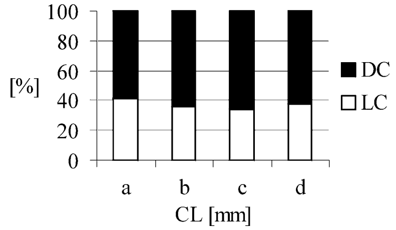

Figure 1. Percentage of light (LC) and dark (DC) crabs according to their size. Number of specimens per size class: a = 17; b = 32; c = 59; d = 21.

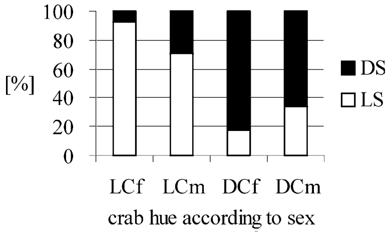

Figure 2. Percentage of dark (DC) and light (LC) crabs occupying light (LS) and dark (DS) shells according to the sex (f: females, m: males). Number of specimens: LCf = 30; LCm = 14; DCf = 53; DCm = 32.

The lack of association between morph and size shows that in *C. tubularis* colour is also not age-related, as suggested by Wolf, (1998) for *C. maenas*.

Reid et al. (1997) underlined that red *C. maenas* specimens are adult when reproductive, characterised by a long intermoult period; on the contrary, the green crabs are young in growth phase (see also Wolf, 1998). In *C. tubularis* we could not verify the same case because the examined specimens were all adult, potentially all in the reproductive phase. As a consequence, the chromatic patterns of *C. tubularis* seem to depend neither on intermoult period nor on reproductive status.

According to Jones et al. (1996), the different chromatic pattern could also be influenced by food type. We noted that both LC and DC reared in the laboratory show the tendency to fade. If diet could be an explanation for specimens reared in the laboratory (fed exclusively on mussels), it is unlikely that wild crabs living in the same site feed continuously on distinct resources to such an extent as to develop two different morphs.

A change of colour during the reproductive season occurs in *Uca tangeri* (Eydoux, 1835) (Wolfrath, 1993). This hypothesis is not confirmed for *C. tubularis*, because there is no significant difference between DC and LC numbers during reproductive and non-reproductive season (G test: $G = 0.346$, $df = 1$, $p = ns$).

In both sexes the two chromatic patterns were present in equal proportion (2/3 DC and 1/3 LC). As reported by Pessani & Premoli (1992) for red and green morphs observed in *C. timidus*, the two morphs ascertained in *C. tubularis* might be supposed to be due to a genetic polymorphism.

Eighty per cent of the crabs occupy *C. vulgatum* shells. This species lives preferably both on the upper part of Zosteraceae beds and at low depths on bottoms of rocks mixed with sand (Mars, 1954). According to Stachowitsch (1980) and Hazlett (1984), as long as the shell is occupied by the gastropod, it is free from epibionts, which begin its colonization when it is occupied by the crab. Therefore, in their habitat, *C. vulgatum* shells, beige coloured and slightly marbled, are perfectly mimic. Considering the depth difference between the habitat of *C. vulgatum* (less deep) and that where *C. tubularis* specimens were collected, we could suppose that the empty shells, still free from epibionts, have been conveyed deeply either by water movements or by crabs themselves. The epibionts settle after shell occupation by the hermit crab (Hazlett, 1984), giving the shell the dark or light hue. As a consequence, the crabs are mimic only if their body hue is close to that of the shell; both LC in DS and DC in LS are less cryptic than specimens occupying shells with colours resembling those of the body. As previously said, the habitat from which crabs were collected was characterized by the presence of green algae, the red *C. elongata*, and to a minor extent by concretions of polychaetes and bryozoans. Therefore, the hermit crabs succeeding in being cryptic not only with their shell but also in their habitat seem to have a greater possibility of escaping from predators that hunt by sight (*O. vulgaris* and *Diplodus* spp.). In fact, most of the crabs (79.3%) occupy a shell with a chromatic pattern resembling that of their body.

The preference for a shell with a hue close to that of the crab body is particularly evident in females. The need of being well cryptic in the environment (shell and habitat) may depend on their attempt to maximise the reproductive success, as reported e.g. by Merilaita & Jormalainen, (1997), in *Idotea baltica* (Pallas). In fact, being cryptic, the females possibly can avoid predation and have more chances to reproduce.

In the laboratory, preliminary experiments have shown that, when presented with DS and LS, *C. tubularis* chooses shells having a hue similar to that of its body (unpublished data).

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Trawl selectivity trials on the deep-water rose shrimp (*Parapenaeus longirostris*) in Sicilian waters

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Key words: trawl net selectivity, rose shrimp, *Parapenaeus longirostris*, Mediterranean Sea

Abstract

The selectivity of the traditional commercial bottom trawl net employed in Sicily to catch the Mediterranean deep-water rose shrimp, *Parapenaeus longirostris*, has been assessed. Two fishing campaigns were carried out in the Strait of Sicily and in the Southern Tyrrhenian Sea, using the covered cod-end method (mesh 20 mm vs. 31 mm). Of a total catch of 11,601 individuals, 23.4% escaped in the cover; the sample length structure from the Strait is unimodal, while that from the Tyrrhenian, polymodal. A logistic curve, fitted with a maximum likelihood criterion, has been used to model the selectivity data, in order to obtain the parameters $CL_{c50\%}$ (50% retention size), $SR_{75-25\%}$ (selection range) and SF (selection factor, i.e. $CL_{c50\%}$/mesh). The two sets of data, besides a larger selection range for the Strait sample (5.2 mm vs. 2.3 mm), produced very similar estimates (retention sizes of 13.0 mm vs. 12.8 mm CL), fitting the logistic curve well. Almost no shrimp larger than 20 mm does escape from the cod-end; moreover, from the amount of damaged specimens found in the cover, even the evaded shrimps sustain a high fishing mortality. An increase of the present cod-end mesh opening, even above the size required by the EU bylaws (at present, 40 mm stretched) seems necessary for managing the fishery.

Introduction

The deep-water rose shrimp, *Parapenaeus longirostris* (Lucas, 1846), represents one of the most common and sought-after species in the Mediterranean fisheries (Relini et al., 1999). While it is mainly fished between 100 and 400 m of depth, the rose shrimp distribution ranges from 20 to 700 m, on sand-muddy bottoms; size and male presence are positively correlated with depth (Froglia, 1982); although some geographical variations were observed (Sobrino et al., 2000), mature females are present in all seasons, but reproduction begins in winter and maturity is reached in Autumn and early Winter (Relini et al., 1999).

The Mediterranean bottom trawl fisheries traditionally operate using small diamond-shaped meshes in the cod-ends, which tend to retain almost all animals (Ragonese et al., 2000). Yet, smaller specimens are priced per kilogram much less than the larger ones; furthermore the use of such small mesh sizes leads to a by-catch, which is almost entirely discarded (Ragonese et al., 2001; Bianchini et al., 2003). Concerns have been raised about the capabilities of the resources to withstand the present fishing pressure.

The aim of this paper is to assess the trawl selectivity for the deep-water rose shrimp in the Sicilian waters; however, in this study only the cod-end selectivity has been taken in consideration, even if features other than the mesh size in the cod-end do influence the final selectivity of the trawl gear (Mac Lennan, 1992).

Materials and methods

Two fishing campaigns were carried out, respectively in the Strait of Sicily (autumn 1997; 27 hauls) and in the southern Tyrrhenian Sea (spring 1998; 16 hauls, 1 h each), using the covered cod-end method (mesh 20 mm vs. 31 mm, stretched), in depths up to 800 m.

Sampling was conducted with the commercial stern trawler "Sant' Anna" of 197 gross registered tonnage, powered by a 1012 HP engine; a commercial trawl net, made of nylon and polyamide, of Italian design and called "di fondale", was used.

The captured rose shrimps, both retained in the cod-end (IN) or escaped into the cover (OUT), were individually measured to the nearest mm with a watch caliper, taking the dorsal oblique length of the carapace (herein CL), from the orbital sinus to the internal posterior margin of the carapace. Weighing, counting and measuring were performed directly on board on fresh material, on the whole catch without subsampling.

A logistic curve,

$$\mathrm{p_{CL_i}} = \frac{\exp(\mathrm{a + b\ CL_i})}{1 + \exp(\mathrm{a + b\ CL_i})}$$

(where a and b are the intercept and slope coefficients after logit linear transformation and $\mathrm{p_{CLi}}$ is the retained proportion for each length class $\mathrm{CL_i}$), fitted minimizing a maximum likelihood criterion (Leonori & Fiorentini, 1998),

$$\sum_{\mathrm{CL}} \{\mathrm{n_{CL_{in}}} * \log_e(\mathrm{p_{CL_i}}) + \mathrm{n_{CL_{out}}} * \log_e[1 - \mathrm{p_{CL_i}}]\}$$

(where $\mathrm{n_{CLin}}$ and $\mathrm{n_{CLout}}$ denote the number of retained and escaped shrimps, and $\mathrm{p_{CLi}}$ the retained proportion in each length class $\mathrm{CL_i}$), has been employed to model the selectivity data, obtaining the parameters $\mathrm{CL_{c50\%}}$ (50% retention size) and $\mathrm{SR_{75-25\%}}$ (selection range).

A unique selection curve, and the corresponding parameters, was estimated by combining all the hauls, seasons and fishing grounds, assuming that the retention probability for each length class was invariant with time and that the selectivity pattern was the same for each haul, besides sampling variability (Ragonese et al., 2002). The selectivity coefficients were estimated for sexes combined.

The selection factor (SF), also known as the coefficient of selectivity, was computed too. In fact, SF corresponds to the coefficient of proportionality between the $\mathrm{CL_{c50\%}}$ and the corresponding mesh size for a given species and gear; as a rule, a direct linear proportionality ($\mathrm{CL_{c50\%}}$ = SF*mesh) is assumed between the two variables (Chevaillier & Laurec, 1990). To check whether this hypothesis holds in the rose shrimp fishery, present results were regressed together with analogous estimates available from the literature (Table 1).

Results and discussion

The accountable catch has been 11,601 individuals (8878 in the Strait, 2723 in the Tyrrhenian), of which 2712 escaped in the cover. It must be noted that the population structures were quite different between the two campaigns; in fact, the LFD from the Strait is unimodal (at 16-17 mm CL), while the Tyrrhenian LFD is polymodal.

The length compositions (absolute numbers) of the measurable rose shrimps retained in and escaped from the cod-end are reported in Fig. 1; almost no shrimp larger than 20 mm CL (i.e., around one year of age; Froglia, 1982; Nouar, 1985) does pass in the cover. A large fraction of the evaded rose shrimps are damaged or broken and many individuals cannot be measured. In the autumn data, 23.6% of the shrimps escaped, while the proportion was around 22.5% during spring. Juveniles are present exclusively in the spring sample, which displays a well-discriminated juvenile mode; only specimens belonging to this first component were able to escape. In fact, juveniles recruit during spring in the Tyrrhenian and Ionian seas (D'Onghia et al., 1998), but they peak in winter in the Strait of Sicily (Levi et al., 1995), confirming the adaptability of the life cycles in the rose shrimp (Sobrino & Garcia, 1994).

Considering the two campaigns, the selectivity parameters were $\mathrm{CL_{c50\%}} = 13.0 \pm 0.09$ mm, $\mathrm{SR_{75-25\%}} = 5.2 \pm 0.14$ mm, SF = 0.42 and $\mathrm{CL_{c50\%}} = 12.8 \pm 0.09$ mm, $\mathrm{SR_{75-25\%}} = 2.3 \pm 0.11$ mm, SF = 0.42,

Table 1. Selectivity parameters for different *Parapenaeus longirostris* stocks available in the literature

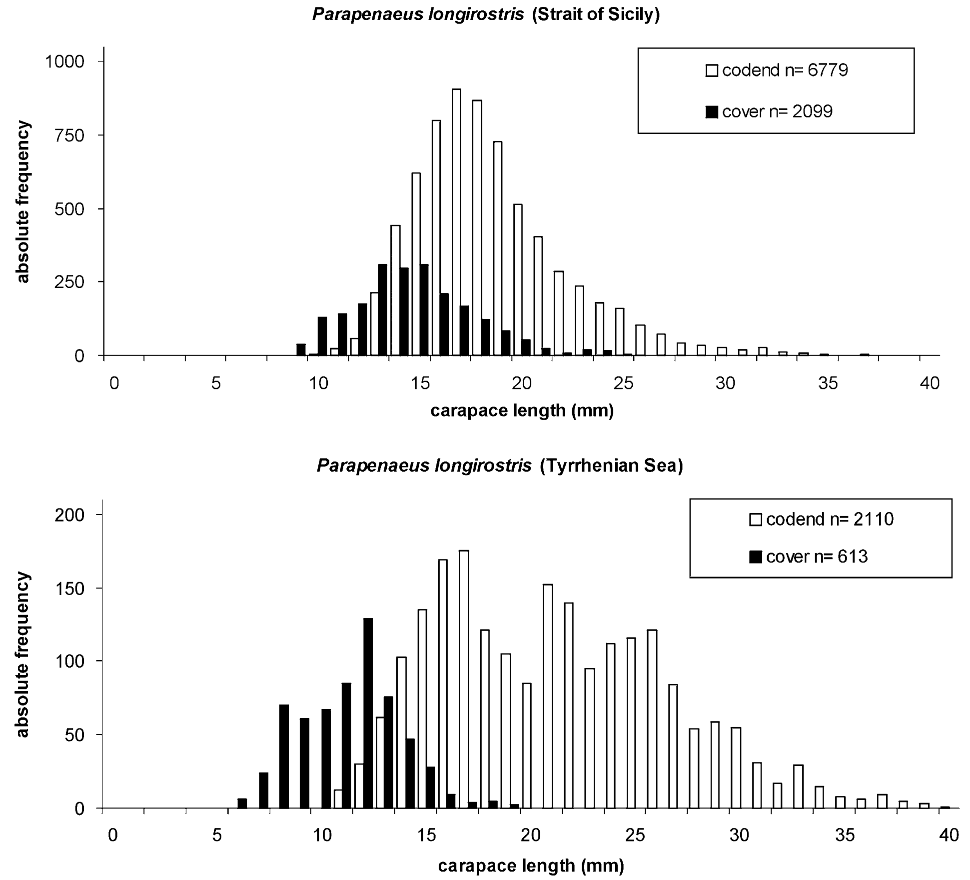
Mesh	$CL_{c50\%}$	SF	Area	Reference
31	13.0	0.42	Strait of Sicily	Present note
31	12.8	0.42	Southern Tyrrhenian Sea	Present note
36	14.1	0.39	Southern Tyrrhenian Sea	Unpublished data
40	14.2	0.36	Central Tyrrhenian Sea	Lembo et al., 2002
60	20.0	0.33	Central Tyrrhenian Sea	Lembo et al., 2002
40	16.8	0.42	Algeria	Nouar, 1985
34	12.9	0.38	Southern Ligurian Sea	GRU.N.D., 1999
38	10.6	0.28	Northern Tyrrhenian Sea	GRU.N.D., 1999
30	11.4	0.37	Central Tyrrhenian Sea	GRU.N.D., 1999
36	10.9	0.31	Southern Tyrrhenian Sea	GRU.N.D., 1999
40	13.4	0.34	Ionian Sea	GRU.N.D., 1999
36	14.3	0.40	Gulf of Cádiz	Sobrino et al., 2000
40	12.8	0.32	Gulf of Cádiz	Sobrino et al., 2000
40	14.7	0.37	Gulf of Cádiz	Sobrino et al., 2000
40	15.2	0.38	Gulf of Cádiz	Sobrino et al., 2000
40	15.8	0.39	Gulf of Cádiz	Sobrino et al., 2000
43	20.9	0.49	Gulf of Cádiz	Sobrino et al., 2000
46	19.2	0.42	Gulf of Cádiz	Sobrino et al., 2000
53	19.3	0.37	Gulf of Cádiz	Sobrino et al., 2000
60	26.3	0.44	Gulf of Cádiz	Sobrino et al., 2000
66	29.6	0.45	Gulf of Cádiz	Sobrino et al., 2000
55	24.7	0.45	Portugal	Pestana & Ribeiro-Cascalho, 1991
39	20.0	0.51	Morocco	Goñi, 1985
60	25.1	0.42	Morocco	Goñi, 1985

respectively for the Strait of Sicily and for the southern Tyrrhenian. The observed retained proportions and the estimated selection ogives are reported in Fig. 2; the maximum likelihood procedure shows a greater approximation in the upper range of selection, a result that can be explained by the weighed (by frequency) regression employed by ML, which gives a higher importance to the larger size classes. Notwithstanding the almost identical $CL_{c50\%}$s, the differences in the SR values determine profiles of dissimilar shape.

The selection parameters values are close to those already estimated with comparable mesh sizes in other Italian areas (Table 2), and particularly in the Central Tyrrhenian ($CL_{c50\%} = 14.2$, SR = 2.9 mm, SF = 0.36; Lembo et al., 2002) and in the Southern Tyrrhenian ($CL_{c50\%} = 14.1$, SR = 4.0 mm, SF = 0.39; unpublished data); this is also true with the estimates from Algeria, i.e. $CL_{c50\%} = 16.8$ mm and SR = 4.4 mm (Nouar, 1985).

Given the gradient in sizes, the length at first capture is often higher on the slope than on the shelf, as shown in the southern Tyrrhenian (unpublished data). This phenomenon is evident in the Strait too, where the selection process is more pronounced at depths between 50 m and 200 m, in which 26.7% of the rose shrimps escapes from the cod-end; the proportion is only 16.4% between 200 m and 300 m, and lowers to 1.6% in deeper hauls.

The straight-line regression fitted to the whole known set ($n = 24$) of selection parameters presented in Table 1 has a slope, i.e. the SF, of 0.459 (SE = 0.048; standard error of the estimate (SEE) = 2.373; mean square error (MSE) = 5.6; $p = 0.000$; df = 22), but the intercept does not differ from zero (−2.758 mm; SE = 2.124; $p = 0.208$; df = 22) (Fig. 3). Repeating the regression through the origin (i.e., applying the theoretical model: $L_{c50\%} = SF{*}mesh$), the estimation of the SF lowers to 0.398 (SE = 0.011; SEE = 2.408; MSE = 5.8;


Parapenaeus longirostris (Strait of Sicily)

Parapenaeus longirostris (Tyrrhenian Sea)

Figure 1. Overall length structures of *Parapenaeus longirostris* retained (cod-end) and escaped (cover), by fishing area.

$p = 0.000$; df = 23). Considering that the size at 50% of maturity ranges between 20 mm and 28 mm (Sobrino & Garcia, 1994; Relini et al., 1999), the corresponding suggested range for a "biologically-sound mesh size" is 50.2–70.3 mm. Even considering the lowest limit, such opening is well above the requirements of the European legislation (40 mm). Mesh sizes below 50 mm, consequently, tend to reduce the spawning potentiality of the stock, determining the direct (by capture) and indirect (individuals lost to fishermen, but damaged after escaping) death of a great amount of shrimps, the most part still immature.

Conclusions

The sustained exploitation of any marine living population requires management procedures, among which the regulation of the cod-end mesh size is one of the most appealing. Selectivity studies toward a suitable mesh size in the cod-ends are attractive for their relative conceptual and experimental simplicity, with easy implementation (Fiorentino & Ragonese, 2000).

General characteristics of the shrimp trawl fisheries are a very high fishing pressure (Gulland & Rothschild, 1984) and the fine mesh employed. However, the exploitation of young under-sized shrimps produces a condition of "economic growth overfishing", given that the market value increases markedly with individual size, and a growing attention has been focused at improving the selectivity of the shrimp gear.

The present selectivity trials show that, as a rule, the net utilized retains almost every rose shrimp encountered; even the animals which manage to sieve out are damaged, and many could not be measured. It is evident that the cod-end presently in use is insufficiently selective; more-

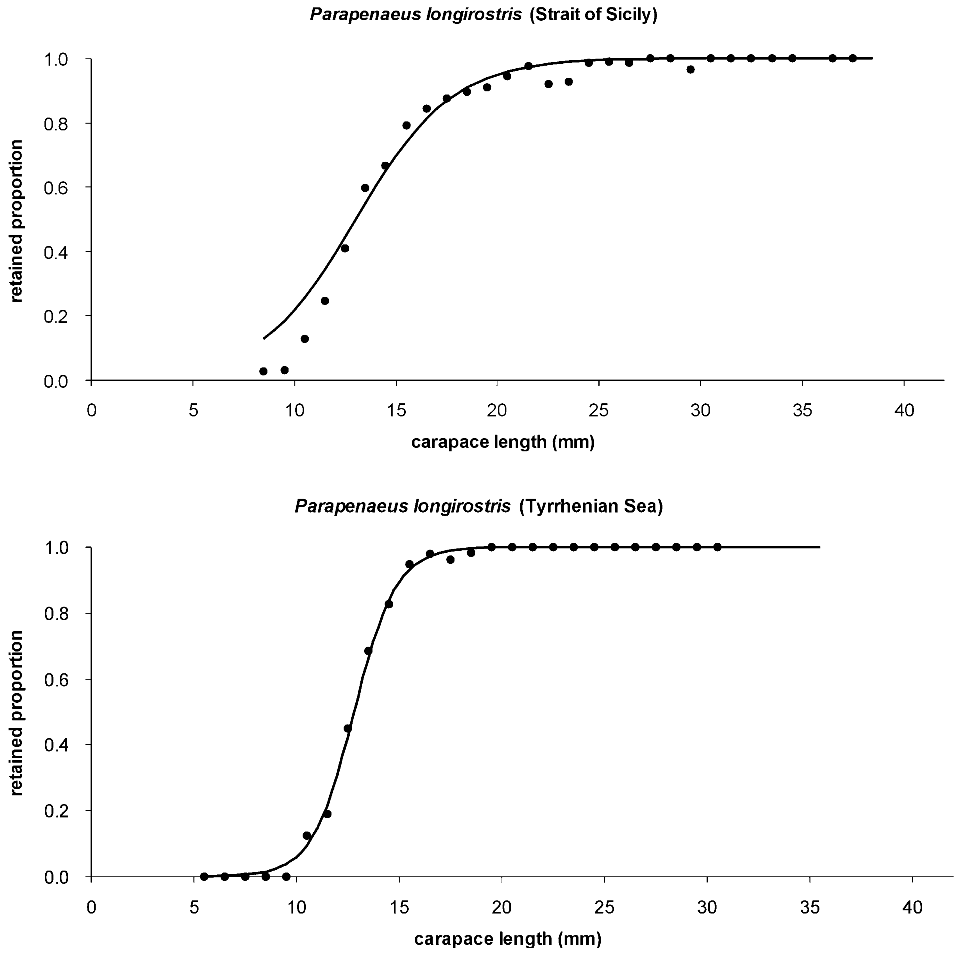


Figure 2. Observed retained proportions (dots) and estimated selection ogive for *Parapenaeus longirostris* according to the maximum likelihood procedures, by fishing area.

over, previous results clearly indicate that even the legal measure (40 mm) is not large enough (GRU.N.D., 1999). In particular, Levi et al. (1995) estimated that a mesh of 43 mm should optimize the yield maintaining the current level of effort in the Strait of Sicily. Further increasing the

Table 2. Selectivity parameters for *Parapenaeus longirostris* from different Italian fisheries, obtained using the same methodology (cover of 20 mm mesh; data from GRU.N.D., 1999)

U.O.	cod-end mesh (mm)	Retained # IN	Escaped # OUT	$CL_{c50\%}$ mm ± SE	$SR_{75–25\%}$ mm ± SE	SF
2	34	2537	72	12.9 ± 0.4	3.2 ± 0.4	0.38
3	38	7860	22	10.6 ± 0.6	3.6 ± 0.4	0.28
4	30	15236	341	11.4 ± 0.0	3.5 ± 0.0	0.37
5	36	16057	1583	10.9 ± 0.1	4.1 ± 0.1	0.31
6	36	5816	663	14.1 ± 0.1	4.0 ± 0.1	0.40
10	40	27475	3332	13.4 ± 0.0	2.4 ± 0.0	0.34

U.O. 2 = southern Ligurian Sea; U.O. 3 = northern Tyrrhenian Sea; U.O. 4 = central Tyrrhenian Sea; U.O. 5 = southern Tyrrhenian Sea; U.O. 6 = southern Tyrrhenian Sea; U.O. 10 = Ionian Sea.

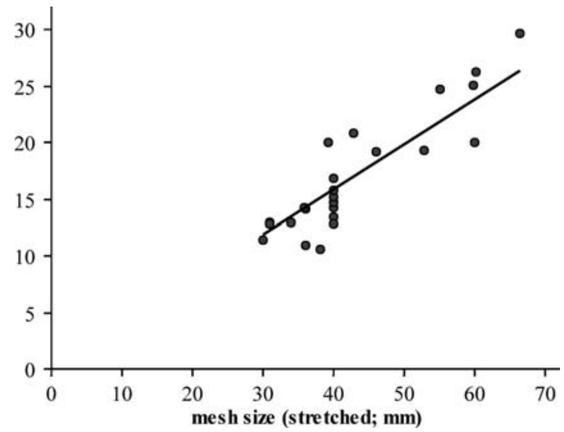


Figure 3. Regression of the size at capture (50% retention, CL in mm) vs. the mesh size (stretched, in mm) known from the literature (see Table 1) for different *Parapenaeus longirostris* stocks.

mesh size should allow some more escapement, with likely benefits for the environment and for the fishery, both biologically (reduction of the "size overfishing") and technologically (e.g., by minimizing handling time and improving the catch quality), and probably only minimal short-term economic losses, as demonstrated with other shrimps (Bianchini et al., 2002); in any case, the economic loss will be recovered in the long term (Sobrino et al., 2000; Lembo et al., 2002).

Moreover, considering that the rose shrimp fishery, like most of the Mediterranean fisheries, is multi-species, its management requires, especially on the shelf bottoms, a careful consideration of the simultaneous presence of the juveniles of other demersal resources; therefore, there is general agreement on the need for increasing the mesh sizes used in shrimp fisheries, and an acceptable mesh size should not be smaller than 48 mm.

Nevertheless, the change of mesh size cannot be used by itself as the only management tool and other interventions (e.g., temporal and spatial closures) must be considered to maintain both the future resources and a sustainable fishery.

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Catch composition and damage incurred to crabs discarded from the eastern Ligurian Sea "rapido" trawl fishery

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Key words: damage, crustaceans, discard, trawling, fishing impact, western Mediterranean

Abstract

Catch composition and damage incurred to the populations of three crabs (*Liocarcinus depurator*, *Medorippe lanata* and *Goneplax rhomboides*), discarded by the "rapido" trawl fishery of Viareggio (eastern Ligurian Sea), was investigated. Data were collected on a monthly basis (January–December 2001) through embarks on board of a commercial fishing vessel, so as to reflect commercial fishing practice. The three species represented from 7 to 31% of the discarded biomass, varying with season. Density and biomass indices were characterised by a clear seasonality, each species showing a peak in a different period of the year. A six level scale, based on macroscopic external injuries was used to assess the damage sustained by each species after fishing and sorting operations. For the three species, very similar percentages (56–58%) of individuals were macroscopically intact, while inspection of damaged individuals revealed some differences in the distribution of injuries. *G. rhomboides* showed the highest proportion of serious damage, followed by *L. depurator* and *M. lanata*. The severity and frequency of damage was mainly correlated with interspecific and intraspecific (sexual dimorphism) morphological and behavioural characteristics. For example, specimens with longer and thin appendages, like males of *G. rhomboides*, seemed to be particularly vulnerable to the fishing and sorting operations; this could also explain the positive correlation between damage level and size found in this species. In *M. lanata*, small individuals proved to be more vulnerable, while for *L. depurator* no significant correlation was found between damage and size. Although these estimations did not take other injury typologies into account (e.g. internal and physiological damage), these results can be seen as a first indirect estimation of discard mortality for these species and can represent an important term of reference both for similar studies performed in other areas and for the monitoring of this fishery.

Introduction

In recent years there has been a general perception that classical stock assessments, based on single species approaches, are often ineffective tools for fisheries management policies and may lead to uncertainty when choosing management options in a multispecies fishery context (Brugge & Holden, 1991). A more holistic approach to resource management is needed, with special emphasis on the ecosystem level. Therefore, studies on the species assemblages subjected to exploitation, on by-catch and discards, as well as on the ecological impact of the fishing activity have recently been added to the classical investigations that tended to focus on the target species (Alverson et al., 1994; Gislason, 1994; Jennings & Kaiser, 1998; Hall, 1999). In this perspective, increasing scientific effort is being devoted to the studies of the discarded fraction of by-catch and in particular on the fate of this important component of the catch.

More attention has recently been drawn to estimation of the mortality of discarded species, both with a direct approach (e.g. survival experiments, Kaiser & Spencer, 1995; Bergmann & Moore, 2001; Bergmann et al., 2001b) and indirect assessment (e.g. evaluation of external damage, Kaiser, 1996; Farmer et al., 1998; Bergmann et al., 2001a; Pranovi et al., 2001; Veale et al., 2001). Although survival experiments represent the best way to estimate mortality, this approach is often expensive and time-consuming. The alternative approach is based on the macroscopic evaluation of injuries sustained by the discarded species due to their sensibility (MacDonald et al., 1996) and can represent a first estimate of the mortality produced during the fishing and sorting operations. Several damage scales have been proposed, according to different taxa (e.g. Wassenberg & Hill, 1989; Kaiser & Spencer, 1995; Kaiser, 1996; Bergmann et al., 2001a; Pranovi et al., 2001), standardising this evaluation with simple sampling protocols.

It is obvious that fishing activities not only involve a direct impact on target species but also affects the whole marine community to various degrees (Rice & Gislason, 1996). Mobile demersal gears (e.g. trawling, dredging) undoubtedly produce the major environmental effect, both from the physical and biological point of view (Bergman & Hup, 1992; Jennings & Kaiser, 1998; Kaiser et al., 1998; Kaiser & De Groot, 2000; Sanchez et al., 2000). One towed gear about which little is still known is the "rapido" trawl. This is a bottom trawl gear employed on soft bottoms in Italy mainly to catch sole (*Solea vulgaris* Quensel, 1806) and scallops (*Aequipecten opercularis* (Linnaeus, 1758) and *Pecten jacobaeus* (Linnaeus, 1758)). "Rapido" is a modified beam trawl, a gear at present largely used in many northern European countries, with a rigid mouth fitted on the lower part with iron teeth; it is usually towed at a greater speed than otter trawls, which accounts for its name "rapido" (Giovanardi et al., 1998; Hall-Spencer et al., 1999; Pranovi et al., 2001). At present, this gear is largely used in the northern and central Adriatic Sea, where consistent fleets (both in number and vessel size) are present in several ports (Fabi & Sartor, 2002). In contrast, only a very small number of vessels fish with "rapido" along the western Italian coasts because of the unsuitability of the seabed and to the reduced number of licenses granted for this gear in the area in question. One such example is the fleet of Viareggio (Tuscany), with only two boats, that are lower in size and engine power when compared to those from Adriatic.

Despite the fact that "rapido" trawl is generally considered more selective for the target species than other trawls, several other commercial and non-commercial species of fish, crustaceans and molluscs are incidentally caught with this gear and the discarded biomass can represent an important fraction of the catch (Pranovi et al., 2001; Fabi & Sartor, 2002). Discards are mostly non-commercial species, especially those typical of the soft bottom benthic megafauna (Fabi & Sartor, 2002).

The three crabs *Liocarcinus depurator* (Linnaeus, 1758) (Brachyura: Portunidae), *Medorippe lanata* (Linnaeus, 1767) (Brachyura: Dorippidae) and *Goneplax rhomboides* (Linnaeus, 1758) (Brachyura: Goneplacidae) represent ecologically important species in the soft bottoms communities of continental shelf and slope of the Mediterranean basin (Abelló et al., 1988). Fabi & Sartor (2002) reported high catch rates of these species produced by "rapido" trawlers in two areas of the central Adriatic and eastern Ligurian Sea, where these species have low or no commercial value and are routinely discarded at sea by trawlers.

The main objectives of this study were the estimation of discard quantities and the evaluation of damage sustained by the three crab species produced by the "rapido" trawl fishery of Viareggio (eastern Ligurian Sea). The work endeavours to assess for each species the damage sustained, as a function of sex, size, maturity stage and volume of the total catch, in order to have a first estimate of its ability to survive when discarded at sea.

Material and methods

Description of the study area

The area investigated corresponds to the fishing grounds usually exploited by the Viareggio "rapido" trawl fleet. They are located in a zone of the south eastern Ligurian Sea, between 8 and 12 miles from the coast, from Meloria Bank in the south to the Gulf of La Spezia in the north, over an area of about 800 km^2; the fishing depth ranges from 20 to 60 m (Fig. 1). This zone is strongly influenced by freshwater outflow of the R. Magra (north), R. Serchio and R. Arno (south).

Collection of samples and data analysis

Sampling was carried out on a monthly basis on board of commercial vessels, from January to December 2001. In this period, a total of 91 commercial hauls were monitored by scientific personnel, without interfering with the normal fishing practice. The fishing grounds, duration of the hauls and sorting of the catch were exclusively decided and carried out by the crew of the vessel.

The vessel used was a trawler of the Viareggio fleet (overall length = 18.4 m; engine power = 210 kw; Gross Tonnage = 31). The fishing gear consisted of two "rapido" trawls of 3 m width, provided with 4 skids and one wooden plank placed above the upper side of the iron frame (see Hall-Spencer et al., 1999 for the design). Thirty-three iron teeth (diameter of 12 mm) were fixed to the lower side of the "rapido" mouth and extend 0.5–1 cm behind the tooth bar. The net, about 5 m long, had a cod-end with a mesh size (stretched) of 40 mm. The two "rapido" trawls are usually towed at once at a speed of 9–10 km h^{-1}; fishing was carried out essentially by day for about 12 h, performing 7–8 hauls of about 1.5 h each.

The catch obtained in each haul was subdivided into the following categories, according to the commercial procedures adopted by the fishermen:

- Target species and kept by-catch: all the catch of *S. vulgaris* and the portion of the catch retained and landed with the target species.
- Discarded by-catch: individuals caught but not retained; this fraction comprised commercial

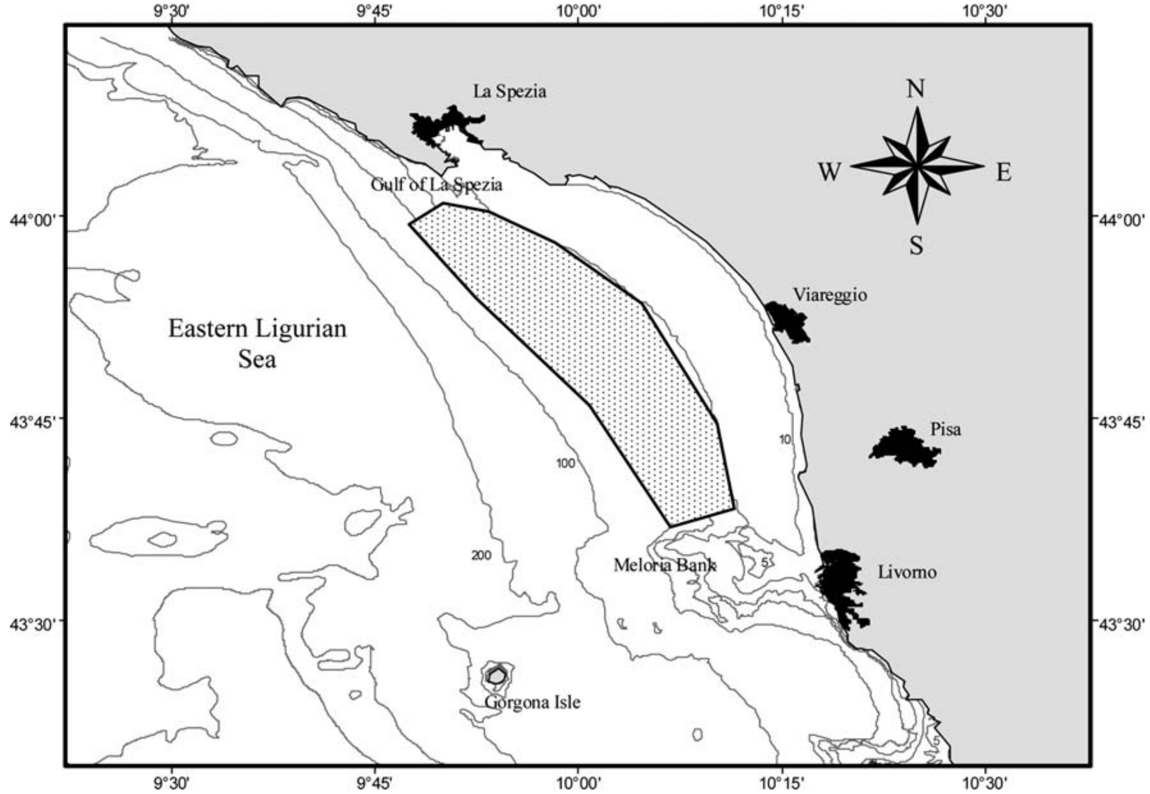

Figure 1. Fishing grounds of the rapido trawl fleet of Viareggio (shaded area).

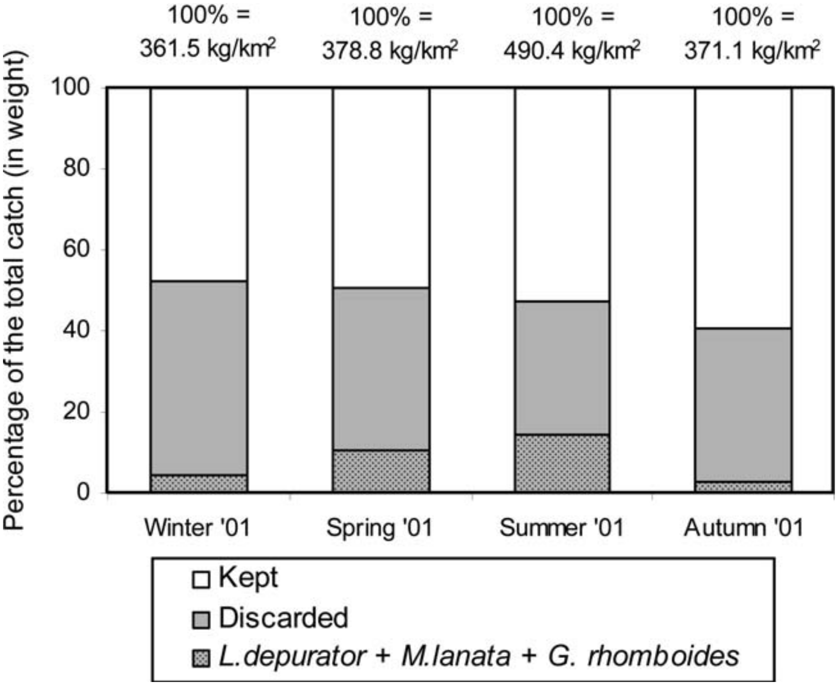

Figure 2. Percentage composition of the total catch obtained in different seasons.

species (specimens either damaged or below the minimum landing size) and non-commercial species (the fraction of the catch without commercial value).

The number of individuals and the total weight (kg) was recorded for each species. For each haul, the abundance and biomass values were standardised as density and biomass indices (number of individuals km^{-2} and kg km^{-2}, respectively). The swept area of each haul was calculated by taking into account trawling speed, haul duration, horizontal opening and number of the gears used.

For each haul, a random sample of *L. depurator*, *M. lanata* and *G. rhomboides* was collected from the discards after the completion of the sorting operations. The size (CL = carapace length, to the nearest millimetre), sex and number of ovigerous individuals were recorded on board of the fishing vessel.

To assess the effect of the "rapido" trawl gear on the individuals caught, a six-level scale (0–5) based on macroscopic visible damages was used, following Wassenberg & Hill (1989) and Pranovi et al. (2001) (Table 1). The mean value of damage level was calculated for each species; maximum level of damage (5) can be also considered as a minimum estimate of discard mortality, in agreement with Kaiser & Spencer (1995), who reported no survival in crabs with damaged carapace. We would like to emphasize that we were unable to distinguish between recent and older injuries, nor was it possible, according to this protocol, to take into account autotomy.

Table 1. Scale based on macroscopically visible injuries used to categorise damage sustained by the three species of crabs

Damage level	Description
0	Intact
1	One limb missing
2	Two or more limbs missing
3	One chela missing
4	One chela and one or more limbs missing
5	Both chelae missing and/or body crushed

Comparisons in the damage extent among species, sexes and, for females, maturity stage (presence of ovigerous females) were performed by a non-parametric Mann–Whitney U-test (Siegel, 1956). For each species, the Spearman's rank correlation (Siegel, 1956) was used in order to test if damage level was correlated with size or weight of the total catch (all species).

Results

The mean seasonal catch with "rapido" trawls recorded in the entire year of study ranged from 361.5 ± 23.1 kg km^{-2} (winter 2001) to 490.4 ± 22.6 kg km^{-2} (summer 2001). Roughly half of this biomass (from 47.8 to 59.5%, in the different seasons) consisted of commercially retained species, the remainder was returned to sea (Fig. 2). Discards consisted of a large number of species, most of them of no commercial value. This fraction was dominated by molluscs (62% of the discarded biomass, on annual average), in particular the gastropods *Turritella communis* Risso, 1826 and *Aporrhais pespelecani* (Linnaeus, 1758), followed by crustaceans (19%), echinoderms (17%, mainly *Astropecten irregularis* (Pennant 1777) and fish (2%). The three species forming the object of this study, *L. depurator, M. lanata* and *G. rhomboides* accounted for practically all of the crustaceans discarded, both in terms of abundance and biomass and, depending on the season, represented between 7% (autumn 2001) and 31% (summer 2001) of the discards in weight.

Monthly average density and biomass indices of the three crabs showed a temporal pattern, with evident seasonality (Fig. 3). Analysis of the weight index showed that *L. depurator* values peaked in June and July (54.8 ± 6.4 kg km^{-2} and 58.8 ± 7.2 kg km^{-2}, respectively), while those of *M. lanata* had maximum values in the subsequent 2 months (50.6 ± 12.6 kg km $^{-2}$ in August and 43.5 ± 9.01 kg km^{-2} in September). Values for *G. rhomboides* were lower than in the other two species and showed two peaks, one in August and another, more important one, in March and April (11.9 ± 3.0 kg km^{-2} and 12.7 ± 1.9 kg km^{-2}, respectively). The monthly trend of the density index essentially reflected that of the biomass values, the highest values being recorded in June–July for *L. depurator* (maximum of 8184 ± 995 ind. km^{-2} in July), in August–September for *M. lanata* (peak of 3368 ± 844 ind. km^{-2} in August) and in April for *G. rhomboides* (2127 ± 245 ind. km^{-2}).

Wide size spectra were observed, especially for *L. depurator* (12–34 and 13–36 mm CL for females and males, respectively) and *M. lanata* (10–29 and 9–29 mm CL for females and males, respectively) (Fig. 4). Sizes of the samples of *G. rhomboides* ranged from 7 to 20 mm CL for females and from 7 to 21 mm CL for males.

In the sampled population of *G. rhomboides* males (630) clearly outnumbered females (94), giving a sex ratio significantly divergent from 1:1 ($p = 0.000$, Chi-square test), while in the other two species a slight predominance of females was observed (p = 0.016 in *L. depurator* and $p = 0.022$ in *M. lanata*) (Fig. 4).

Values indicating the percentage of specimens free from visible external damages were not markedly different among the various species, ranging from 55.5% in *G. rhomboides* to 58.4% in *M. lanata*. With regards to the injured individuals, on the other hand, some differences were detected among the three species (Table 2; Fig. 5). Thus, in *M. lanata* the most frequent damage was of level 1 and 2, while in *L. depurator* and especially in *G. rhomboides*, damage of type 3–4 and 5 was frequently observed. Overall, the mean level of damage observed in *M. lanata* (0.76) was significantly lower ($p < 0.05$, Mann–Whitney U-test) than those observed in *L. depurator* (1.17) and in *G. rhomboides* (1.22). Furthermore, when the frequency of type 5 damage (both chelae missing and/or body crushed) was considered, evident differences among the three species were detected, with the highest values obtained in *G. rhomboides* (8.8%), followed by *L. depurator* (5.1%) and *M. lanata* (1.1%).

These differences were confirmed also by analysis of the distribution of these injuries as a function of the different appendages (Table 3). Loss of the chela was the most frequent damage sustained by *G. rhomboides* and *L. depurator*, while for *M. lanata* the most frequent loss of limbs concerned pereiopods 2 and 3. For all the three species, no statistically significant differences were found in frequency of the injury sustained by the same appendage, if placed on the left or the right side of the body (Chi-square test, $p > 0.05$).

The incidence of damage was also analysed as a function of sex. Highly significant differences, for *G. rhomboides*, and significant differences, for

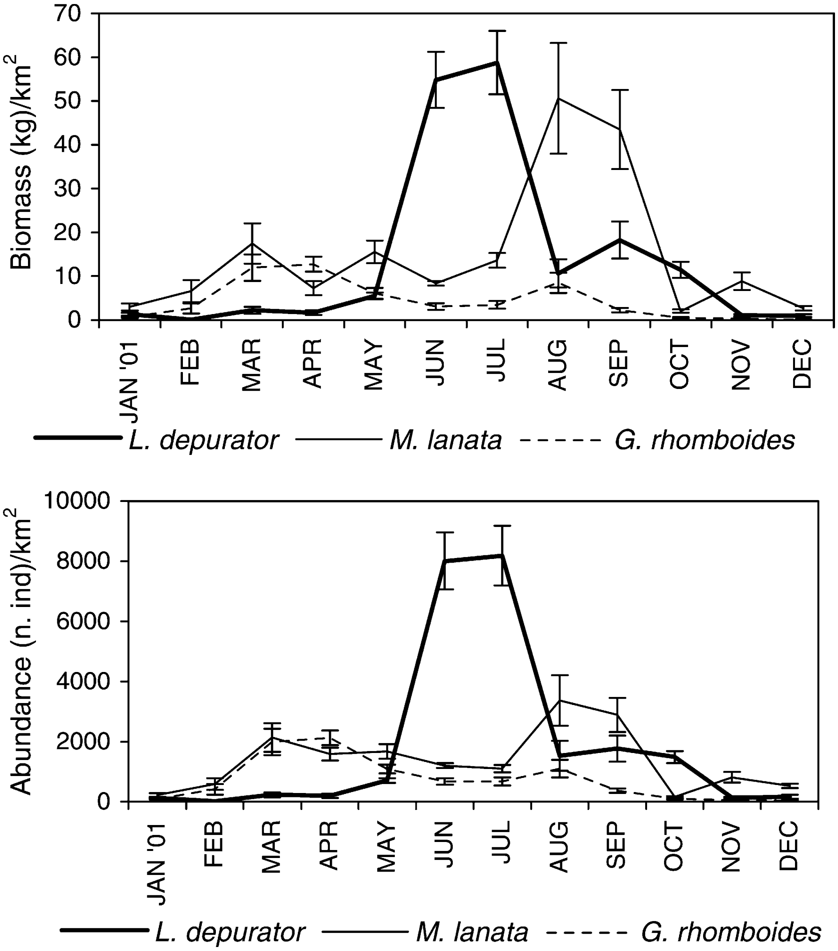

Figure 3. Mean monthly indices in biomass (above) and density (below), by species.

L. depurator, were found between the two sexes, showing a mean damage level higher in males than in females in both species (Table 4), while in *M. lanata* the injuries sustained by females did not statistically differ from those sustained by males. Ovigerous females of *M. lanata* were significantly less injured than females without external eggs, while in *L. depurator* no statistical differences were found when comparing the damage sustained by females in the two maturity stages (Table 5). For *G. rhomboides* it was impossible to perform this analysis due to the scanty number of ovigerous females collected.

Frequency of damage was found to be significantly correlated with body size in *M. lanata* (higher damage level found in small specimens) and in *G. rhomboides* (higher damage level in the larger specimens) (Table 6). Finally, for all three species no significant correlation was found between the amount (weight) of the catch and the level of damage (Table 7).

Discussion and conclusion

It is well known that fishing involves direct and indirect effects not only on the target species and by-catch, but also on the habitat, biodiversity and productivity of benthic communities (Jennings & Kaiser, 1998). Trawling is unquestionably one fishing activity with important impacts on both the exploited resources and the benthic ecosystems, in

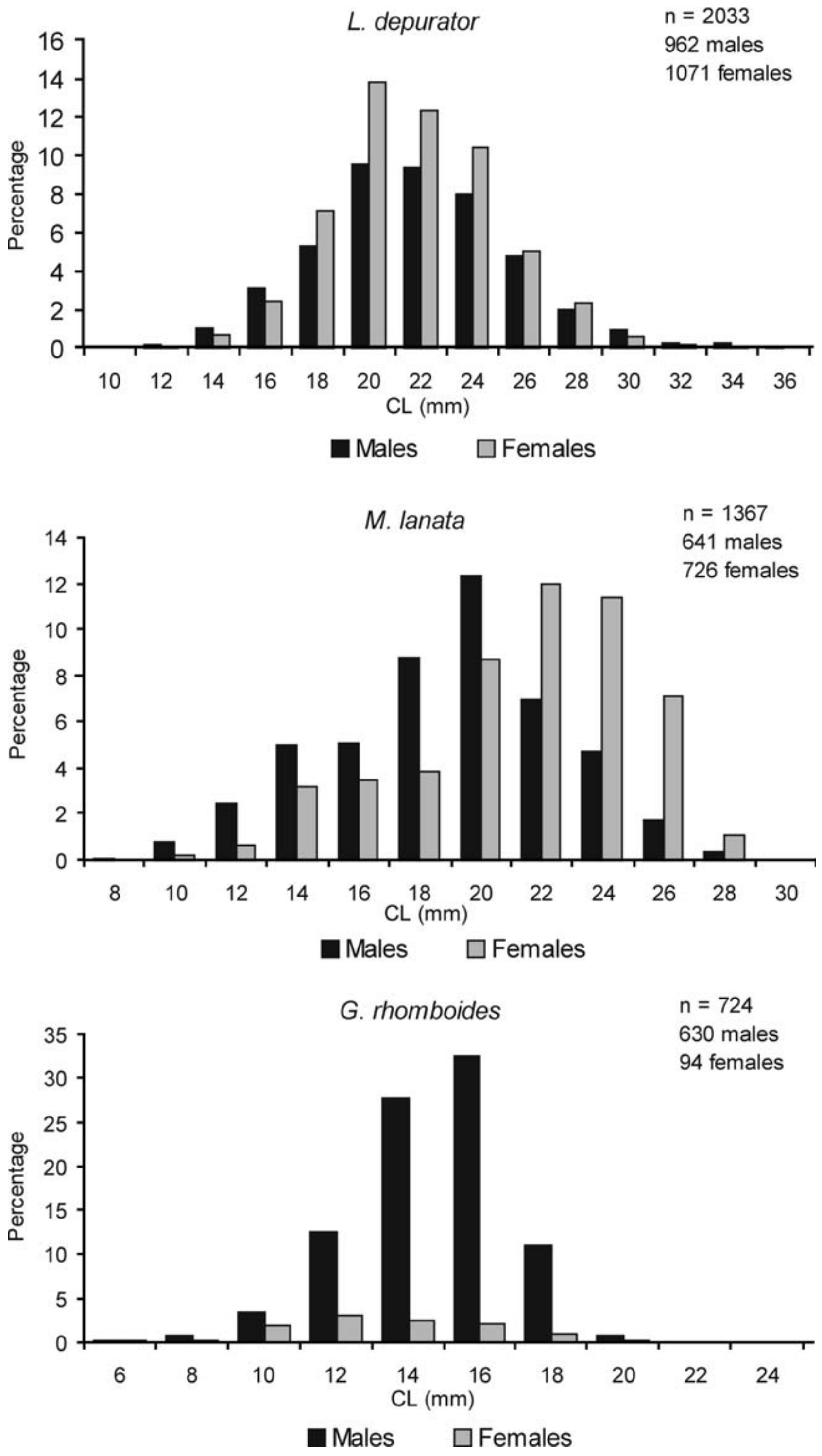

Figure 4. Size frequency distribution of the catch obtained in the whole period of study, by species.

Table 2. Mean damage level, percentage of individuals without macroscopic injuries and percentage of individuals showing the maximum level of damage, calculated for each species (*n* = number of individuals; SE = standard error)

Species	*n*	Mean damage level	SE	Without visible damage (%)	Maximum damage (%)
L. depurator	2033	1.174	0.036	57.6	5.1
M. lanata	1367	0.756	0.031	58.4	1.1
G. rhomboides	724	1.218	0.063	55.5	8.8

particular the epifauna and the organisms inhabiting the upper sediment layers (Kaiser & De Groot, 2000). It has been estimated that 20–40% of the total catch is discarded by Mediterranean trawlers, including "rapido" (Carbonell et al., 1997; Machias et al., 2001; Pranovi et al., 2001; Fabi & Sartor, 2002).

Previous studies on towed gears have clearly detected external injuries on discarded individuals. Damage is more frequently found in certain groups, such as echinoderms (echinoids and ophiuroids) and crabs, in comparison to gastropods and hermit crabs, with extent of damage depending on the fragility and sensitivity of each species (Wassenberg & Hill, 1993; Kaiser & Spencer, 1995; Bergmann et al., 2001a; Pranovi et al., 2001).

From our observations it was found that, apart from fishes, most of the species discarded by the "rapido" trawling were still alive when discarded, although often displaying different levels of external damage (Fabi & Sartor, 2002). The results showed that about 45% of the specimens of *L. depurator*, *M. lanata* and *G. rhomboides* sustained external damage after fishing (trawling and sorting on deck), the remaining fraction being composed of live and apparently intact specimens. Only a restricted portion, 9% of the specimens, as a maximum in *G. rhomboides*, showed the highest level of damage corresponding to loss of both chelae and/or to injured or crushed carapace.

To date, several studies have been carried out to estimate damage sustained by discarded species after fishing, especially in the Atlantic (Kaiser, 1996; Bergmann et al., 2001a; Pranovi et al., 2001; Veale et al., 2001). However, regarding the species that are the focus of this study, only for *L. depurator* a consistent series of results is available. For this species, results of the present study substantially agree with the findings of Kaiser & Spencer (1995) for beam-trawling (52% of specimens with missing legs) and Bergmann et al. (2001a) for the Scottish Norway lobster otter-trawl fishery (47% of damaged specimens). The latter authors also reported a similar value of damage for the long-clawed squat lobster *Munida rugosa* (Fabricius, 1775) (57%).

On the other hand, studies performed on "rapido" trawling in the northern Adriatic reported higher damage levels than those recorded in the present study. Pranovi et al. (2001) used a damage scale similar to that employed in this study and found that about 90% (88% for the flatfish fishery, 95% for the scallop fishery) of *Liocarcinus* spp. sustained damage, and ca. 30% of the individuals incurred the highest level of injuries. Similarly, Hall-Spencer et al. (1999) found that 16% of *L. depurator* incurred severe damage by the "rapido" trawl fishery for scallops in northern Adriatic.

These differences could be due to the different technical characteristics of the gears employed in the two different study areas, as well as due to differences in catch composition. "Rapido" trawls in the Adriatic are considerably larger in size as compared to those used in use in the eastern Ligurian sea; this may have produced notable differences in the volume of the catch obtained in each haul; particularly striking is the difference concerning the amount of debris, the fraction especially composed by dead shells, stones and wood (Pranovi et al., 2001; Fabi & Sartor, 2002). Bergmann et al. (2001a) have pointed out that large heavy catches, especially when the contribution of "hard" material is considerable, increase the probability of injury during the haul itself, as well as the compression upon hauling and whilst on deck. In the case of "rapido" trawl fisheries this is particularly true when the gear is employed on bottoms that harbour hard biogenic structures, often producing huge amounts of hard

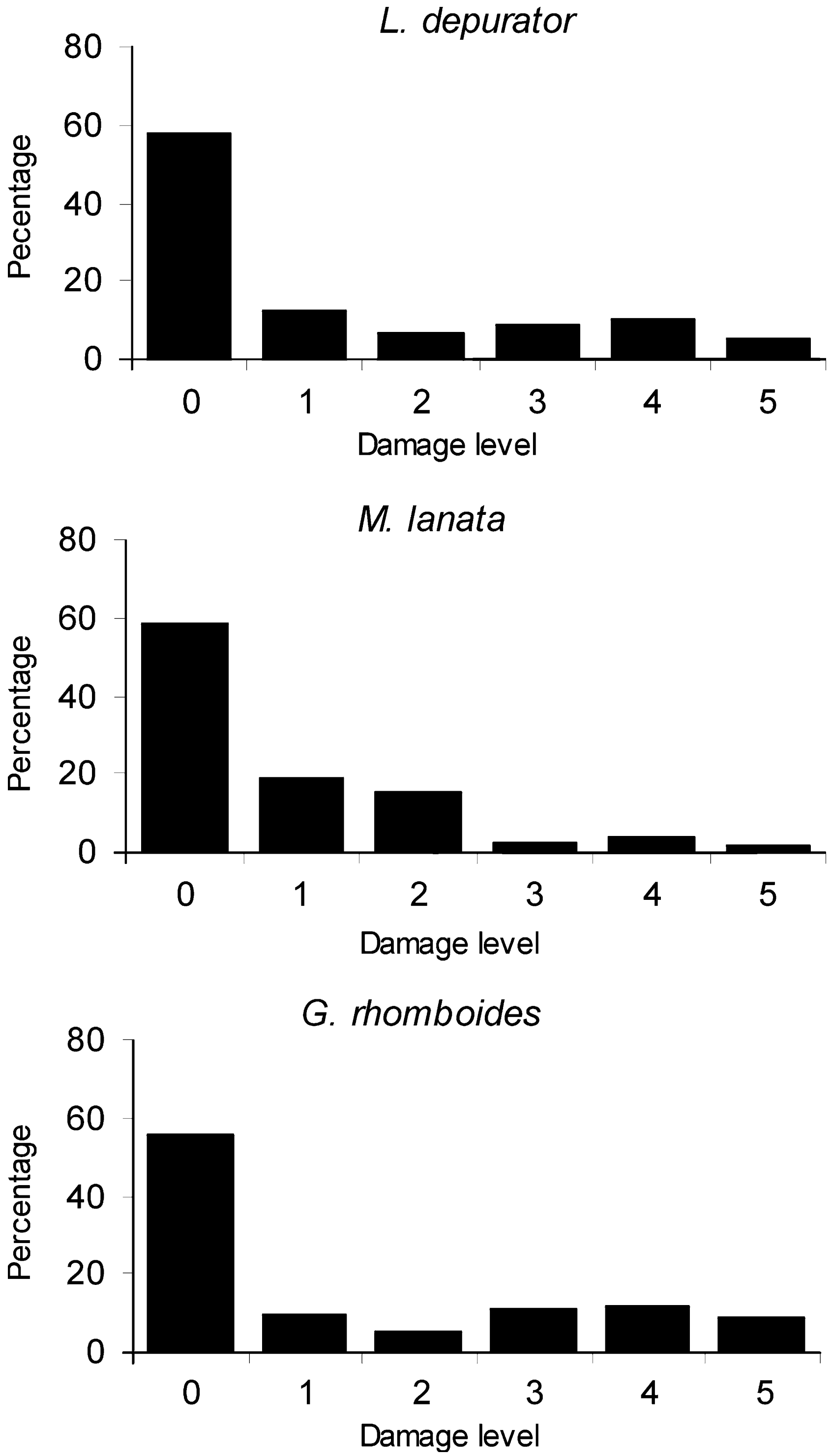

Figure 5. Frequency of damage level recorded in the three species after the on-deck sorting operations.

Table 3. Number of individuals, for each species, with missing appendages (P2–P5 = pereiopods 2–5)

Species	*n*	Intact	Number of damaged individuals					
			Position	Chela	P2	P3	P4	P5
L. depurator	2033	1171	Right	317	183	168	183	88
			Left	282	171	188	177	96
M. lanata	1367	798	Right	57	147	142	84	82
			Left	56	166	162	79	90
G. rhomboides	724	402	Right	123	42	40	52	55
			Left	118	38	48	52	39

Table 4. Mean damage level calculated by sex, for each species (*n* = number of individuals; SE = standard error; U = value of the Mann–Whitney U-test; *p* = significance level, * = significant, NS = not significant)

Species	Sex	*n*	Mean damage level	± SE	U	*p*
L. depurator	Females	1071	1.120	0.033	474675.5	0.043 *
	Males	962	1.240	0.041		
M. lanata	Females	726	0.744	0.028	229866.0	0.699 NS
	Males	641	0.769	0.030		
G. rhomboides	Females	94	0.798	0.082	25147.5	0.008 *
	Males	630	1.280	0.051		

Table 5. Mean damage level calculated for ovigerous and non-ovigerous females, for each species (*n* = number of individuals; SE = standard error; U = value of the Mann–Whitney U-test; *p* = significance level, * = significant, NS = not significant)

Species	Maturity stage	*n*	Mean damage level	± SE	U	*p*
L. depurator	Ovigerous	136	1.090	0.093	66100.0	0.804 NS
	Non-ovigerous	985	1.110	0.035		
M. lanata	Ovigerous	298	0.389	0.023	45156.0	0.000 *
	Non-ovigerous	428	0.991	0.048		

Table 6. Spearman's rank correlation between carapace length and damage level (*r* = correlation coefficient; *p* = significance level; * = significant, NS = not significant)

Species	*n*	*R*	*p*
L. depurator	2033	−0.083	0.288 NS
M. lanata	1367	−0.170	0.000 *
G. rhomboides	724	0.079	0.039 *

Table 7. Spearman's rank correlation between total catch weight and damage level (*r* = correlation coefficient; *p* = significance level; * = significant, NS = not significant)

Species	*n*	*R*	*p*
L. depurator	2033	0.111	0.615 NS
M. lanata	1367	−0.083	0.071 NS
G. rhomboides	724	−0.023	0.555 NS

material in the debris, in particular stones and dead shells.

The differences in damage levels detected in this study, apparent at species but also at sex and size level, are mostly attributable to the morphological characteristics of each species as well as to the sexual dimorphism and the species behaviour. Thus, the length of the chela and the fragility of the other appendages could be the most important factor explaining the higher proportion of damage incurred by *G. rhomboides* and *L. depurator*. Sex-related differences in the injury level were particularly pronounced for species showing clear sexual dimorphism, such as *G. rhomboides*, where the chelae are markedly longer in males than in females.

Differences in the extent of damage as a function of body size can likewise be explained by the morphological characteristic of the three species. The higher proportion of damages found in the larger specimens of *G. rhomboides* is mainly due to the fact that the bigger-sized males constitute the bulk of the population; in contrast, the smaller individuals of *M. lanata* are probably more sensitive, due to the fragility of their thin appendages. Similarly, Bergmann et al. (2001a) found that smaller-sized *Liocarcinus holsatus* were more prone to damage, possibly as a result of their thinner carapaces.

Ovigerous females of *M. lanata* were observed to sustain less damage than non-ovigerous females; Bergmann et al. (2001a) reported the same for *M. rugosa* and *L. depurator*, but the opposite for *L. holsatus*. Despite this variability of results, it may be surmised that, in some species, ovigerous females attempt to protect the egg masses with the appendages or bury themselves in the sediment rendering them less vulnerable to the gear.

No significant correlation between damage and total weight of the catch was found for the three species investigated. In this context, it is notable that the total weight of the catches observed during this study was rather constant, as the fishing activity was carried out on the same bottoms and duration of the hauls was very similar (Fabi & Sartor, 2002). Note, however, as mentioned above, that a positive correlation between the level of damage and volume of the catch has been reported for *L. depurator* in the Clyde area (Bergmann et al., 2001a) and for many by-catch species in the Irish Sea (Veale et al., 2001).

Evaluation of the damage sustained during the fishing operations can provide an indirect estimate for the mortality produced in the species, as demonstrated by short-term survival experiments (Kaiser & Spencer, 1995). Bergmann & Moore (2001) reported long-term mortality for 30–50% of injured *L. depurator*, while the survival of specimens with damaged carapace was assumed to be zero (Kaiser & Spencer, 1995).

It is important, however, to take into account that survival evaluations based on external damages are certainly underestimates. Several crucial additional factors were omitted, such as internal and physiological damage produced both during fishing and sorting operations. Although the species may appear alive when discarded they may suffer post-trawling mortality in the field, as shown by Ramsay & Kaiser (1998). In Atlantic Sea Bergmann et al. (2001b) detected physiological stress experienced by *L. depurator* and squat lobsters during commercial fishing; these authors reported changes in the heamolymph composition as a result of anoxia during air exposure on deck, as the main causes of physiological stress. This might be even more pronounced in Mediterranean, where the temperature difference between the sea bed and the air is higher. Furthermore, even in cases where a species appears to show a good level of resilience -e.g. *L. depurator* is omnivorous, has a high fecundity and attains the sexual maturity in the first year of life (Mori & Zunino, 1987; Abelló, 1989; Fernández et al., 1991) – it is likely that regeneration or repair of damaged appendages imposes additional energetic costs and implies reduction of fitness, e.g. decreases subsequent moult increments, foraging efficiency, mating success, and increases the vulnerability to predators and to inter- and intra-specific attacks (Bennett, 1973).

The results of this study can be interpreted as providing minimum estimates of mortality for these species and could therefore constitute an important tool in monitoring this fishery and in the evaluation of any management measure. At present, no specific regulation is in force to manage this fishing activity, the existing regulation being the same as that adopted by otter trawls. Although the current fishing effort of rapido trawlers in the area investigated is undoubtedly low (at present only two vessels), in order to know if the impact of this activity is ecologically sustainable, it is important to perform exhaustive monitoring activities, with particular attention to the ecology and biology of the species belonging to the discard.

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Fishery of the deep-water rose shrimp *Parapenaeus longirostris* (Lucas, 1846) (Crustacea: Decapoda) in the northern Tyrrhenian Sea (western Mediterranean)

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Key words: *Parapenaeus longirostris*, deep-water rose shrimp, fishery, selectivity, discard, western Mediterranean

Abstract

The aim of this study is to provide information on the fishing pattern of *Parapenaeus longirostris* in the northern Tyrrhenian Sea (western Mediterranean), in order to improve the management of the resource in the area. Data were obtained from commercial fishery, covering the period 1991–2002. Catch rates revealed notable inter-annual fluctuation of the *P. longirostris* landing, probably due to the life cycle of the species. The highest yields (kg per hour of trawl) of the commercial fishery were obtained at depths ranging between 180 and 300 m, the lower ones at depths between 100 and 180 m, where small specimens are more abundant. Commercial catches were characterised by a very low presence of specimens smaller than 20 mm carapace length (CL), mainly due to the deeper localisation of the fishing grounds when compared to the recruitment areas of the species. The size at which 50% of the specimens were discarded by the fishermen was 15 mm CL, while the estimated size at first capture of the individuals caught by the commercial net was 12.4 mm CL.

Introduction

The deep-water rose shrimp *Parapenaeus longirostris* (Lucas, 1846) is distributed in the eastern Atlantic from the north of Spain to the south of Angola and throughout the whole Mediterranean (Olaso, 1990). The Atlantic fishery targeting this resource operates mainly off the coast of southern Portugal and in the Gulf of Cadiz (Sobrino et al., 1994; Figueiredo et al., 2001), as well as in many areas along the coast of the west African countries (Cervantes et al., 1991; Sobrino & Garcia, 1991, 1992a, 1992b). Of all the species caught in the Mediterranean, in the period 1972–1991, the deep-water rose shrimp was (in terms of biomass landed) the fifth largest (Stamatopoulos, 1993). Along the Italian coasts this species is mostly caught in the Sicilian Channel and the Ionian Sea (Levi et al., 1995; D'Onghia et al., 1998). However, in terms of biomass and economic value, it also represents an important component of commercial landings in some areas of the Tyrrhenian Sea (western Mediterranean) (Ardizzone & Corsi, 1997; Sbrana et al., 2003). Although this species shows a wide bathymetric distribution at depths of between 20 and 750 m (Tom et al., 1988), trawl survey results suggest that in the Italian waters the greatest biomass values are to be found at depths of between 150 and 400 m, i.e. on the continental shelf and in the upper part of the slope on muddy or sandy muddy bottoms (Ardizzone et al., 1990; Ardizzone & Corsi, 1997).

Because of the commercial importance of this resource, numerous studies have investigated the species biology, ecology and exploitation, in the areas where it is abundant (Froglia, 1982; De Ranieri et al., 1986, 1998; Mori et al., 1986, 2000a, 2000b; Ribeiro Cascalho & Arrobas, 1987; Chaouachi & Ben Hassine, 1988; Ardizzone et al., 1990; Arculeo et al., 1993; Sobrino & García, 1994; Spedicato et al., 1996; Carbonara et al., 1998; Dos Santos, 1998; Tserpes et al., 1999). In contrast, assessments of the resource are available only for Portuguese waters (Pestana, 1991; Pestana & Ribeiro Cascalho, 1991; Cadima et al., 1995), Gulf of Cadiz (Sobrino et al., 2000) and central Mediterranean (Levi et al., 1995; D'Onghia et al., 1998).

In the northern Tyrrhenian Sea, *P. longirostris* is an important target species for bottom trawling (Sbrana et al., 2003); fishing is carried out all year round by the Porto Santo Stefano and Porto Ercole fleets. The aim of this study is to improve the knowledge of some aspects of the fishery of this shrimp in order to identify a more efficient management strategy. To this end, landing time series and the demographic structure of the commercial catches (landed and discarded fractions) were reported, as well as the selectivity of the commercial trawl gear.

Materials and methods

The study was performed in the northern Tyrrhenian Sea (Fig. 1), where an important traditional trawl fishery targeting the deep-water rose shrimp is already established. Porto Santo Stefano was chosen to carry out the research because of the presence in this port of a significant number of vessels specialising in the crustacean fishery. In this port, the fleet exploiting *P. longirostris* is composed of otter-trawlers using traditional trawl nets, characterised by a vertical opening of about 1 m and nominal cod-end stretched mesh size of 40 mm.

From 1991 to 2002, landing data were collected monthly over a 3- to 5-day observation period at the local auction of Porto Santo Stefano. On each of these days, data on specific composition of the landing (total weight by species or commercial category) and fishing effort (number of fishing days) were collected for each vessel, which used a traditional trawl net. Monthly landings of *P. longirostris* were thus estimated by raising the sampled data to the total fishing days of the fleet during the same month. The total monthly fishing days were obtained by consulting the archives of the auction (sale notes). The landing rates (landing per unit of effort, LPUE) were calculated by taking into account the fishing day as the effort unit (kg/day/boat).

Between 1995 and 2001, fishing activity was also monitored by scientific personnel on board commercial bottom trawlers, gathering information on roughly 700 trawling hours. These trips were performed all year round, on board of vessels with different technical characteristics (length, engine power, tonnage) and operating in a wide bathymetric range. Monitoring activities did not interfere with the fishing routine or the *modus operandi* of the crew (e.g. allocation and duration of the haul, sorting of the catch). Data on species composition of the catch, subdivided by the fishermen into commercially retained and discarded fractions, were collected for each haul. In addition, from 1998 to 2000, a representative sample of specimens of each fraction was measured (carapace length, CL, rounded down to the nearest mm). Data collected on board commercial vessels were analysed as hourly yields (kg/h).

Geo-referenced data collected by observations on board commercial vessels (kg/h) were analysed by means of the Geographic Information System using a suitable algorithm (Weight Inverse Distance) in order to produce a map of the fishing grounds of *P. longirostris* (Meaden & Do Chi, 1996).

In addition, a selectivity study on the commercial trawl net was performed using the covered cod-end method (Pope et al., 1975). The surveys were carried out in April and July 1999, utilising a commercial trawler of the Porto Santo Stefano fleet. During 21 days at sea, a total of 98 hauls (each one of 30 min duration and at 3 knots towing speed) were carried out. The stretched mesh size of the cod-end, 35.93 mm (±0.58), was determined using the ICES mesh gauge calibrated to 2 kg, as recommended by Pope et al. (1975). This mesh size corresponds to the nominal mesh size of 40 mm measured using a standard EU wedge gauge. A cod-end cover with 15 mm stretched mesh size was utilised. Data obtained from different hauls were pooled together. The probability of retention in the cod-end (p) was estimated by means of the following logistic selectivity curve:

$$p = \frac{1}{1 + e^{-(a+b\mathrm{CL})}}$$

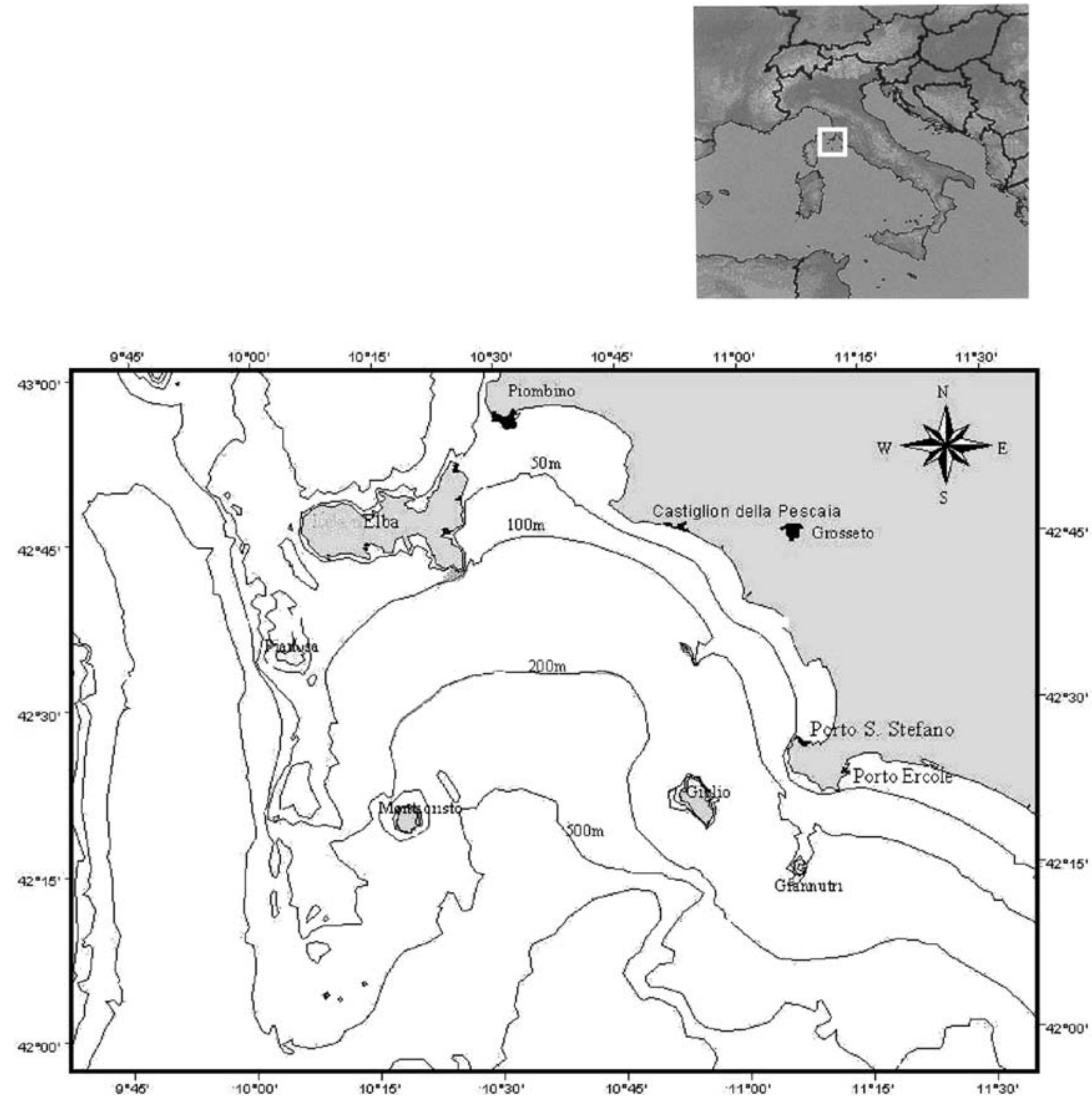

Figure 1. Northern Tyrrhenian Sea. Study area with the most important harbours for trawlers.

where, CL is the carapace length (mm), *a* and *b* are the selectivity parameters (Pope et al., 1975).

The same model was utilised to estimate the size at 50% discard level, considering the fractions of specimens discarded and retained by the crew (Stratoudakis et al., 1998).

Results

From 1991 to 2002, the annual total landing of *P. longirostris* in Porto Santo Stefano ranged from a minimum of 10 tons in 1996, to a maximum of 66 tons in 1999, corresponding to a first sale income of 120,000–800,000 Euros. Mean yearly values of LPUE during the studied period are shown in Figure 2. The index was constant from 1991 to 1997, ranging from 3.6 to 4.8 kg/day/boat, with the exception of 1992, when the mean yield reached 8.5 kg/day/boat. A sharp increase was observed starting in 1998, reaching the maximum value during the period 1999–2000 (around 19 kg/day/boat) followed by a decreasing trend in the last 2 years. The catch of a single boat reached the maximum value of 104 kg/day during spring 2000. In the study period, the fleet of Porto Santo Stefano, using the traditional trawl net and habitually fishing *P. longirostris*, has undergone an evident decrease, with the size of the fleet falling from 29 vessels in 1991 to 12 in 2002 (Fig. 3).

The observations carried out on board commercial trawlers of Porto Santo Stefano allowed the identification of the fishing ground of this resource (Fig. 4a). The highest commercial yields were obtained at depths ranging from 180 to 300 m, in the region between the islands of Elba and Montecristo. The maximum value (10.3 kg/h) was observed in February 2001 at a depth of 220 m.

Discards of *P. longirostris* were quite negligible, ranging from 0.35 to 1.24% of the total catch of the species (Table 1). Discards occurred at depths of less than 200 m, where juvenile specimens were more abundant (Fig. 4b). The maximum value (0.2 kg/h) was observed in August 1999, at 130 m depth. Length frequency distributions of the commercial fraction obtained in the period 1998–2000 from observations on board were characterised by a

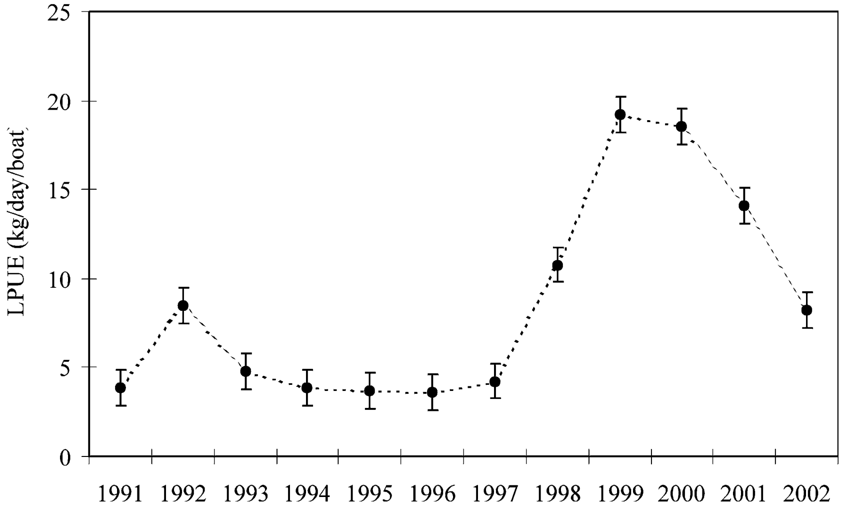

Figure 2. Landings per unit of effort (LPUE) trend (mean value per year ± SE) of *P. longirostris* obtained by the traditional trawl fleet of Porto Santo Stefano in the period 1991–2002.

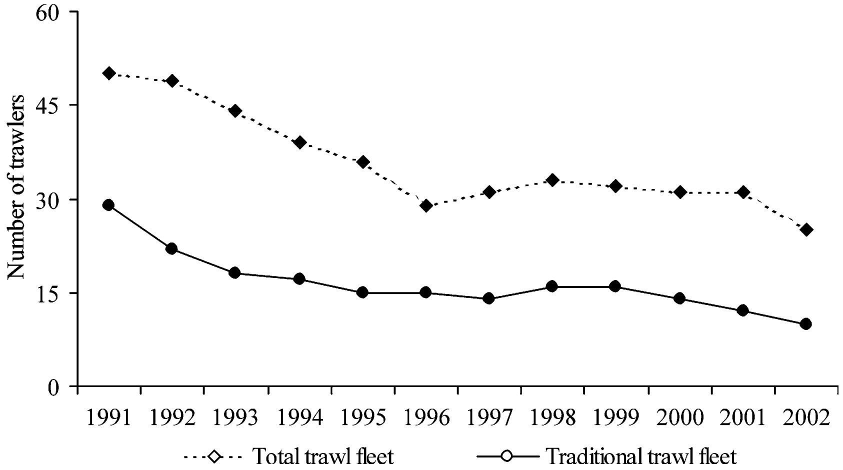

Figure 3. Evolution of the trawl fleet of Porto Santo Stefano in the years 1991–2002. The trend of the vessels fishing with traditional trawl is also reported.

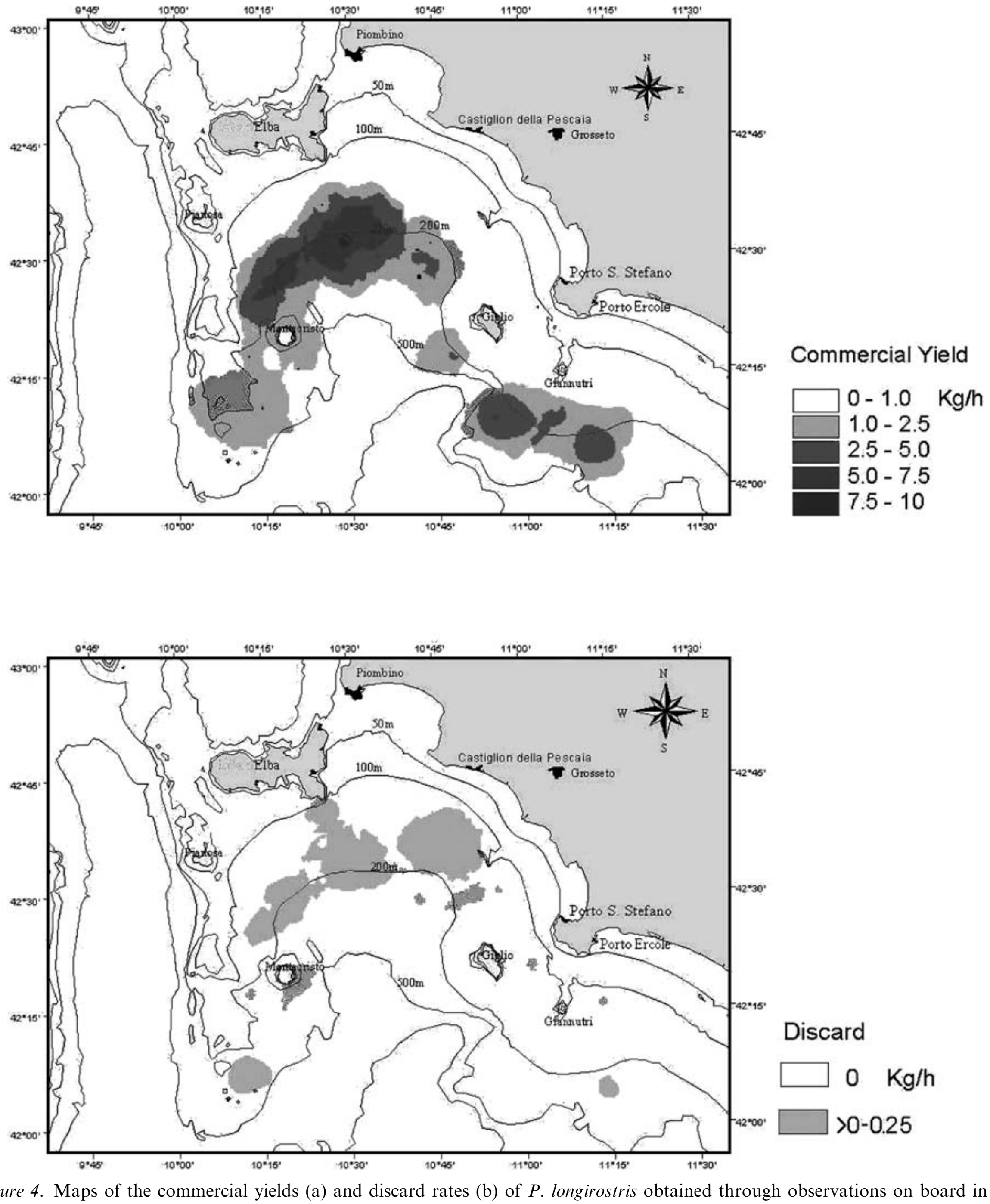

Figure 4. Maps of the commercial yields (a) and discard rates (b) of *P. longirostris* obtained through observations on board in the northern Tyrrhenian Sea during the study period.

wide size range (11–44 mm CL), but a very low number of specimens under 20 mm CL were commercialised (Fig. 5, left side). The modal class was quite similar during the 3 years analysed, ranging from 22 mm CL in 1999 to 24 mm CL in 1998 and 2000. The Kolmogorov–Smirnov test only showed significant differences ($p < 0.05$) in the size-frequency distributions when comparing the years

Table 1. Mean commercial yields and mean discard (kg/h ± SD) and percentage of discarded biomass in the commercial catches of *P. longirostris*

	1995	1996	1998	1999	2001
Commercial yield (±SD)	0.96 (1.00)	0.85 (1.28)	1.31 (1.53)	4.35 (2.60)	2.34 (2.77)
Discard (±SD)	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)	0.03 (0.05)	0.02 (0.03)
Percentage of discard	1.24	0.35	0.53	0.64	0.89

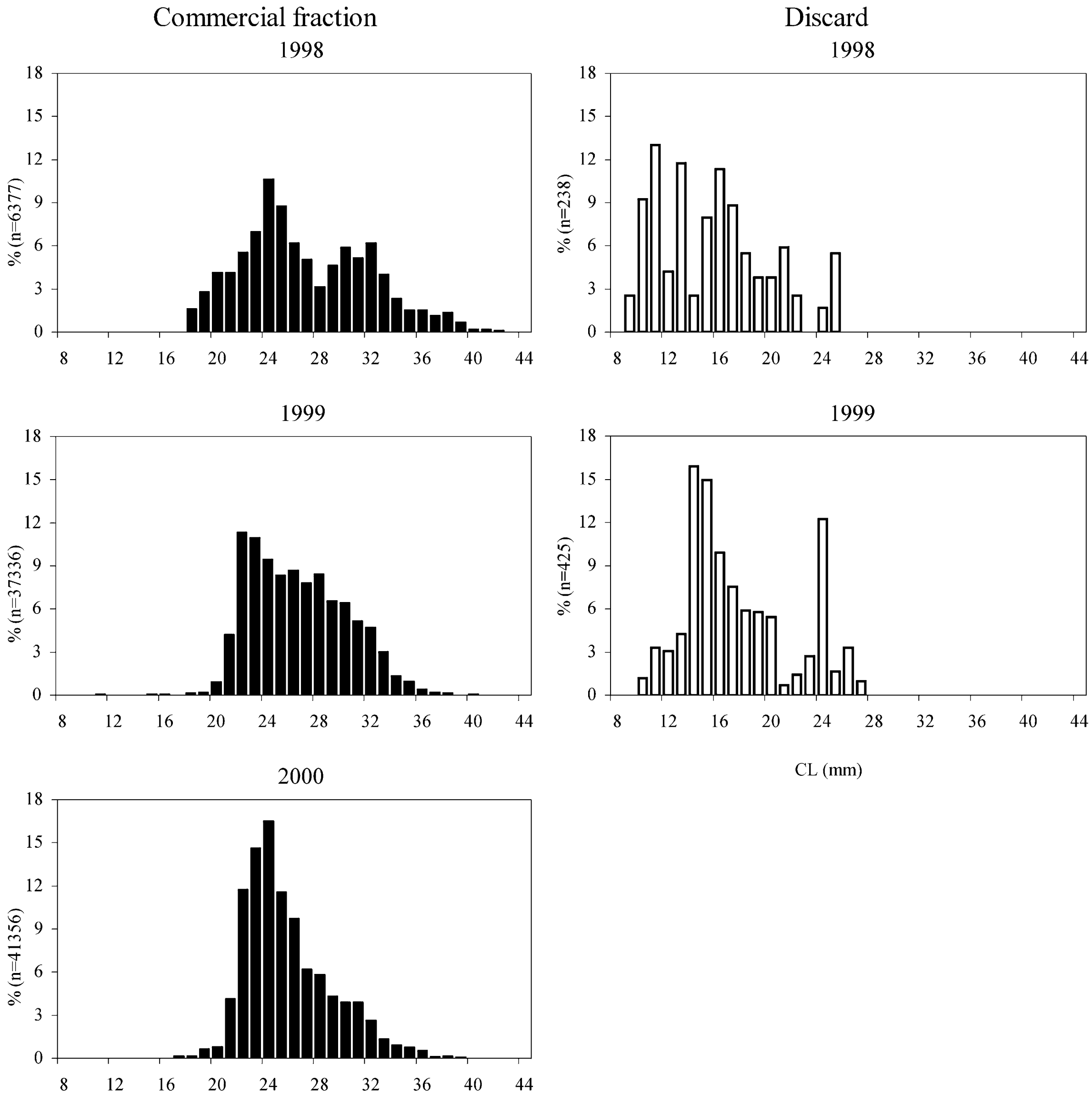

Figure 5. Annual length frequency distributions of the catches of *P. longirostris* obtained from commercial fishery (subdivided into landing and discard fractions) in the different years. In the year 2000 the sizes of the specimens discarded were not available.

1998 and 2000. The size range of the specimens discarded by fishermen was found to be between 9 and 27 mm (Fig. 5, right side), with the majority of the individuals smaller than 22–23 mm CL. An evident main modal class at 14 mm CL was detected in 1999, while sizes were more homogeneously distributed among different classes in the previous year. The size at which 50% of the specimens were discarded was 14.99 mm CL, whilst almost everything equal to or greater than 20 mm CL was commercialised. Table 2 and Figure 6 show the results of the analyses performed to evaluate the discard practices operated by the fishermen on this resource and the results of the selectivity experiment carried out on the commercial net.

During the selectivity study, a total of 14,571 specimens was caught. The number of the specimens retained in the cod-end was 14,381, with sizes ranging from 10 to 39 mm CL; the cover retained 190 specimens, ranging from 6 to 19 mm CL. The estimated size at first capture (CL_{50}) of the individuals caught by the commercial net was 12.37 mm; specimens of 20 mm CL and above were kept in the cod-end at a rate of 100%.

Discussion and conclusions

A significant inter-annual fluctuation of LPUE of *P. longirostris* of the northern Tyrrhenian Sea was detected during the studied period. The trend was similar to the variations of the biomass index (kg/km^2) estimated for the species by means of experimental trawl surveys carried out in the same area in the period 1994–1999 (Abellò et al., 2002). Apart from environmental conditions that may markedly affect the stock, this variability is probably related to the biological characteristics of the species – characterised by short life span and fast growth rates – and to different exploitation rates over the years (Ribeiro-Cascalho & Arrobas, 1987; Abellò et al., 2002).

Table 2. Parameters of the logistic curves of the commercialisation by size and of the commercial net selectivity

	Commercialisation by size	Selectivity of commercial net (35.93 mm)
Total catch	42631	14571
PR (%)	98.91	98.65
R^2	0.85	0.89
a	−8.79	−8.84
b	0.59	0.72
CL_{25} (mm)	13.11	10.83
CL_{50} (mm)	14.99	12.37
CL_{75} (mm)	16.86	13.91
SF	0.416	0.344
SR (mm)	3.75	3.08

Fishing effort is a complex variable, which is difficult to quantify, and which is influenced by many different factors. Moreover, the fishery capacity to evolve rapidly in response to favourable or unfavourable external incentives increases the complexity of the system (Farrugio et al., 1993). For example, during the period in question, a huge decrease in the number of vessels occurred throughout the region being investigated; Porto Santo Stefano and adjacent ports were the most affected, mainly due to the reduction of the fishing effort paid for and encouraged by the European Union (1998).

In addition to fleet size, the intensity of fishing effort is affected by other factors. The choice of fishing grounds is made on a daily basis, effort being concentrated by the fleet in proportion to the local biomass distribution i.e. the more the landing of *P. longirostris*, the more the vessels will fish in a certain area. Traditionally, this behaviour is characteristic of Mediterranean fishing, where trawling could be considered a ‘coastal’ or ‘semi-industrial’ activity (Farrugio et al., 1993), normally carried out within a few hours off the port and not necessitating long periods at sea (maximum 2 or 3 days). Models related to the spatial allocation of the fishing effort in the area (Caddy & Carocci, 1999), indicated that distance from the port to peak biomass is also likely to play an important part in explaining the distribution of fishing effort.

Differences in the spatial distribution of the commercialised and discarded biomass of *P. longirostris* are the consequence of the segregation of bigger specimens at greater depths. In this context, it is worth pointing out that a relationship between the size of *P. longirostris* and bathymetry was reported previously for Italian waters, larger specimens being distributed at greater depths; juveniles of the deep-water rose shrimp settle at around 100 m depth, but

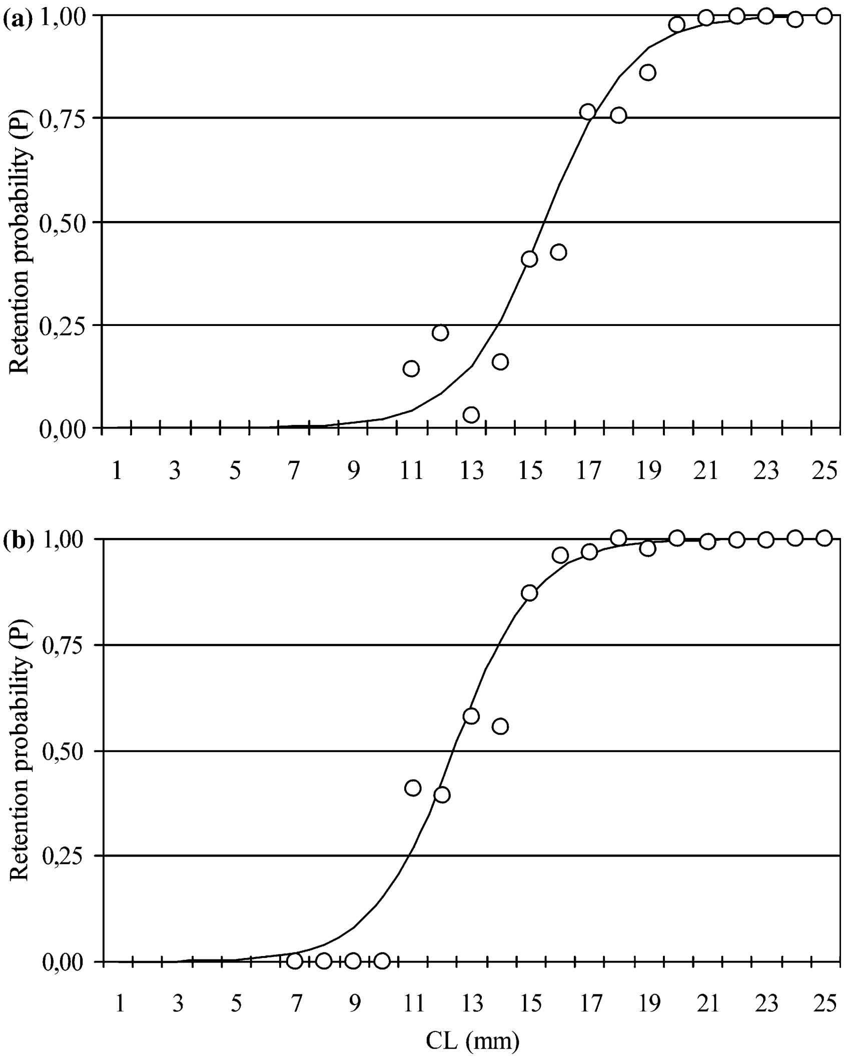

Figure 6. Selectivity fits for the different data sets. (a) curve of commercialisation by size; (b) selectivity curve of the commercial net. Dots, observed retained proportion; continuous lines, estimated selection curves.

move towards deeper waters as they increase in size (Froglia, 1982; Mori et al., 1986; Ardizzone et al., 1990, D'Onghia et al., 1998). The demographic structure of the catch, characterised by a very low presence of specimens under 20 mm CL, was mainly due to the fishing grounds being in deeper waters, where specimens of commercial sizes were more abundant.

A further factor influencing the size composition of the landings was the selectivity of the net utilised by the fleet and the discarding of the smaller specimens. The selectivity trials carried out on the commercial net showed a very small size at first capture ($CL_{50} = 12.37$ mm). Taking into account that, in the study area, the estimated size for females at the onset of maturity was about 22.0 mm CL (Mori et al., 2000b), this means that a significant part of the commercial catch could consist of immature individuals. Our results showed a size at first capture generally smaller than that observed in a previous investigation performed in Atlantic waters with a similar trawl net (nominal value of 40 mm cod-end mesh size), where a value of approximately 15 mm CL was found (Sobrino et al., 2000). In addition, these authors reported a size at first capture of

19.33 mm CL for a cod-end mesh size of 52.7 mm, while in a study carried out in adjacent waters, a size of 20.8–21.8 mm CL for a cod end mesh size of 55 mm was found (Campos et al., 2002). Although discards of *P. longirostris* were quite low in our study, increasing the cod-end mesh size would produce an improvement in the exploitation of the resource, increasing the spawning stock biomass. As an increase in the cod-end mesh size could lead to excessive economic losses, due to the multispecificity of the trawl net, other technical changes could be explored. Arrangements of square meshes in panels in the body of the net or in the cod-end could be an alternative, as outlined in the experiments of Campos et al. (2002) with the same species off the Portuguese south coast. In such a configuration, knowledge of the survival rate of the specimens, which escape through the meshes, would be desirable. Amongst other things, such a system would avoid the capture of juveniles of *Merluccius merluccius* concentrated in nursery areas at depths ranging from 100 to 300 m (Belcari et al., 2001), which are also the primary fishing grounds of the deep-water rose shrimp.

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Selectivity parameters and size at first maturity in deep-water shrimps, *Aristaeomorpha foliacea* (Risso, 1827) and *Aristeus antennatus* (Risso, 1816), from the North-Western Ionian Sea (Mediterranean Sea)

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Key words: trawl selectivity, size at first capture, size at first maturity, *Aristaeomorpha foliacea*, *Aristeus antennatus*, Mediterranean Sea

Abstract

Selectivity experiments were carried out during trawling targeting deep-water shrimps *Aristaeomorpha foliacea (*Risso, 1827) and *Aristeus antennatus* (Risso, 1816) (Crustacea, Decapoda, Aristeidae) in the North-Western Ionian Sea (Eastern-Central Mediterranean). Different criteria were employed to analyse maturity; however, the proportion at 50% of retained, mated and mature specimens was always used to indicate the size, expressed as Carapace Length (CL, mm), at first capture (CL_c), mating (CL_{sp}) and at first maturity (CL_m), respectively. In order to estimate the size at 50% maturity (CL_m) for females of both species, three criteria were adopted. In particular, CL_m was computed for the mature females not considering the presence of spermatophores, for the mature females with spermatophores and for the mature females intersected by the decreasing proportion with size of females without spermatophores. Three diamond stretched mesh codends of 40, 50 and 60 mm were tested using a cover of 20 mm.The 40-mm stretched mesh size (European Union legal size in the Mediterranean) was not selective for the sampled population of each species. The size at first capture (CL_c), calculated in both species for the two sexes combined, increased significantly with mesh size. Even for the mesh size of 60 mm, the size at first capture was still smaller than the sizes at 50% maturity, whatever the criterion adopted. Since the differences between the size at first maturity and the sizes at first capture are greater in *A. foliacea* than *A. antennatus*, the former species appears in this respect to be more vulnerable to trawling than the latter.

Introduction

The deep-water shrimps *Aristaeomorpha foliacea* (Risso, 1827) and *Aristeus antennatus* (Risso, 1816) represent important economic demersal resources of the Mediterranean Sea. As observed in various geographic areas, the abundance of these shrimps can be subject to marked fluctuations (Relini & Orsi Relini, 1987; Orsi Relini & Relini, 1988; Sardà, 1993; Bianchini & Ragonese, 1994; Carbonell et al., 1999; Cau et al., 2002). Since the exploitation of *A. foliacea* and *A. antennatus* concerns both juveniles and adults, the regulation of the mesh size codend would appear to be an effective management measure to assure the sustainability of the catches of these resources (Ragonese et al., 2001, 2002). The adoption of a mesh size greater than 40 mm stretched (European Union legal size in the Mediterranean) should be attempted, taking into account the reproductive patterns of these shrimps and the selection process of the trawl net.

In the Ionian Sea, data on selectivity parameters for *A. foliacea* and *A. antennatus* are only available for the Calabrian sector (South-Western Ionian Sea) where these resources are intensively exploited (D'Onghia et al., 1998b). Size at maturity was only estimated for *A. foliacea* in the whole Ionian basin (D'Onghia et al., 1998a).

Along the Apulian coast of the Ionian basin, the Gallipoli fishery targets primarily deep-water shrimps throughout the year (D'Onghia et al., 1998c). For this fishery, which is characterized by small trawlers (GRT <10 tons, engine power <100–150 kW) operating daily for five days a week (Carlucci et al., 2003), no data on the selectivity parameters and maturity are available.

In this paper, basic information on the selectivity of the bottom trawl net and on the sizes at 50% maturity of *A. foliacea* and *A. antennatus* females in the Gallipoli fishery (North-Western Ionian Sea) is reported.

Materials and methods

Selectivity experiments during trawl fishing on deep-water shrimps *A. foliacea* and *A. antennatus* were conducted during June–July 2000 and June–July 2001 in the Gallipoli fishery (North-Western Ionian Sea) (Fig. 1).

A professional trawler of 9.9 tons gross tonnage (161.8 kW), equipped with a nylon otter trawl net, was hired. The experimental survey was carried out on the slope, in the depth range from 400 to 700 m, where trawl fishing on deep-water shrimps occurs.

The selectivity of 40- (European Union legal codend in the Mediterranean), 50- and 60-mm stretched mesh size codend was evaluated by means of the covered-codend method as reported in Sparre & Venema (1998).

A total of 36 hauls of 3 h in duration were carried out, from dawn to dusk, emulating the commercial trawling in the area. A cover with 20-mm stretched mesh size and proportionally 30% longer and larger than each codend was employed. Three arrays of plastic floats were attached to its top to prevent it from collapsing.

For both species, the carapace length (CL, mm) of the specimens collected both in codend and cover was measured.

The estimates of the selectivity parameters (selection range SR, selection factor SF and size at first capture CL_c) using different mesh sizes were computed for the two sexes combined using both the antisymmetric logistic (Sparre & Venema, 1998) and the asymmetric (Sardà et al., 1993) model. The former, which is the most widely used model, is based on the assumption that the selectivity curve has a sigmoidal shape with a flex point in CL_c. On the contrary, the asymmetric model does not admit an inflection point and the curve is plotted using the equation $S_{CL} = 1-1/\exp^{a(CL-CL0)}$ where S_{CL} is the probability of retention, a is a constant value and CL_0 the smallest size retained. The CL corresponding to $S_{CL} = 0.5$ represents CL_c.

Data on the maturity of *A. foliacea* and *A. antennatus* females were collected. Results refer to all specimens caught both in the cover and codend independently of the stretched mesh size used. The gonads were macroscopically examined using the scale proposed by Levi & Vacchi (1988) and Orsi Relini & Relini (1979) for *A. foliacea* and *A. antennatus*, respectively. For both species, specimens at stages III and IV were considered mature. Furthermore, the presence/absence of spermatophores on thelycum was recorded. The logistic model was applied to estimate the size at which 50% of females had mated (CL_{sp}). Three criteria were adopted in order to estimate the size at 50% maturity (CL_m) for females of both species. In particular, such a size was computed applying the logistic model to:

1. proportion (p) of mature females, not considering the presence of spermatophores
2. p of mature females with spermatophores
3. p of mature females intersected by the decreasing with size proportion of females without spermatophores.

This latter criterion combines the gonadic maturity curve and the mating complementary curve (absence of spermatophores) according to the functional criteria indicated by Morizur (1983) and to the empirical approach proposed by Ragonese & Bianchini (1995). The size of the "maximum reproductive potential" (CL_{mrp}) was obtained following this approach.

Both selectivity parameters and sizes at maturity were estimated for the pooled data of the two years.

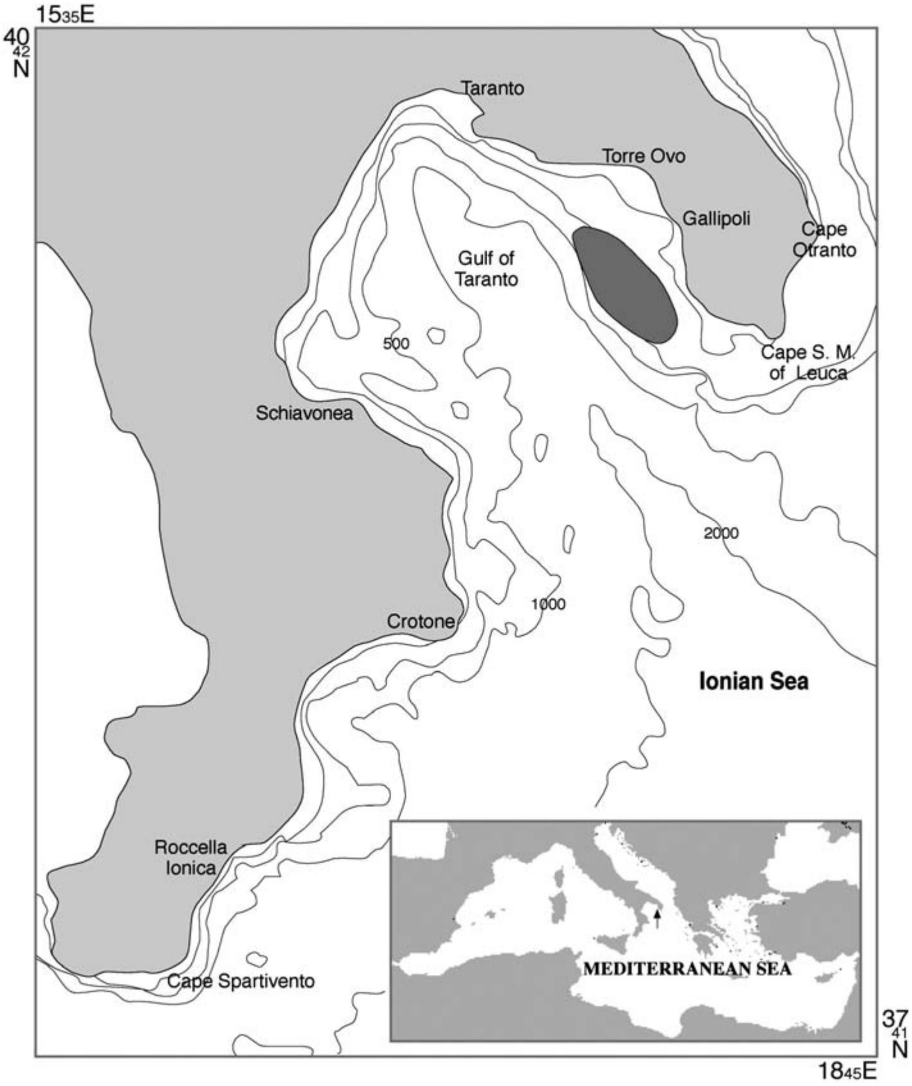

Figure 1. Map of the North-Western Ionian Sea (Mediterranean Sea) with indication of the area investigated (black) during June–July 2001.

Results

Aristaeomorpha foliacea

The length structure of the catch in the codend and the number of specimens escaped in the cover using the different mesh sizes are shown in Figure 2. Only 16 small specimens (0.6% of the total) were collected in the cover employed on the 40-mm mesh size codend. A slight increase in escaped specimens (3.4%) was observed for the 50-mm codend, while 1562 specimens (27.5%) were collected in the cover using the 60-mm codend. The largest specimens that escaped into the cover measured 30 mm CL for the 50-mm and 36 mm CL for the 60-mm codend.

Using the logistic and the asymmetric function for the 50-mm codend, the sizes at first capture were 16.2 mm CL (SR = 6.30 mm and SF = 0.32) and 15.2 mm CL (SR = 3.51 mm and SF = 0.30), respectively. Concerning the 60-mm codend, the values were 21.2 mm CL (SR = 10.66 mm; SF = 0.35) and 20.5 mm CL (SR = 8.72 mm; SF = 0.34), respectively (Table 1).

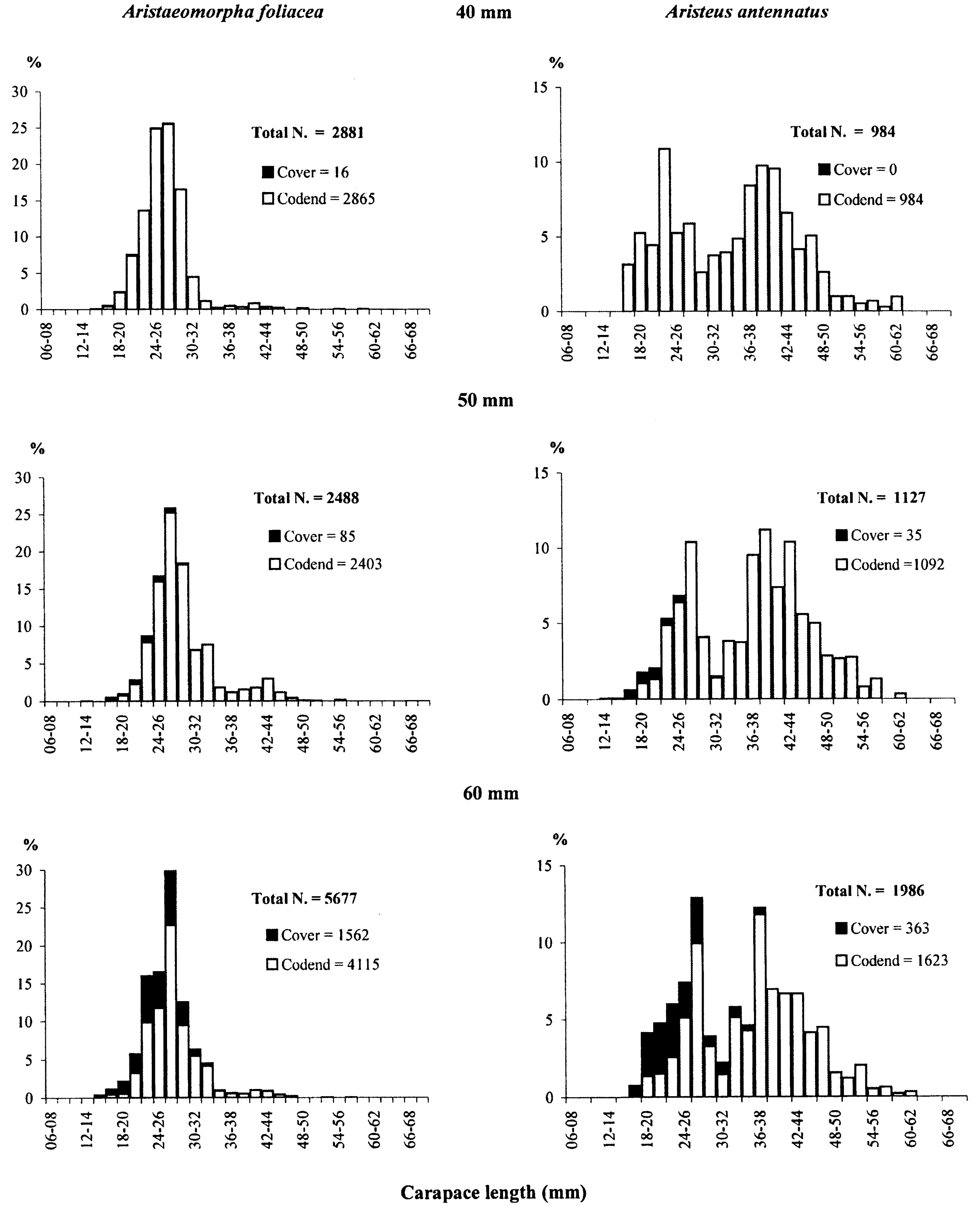

Figure 2. Length-frequency distribution in the codend and cover of *Aristaeomorpha foliacea* and *Aristeus antennatus* specimens caught with 40-, 50- and 60-mm mesh size codends.

Regarding maturity, a total of 5442 females of *A. foliacea* were collected during this study. The smallest mated and the smallest mature (stage III) female measured 21 and 34 mm CL, respectively (Fig. 3). The size at which 50% of the females had mated was 35.9 mm CL (Fig. 4). The female size at first maturity, not considering the presence of spermatophores, was 44.1 mm CL and that of females with spermatophores, 44.14 mm CL (Fig. 4a). The size of the "maximum reproductive potential" defined as the point of intersection of the gonadic maturity curve and the mating complementary curve (absence of spermatophores) was 39.3 mm CL (Fig. 4).

Aristeus antennatus

The number of the individuals that escaped into the cover also increased with mesh size for this species as shown in Figure 2. The 40-mm mesh size codend was not selective. The 50- and 60-mm codends allowed 3% and 18% of the total specimens caught to pass into the cover, respectively. The largest specimens that escaped into the cover employed on the 50- and 60-mm codends was 32 and 38 mm CL, respectively.

The sizes at first capture (CL_c) for the 50-mm codend computed by the logistic and the asymmetric curves were 19.4 and 16.5 mm CL, respectively (Table 1). Moreover, according to the logistic curve the SR was 3.62 mm and the SF = 0.39, while according to the asymmetric curve they were 5.57 mm and 0.33 mm, respectively.

Concerning the 60 mm codend, the CL_c calculated using the logistic and the asymmetric functions were 23.6 mm CL (SR = 9.72 mm; SF = 0.39) and 22.0 mm CL (SR = 7.69 mm; SF = 0.39), respectively.

A total of 2951 females of *A. antennatus* were collected. The smallest mated and mature (stage III) female measured 22 mm CL (Fig. 3).

The size at which 50% of the females had mated was 25.2 mm CL. The size at first maturity (not considering the presence of spermatophores)

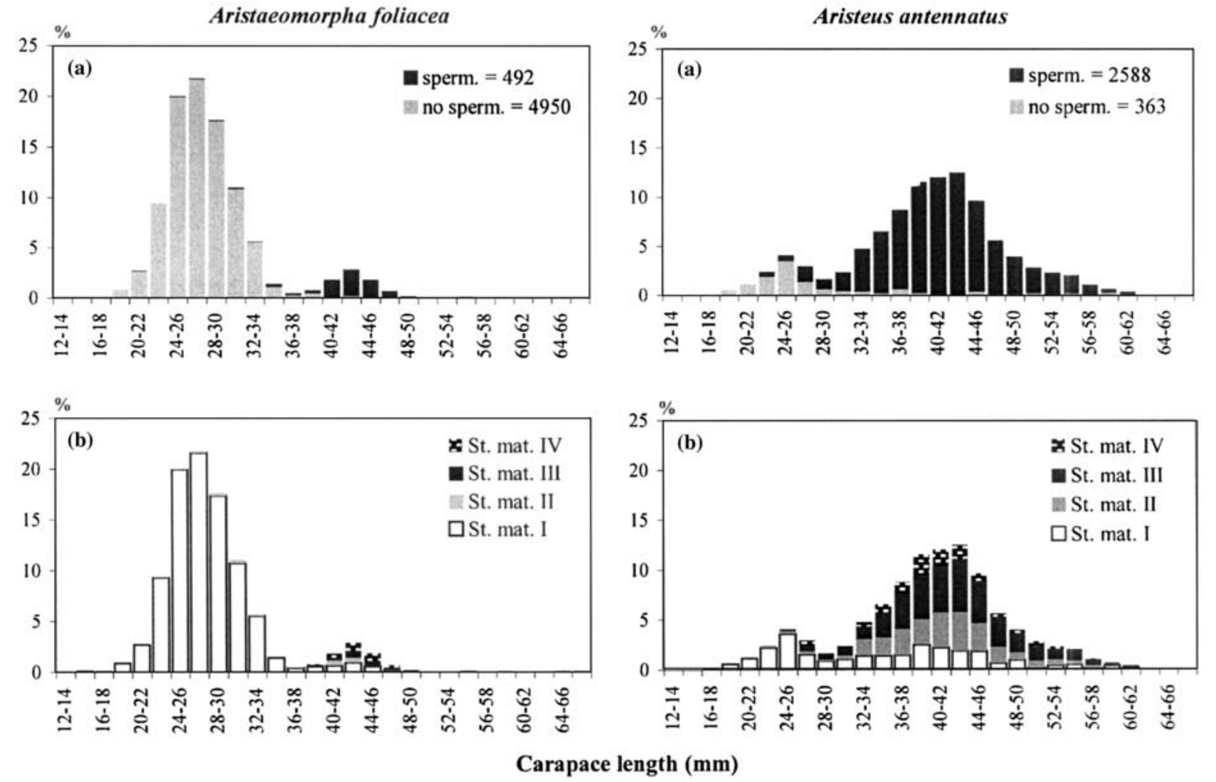

Figure 3. Length-frequency distribution by presence of spermatophores (a) and maturity stage (b) for females of *Aristaeomorpha foliacea* and *Aristeus antennatus* specimens caught in the North-Western Ionian Sea.

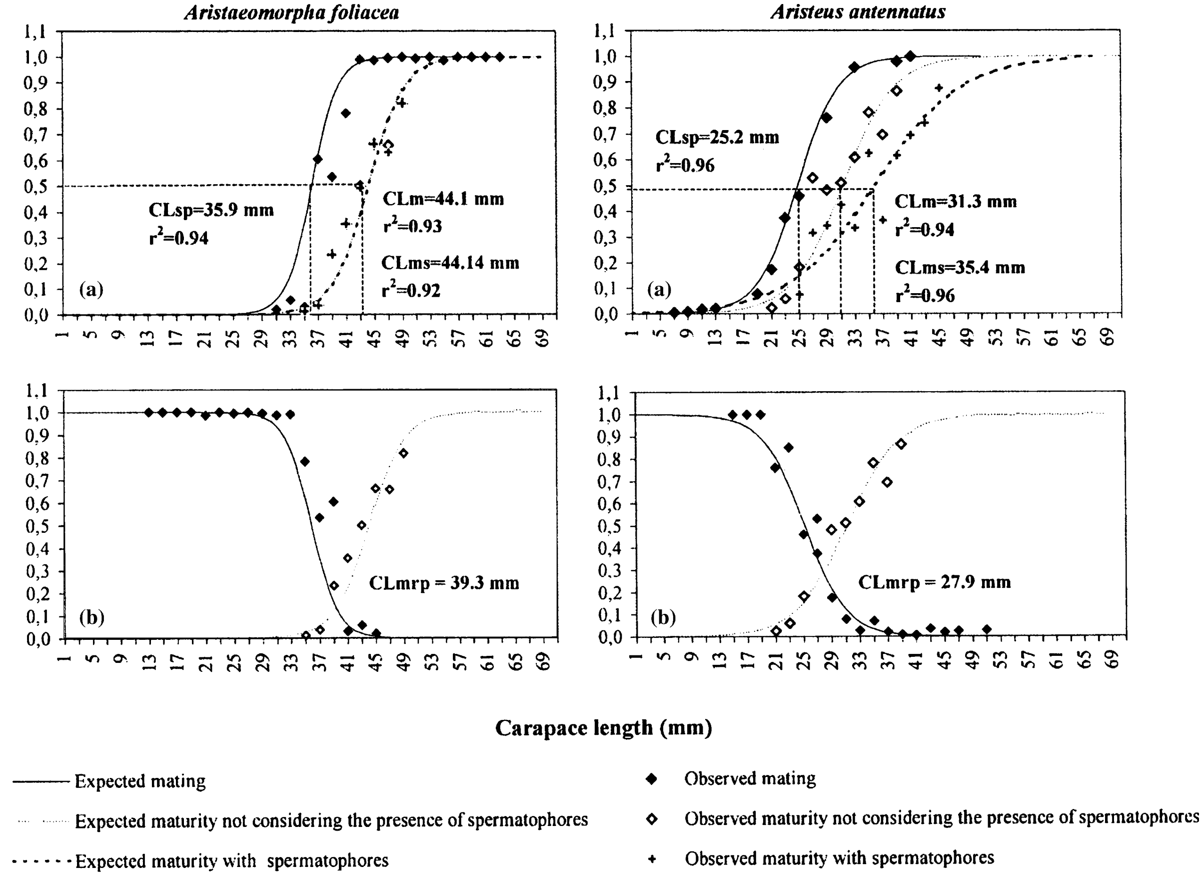

Figure 4. *Aristaeomorpha foliacea* and *Aristeus antennatus* in the North-Western Ionian Sea. (a) expected and observed percentage by size of mated females (CL_{sp}), mature females not considering the presence of spermatophores (CL_m) and mature females with spermatophores (CL_{ms}); (b) size of "maximum reproductive potential" (CL_{mrp}).

Table 1. Size at first capture (CL_c in mm), selection factor (SF), selection range (SR) and index of fitting (r^2) by stretched mesh size (mm) for *Aristaeomorpha foliacea* and *Aristeus antennatus* (sexes combined), estimated in the North-Western Ionian Sea

Mesh size (mm)	Logistic model				Asymmetric model			
	CL_c	SF	SR	r^2	CL_c	SF	SR	r^2
Aristaeomorpha foliacea								
40	–				–			
50	16.2	0.32 ± 0.03	6:30	0.96	15.2	0.30 ± 0.02	3.51	0.97
60	21.2	0.35 ± 0.07	10.66	0.92	20.5	0.34 ± 0.08	8.72	0.90
Aristeus antennatus								
40	–				–			
50	19.4	0.39 ± 0.04	3.62	0.98	16.5	0.33 ± 0.04	5:57	0.94
60	23.6	0.39 ± 0.07	9.72	0.94	22.0	0.39 ± 0.09	7.69	0.91

was 31.3 mm CL and that strictly concerning females with spermatophores was 35.4 mm CL (Fig. 4). The size of the "maximum reproductive potential" was 27.9 mm CL (Fig. 4).

Discussion and conclusions

The values of size at first capture and selection factor estimated for *A. antennatus* in this research are in agreement with those from the Sicily Channel (Ragonese et al., 1994, 2001, 2002) and the Ionian Sea along the Calabrian coast (D'Onghia et al., 1998b, 2003) (Table 2). The values estimated for *A. foliacea* are smaller than those obtained in the Sicily Channel where the selectivity in this shrimp was also shown for the 40-mm codend (Ragonese et al., 1994, 2001, 2002).

In the Gallipoli fishery, although the 40-mm codend allowed a minimal fraction of *A. foliacea* juveniles to pass through, it proved to be substantially not selective for the sampled populations of both species. This could be the consequence of the fact that in both species recruitment occurs during spring (D'Onghia et al., 1997, 1998a). In fact, the numerical changes of the first modal component and the presence of small individuals in the fished stock play an important role in affecting selectivity (Ragonese et al., 1994, D'Onghia et al., 1998b).

Data from the 50- and 60-mm fit both models used, though the logistic model provides CL_c values and determination coefficients slightly greater than the asymmetric one in both species and for both stretched mesh sizes. Moreover, from the SF values obtained, it seems that the selection processes did not differ between the 50- and 60-mm codends, especially in *A. antennatus*.

Regarding reproductive aspects, the sampled population of *A. foliacea* and *A. antennatus* showed different percentages of mated and mature females. The bulk of the *A. foliacea* stock consisted of immature and unmated specimens. In both species, mating in the population occurs before reproduction, as reported in D'Onghia et al. (1997, 1998a).

In *A. foliacea*, reproduction concerns exclusively large specimens, as shown from the size at first maturity of females not considering the presence of spermatophores (44.1 mm CL) and that of females with spermatophores (44.14 mm CL). The superimposition of these two sizes is due to the fact that only very few mature females did not bear spermatophores.

In *A. antennatus*, on the contrary, the female size at first maturity with and without spermatophores (31.3 mm CL) was smaller than the size at 50% maturity of females with spermatophores (35.4 mm CL), indicating that many mature females could not be mated.

Table 2. Size at first capture (CL_c in mm) and selection factor (SF) for *Aristaeomorpha foliacea* (AF) and *Aristeus antennatus* (AA) (sexes combined) estimated in the Mediterranean Sea (diamond mesh, stretched size in mm)

Authors	AF		AA	
	CL_c	SF	CL_c	SF
Ragonese et al. (1994)	17.7 (40 mm)	0.44		
	20.1 (48 mm)	0.42	19.9 (48 mm)	0.42
	22.8 (56 mm)	0.41	25.0 (56 mm)	0.45
Ragonese et al. (2002)	18.6 (40 mm)	0.46		
	20.8 (48 mm)	0.44		
	24.5 (56 mm)	0.44		
D'Onghia et al. (1998b)	19.5 (60 mm)	0.32	23.7 (60 mm)	0.40
D'Onghia et al. (2003)			19.0 (50 mm)	0.38
			25.4 (60 mm)	0.42
Carlucci et al. (present study)	16.2 (50 mm)	0.32	19.4 (50 mm)	0.39
	21.2 (60 mm)	0.35	23.6 (60 mm)	0.39

Both sizes at first maturity estimated for *A. antennatus* were smaller than those calculated for *A. foliacea* confirming observations throughout the Mediterranean Sea (Table 3).

In both species, the size of maximum reproductive potential was smaller than the size at 50% maturity estimated using the other two criteria. The size of maximum reproductive potential computed in this study for *A. foliacea* corresponds to that obtained by Ragonese & Bianchini (1995) (39.667 mm CL) and is barely greater than that reported by D'Onghia et al. (1998a) (37.11 mm CL).

For both species and mostly for *A. foliacea*, the sizes at first capture obtained with the 50- and 60-mm sMS were smaller than the size at first maturity estimated by using the three criteria. This points out the marked impact of the trawl net on the population structure of both species. Since the differences between the sizes at first maturity and the sizes at first capture are greater in *A. foliacea* than in *A. antennatus*, the former shrimp can be considered more vulnerable to trawling than the latter. In addition, further bio-ecological features of *A. foliacea* reported in Orsi Relini & Relini (1985) and Matarrese et al. (1997) could explain the marked depletion of the parental stock of this shrimp in the study area.

According to the present study and other observations made until now in the Mediterranean Sea (Tables 2 and 3) it is evident that a larger mesh than 40-mm stretched should be adopted in the fishing of deep-water shrimps in order to reduce mortality in juveniles. The 60-mm codend mostly reduces the capture of specimens smaller than 30 mm CL, which have the lowest market value. The rigid integument and the "backward" escaping pattern should allow a good survival rate for these individuals (Bianchini et al., 1998). In the Gallipoli fishery, the regulation of a greater mesh size might be justified since the fishery targets primarily deep-water shrimps on bathyal bottoms where other resources, such as *Nephrops norvegicus* (Linnaeus, 1758), are exploited and there has been a marked depletion of the adult component in the stocks (Tursi et al., 1998a, b).

Finally, as indicated in this and previous studies (D'Onghia et al., 1998a, b; Ragonese et al., 2001, 2002), the regulation of a mesh greater than 40 mm should be adopted together with the

Table 3. Values of the size at first mating (CL_{sp} in mm) and at first maturity (CL_m in mm) for females of *Aristaeomorpha foliacea* (AF) and *Aristeus antennatus* (AA) estimated in the Mediterranean Sea

Authors	AF		AA	
	CL_{sp}	CL_m	CL_{sp}	CL_m
Mura et al. (1992)		Between 30 and 34		Between 18 and 24
Campillo (1994)*			28.8	
Carbonell (1994)*				26.0
Cau et al. (1994)		39.0		21.0
Demestre (1994)*				26.0
D'Onghia et al. (1994)*		39.0		35.0
Greco et al. (1994)*	34.0			
Martinez Banos & Mas (1994)*				27.0
Mori et al. (1994)*	46.6 ± 3.6	47.0 ± 4.7		
Spedicato et al. (1995)				35.0
Yahiaoui (1994)*				23.0
Ragonese & Bianchini (1995)	36.4	41.8		
Colloca et al. (1998)				27.0
Matarrese et al. (1997)		43.0		38.0
D'Onghia et al. (1998°)	39.3	47.0		
Carlucci et al. (present study)	35.9	44.14	25.2	35.4

*In Bianchinni & Ragonese (1994).

management tool of a closed season during the spring-early summer period.

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Catch composition on red shrimps' (*Aristaeomorpha foliacea* and *Aristeus antennatus)* grounds in the Eastern Ionian Sea

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Abstract

In the present study, the catch composition and the catch per unit effort (CPUE) by weight and numbers in red shrimps' (*Aristaeomorpha foliacea* and *Aristeus antennatus*) grounds was examined in the southern part of the eastern Ionian Sea, in order to collect important information for the Greek waters, where no deep-water fishery exists. In the depth stratum 500–700 m, the catch of the commercial species represented a high proportion (>70%) of the total catch. Red shrimps and several other commercial species were found in important quantities. The present results suggest the possibility of developing a deep-water fishery in Greece. In such a case, attention should be paid because of the high vulnerability of *A. foliacea* – the main deep-water fishing resource in the area – to the fishing pressure.

Introduction

The giant red shrimp, *Aristaeomorpha foliacea* (Risso, 1827) and the blue and red shrimp, *Aristeus antennatus* (Risso, 1816) are encountered at deep-water grounds; the former species may be found at depths ranging between 120 and 1100 m with maximum abundance between 500 and 700 m (e.g. Fischer et al., 1987; Bianchini & Ragonese, 1994 and references therein; Kapiris et al., 2001; Papaconstantinou & Kapiris, 2001; Politou et al., 2004), while the latter occurs in depths between 80 and 3300 m with maximum abundance between 700 and 900 m (e.g. Fischer et al., 1987; Bianchini & Ragonese, 1994 and references therein; Kapiris et al., 2001; Papaconstantinou & Kapiris, 2003; Sardà et al., 2004). *A. antennatus* is a highly commercial species, extensively exploited in the western and central Mediterranean Sea. *A. foliacea* is also commercially valuable, but nowadays it can be found in few areas of the Mediterranean (Cau et al., 2002).

The commercial importance of these species as well as other by-catch deep-water species make the deep-water resources of increasing interest. Several studies show the presence of both red shrimps in important quantities in the Greek Ionian Sea (Kapiris et al., 2001; Papaconstantinou & Kapiris, 2001, 2003; Politou et al., 2003, 2004). So far, only Politou et al. (2003) examined the deep-water resources of the northern Greek Ionian Sea from a clearly fisheries point of view.

In the present study, catch composition on red shrimp grounds of the southern Greek Ionian Sea was studied, in order to collect useful information on the catch of other fisheries resources accompanying that of red shrimps. This information is of special importance for Greece, where no deep-water fishery exists, and the development of such a fishery is now under consideration by both fishers and managers. It is also important for comparison purposes with westernmost areas of the Mediterranean, which are extensively exploited.

Materials and methods

Data were collected by trawling in depths ranging between 500 and 900 m in the southern Greek Ionian Sea, during two experimental trawl surveys carried out in September 2000 and July–August 2001. Two commercial trawlers were used, a Greek and an Italian one, during the first and second survey, respectively. Since no deep-water fishery exists in Greece, a trawl was constructed for the Greek vessel according to the characteristics of the Italian trawl, in order to avoid the effect of gear dissimilarities on the catch. The cod-end mesh size of both trawls was 40 mm (stretched). The towing speed for both vessels was 2.4 nm/h. By using a random stratified sampling design, 83 stations were sampled in the depth strata 300–500 m, 500–700 m and 700–900 m during the first cruise. From these stations, those with more than 100 red shrimp ind./h (considered as red shrimp grounds) were selected for the purposes of the present work ($n = 37$). These stations were distributed in depths ranging from 500 to 900 m. The second cruise comprised only the selected stations of the first exploratory cruise. The haul duration was 1 h.

Catches were identified to the species level. Total weight and number of individuals per species in each station were recorded on board. The analysis of the catch composition was based on the study of the catch per unit effort (CPUE) by weight and by numbers for the total, commercial and non-commercial catch, the crustacean, fish and cephalopod catch and the catch per species. The mean CPUE for each depth stratum and category was estimated according to Cochran (1977), as follows:

$$\mathrm{CPUE} = \Sigma A_i / \Sigma D_i$$

where ΣA_i: sum of weights (kg) or numbers (individuals) of a species or category in the i stations carried out in the depth stratum and ΣD_i: sum of fishing time (hours) of the i stations in the same depth stratum.

The CPUE was expressed in kg/h and ind./h. The significance of the estimated CPUE values in each cruise and depth stratum for the total, commercial and non-commercial catch and the crustacean, fish and cephalopod commercial and non-commercial catch was tested using paired t-tests between the log-transformed CPUE values of the various stations and their log-transformed mean CPUE value. A t-test was also used in order to detect statistically significant differences between depth strata and cruises using the log-transformed CPUE values of the commercial and non-commercial catch.

Results

The CPUE in terms of weight or numbers for the total catch ranged between 25 and 65 kg/h or 441–2526 ind./h depending on the depth stratum and the cruise. The analysis of the catch by weight or by numbers of the commercial species represented a very high proportion (70–85%) of the total catch in both cruises and depth strata, whereas discards of the non-commercial species constituted a relatively low proportion (15–25%). Comparison of the CPUE values of the total, commercial and the non-commercial catch, using the t-test, showed no statistical differences between cruises in the same depth stratum ($p > 0.05$), whereas significant differences ($p < 0.05$) were found between depth strata in each cruise. CPUE values of the commercial species by weight or by numbers were much higher in the depth stratum 500–700 m (49–50 kg/h or 1413–1747 ind./h) than in the deeper zone (20–26 kg/h or 336–523 ind./h). A similar pattern was observed for the non-commercial catch. The use of the paired t-test showed that, in each cruise, stratum and category of commerciality, there was no statistically significant difference ($p > 0.05$) between CPUE values of the stations and their mean CPUE value.

Total catch in terms of weight was composed of fish (≅71%) and crustaceans (≅29%) in both cruises and depth strata. However, in terms of numbers, total catch components were reversely represented (crustaceans: 71%; fish: 30%) in both cruises and depth strata. Cephalopods' proportion was always negligible, ranging between 0.1% and 1% of the total catch. In all cases, CPUE values of commercial and non-commercial fish in terms of weight were higher than those of the corresponding crustaceans, whereas CPUE values in terms of numbers were higher in commercial crustaceans than in commercial fish (Fig. 1). The low CPUE values of the non-commercial crustaceans were probably related to the selected sampling stations, which were red shrimp grounds. The examination of the significance of the CPUE values shown in Figure 1, using the paired *t*-test, showed that no statistically significant differences ($p > 0.05$) existed between the CPUE values of the stations and their mean CPUE value (except in the case of cephalopod CPUE in terms of weight in the deeper depths).

The analysis of the CPUE values per species showed that, in both cruises in the depth stratum 500–700 m, the most important species in terms of weight were *A. foliacea* (11–14 kg/h, representing 17–23% of the total catch), *Helicolenus dactylopterus* (Delaroche, 1809) (10–11 kg/h), *Centrophorus granulosus* (Bloch & Schneider, 1801) (6.5–10.4 kg/h), *Chlorophthalmus agassizii* (Bonaparte, 1840) (4.6–8.1 kg/h) and *Hoplostethus mediterraneus* (Cuvier, 1829) (4–5.2 kg/h). In the deeper waters, the most important species were *A. foliac*ea (4.8–7.4 kg/h, representing 19–25% of the total catch), *Galeus melastomus* (Rafinesque, 1810) (4.7–5.8 kg/h), *Lophius piscatorius* (Linnaeus, 1758) (2.6–3.5 kg/h), *H. dactylopterus* (1.7–2.8 kg/h), *C. granulosus* (1.5–2.9 kg/h) and *A. antennatus* (1.4–1.9 kg/h). In terms of numbers, the main species in both cruises were *A. foliac*ea (868–873 ind./h), *Plesionika martia* (A. Milne Edwards, 1883) (280–5,561 ind./h), *C. agassizii* (219–444 ind./h) and *H. mediterraneus* (79–107 ind./h) in the shallower depth stratum (500–700 m) and *A.* foliacea (213–396 ind./h), *A. antennatus* (71–75 ind./h), *Nezumia sclerorhynchus* (Valenciennes, 1838) (42–46 ind./h) and *G. melastomus* (27–35 ind./h) in the deeper one.

Discussion

The results of the present work showed relatively high CPUE values for the total and commercial catch from the red shrimp grounds. This could be related to the fact that the deep-water fisheries resources in the area are unexploited. In the 500–700 m depth stratum, the total CPUE in terms of weight was near 65 kg/h and the commercial CPUE near 50 kg/h. In the same depth zone, lower values for the total (< 35 kg/h) and the

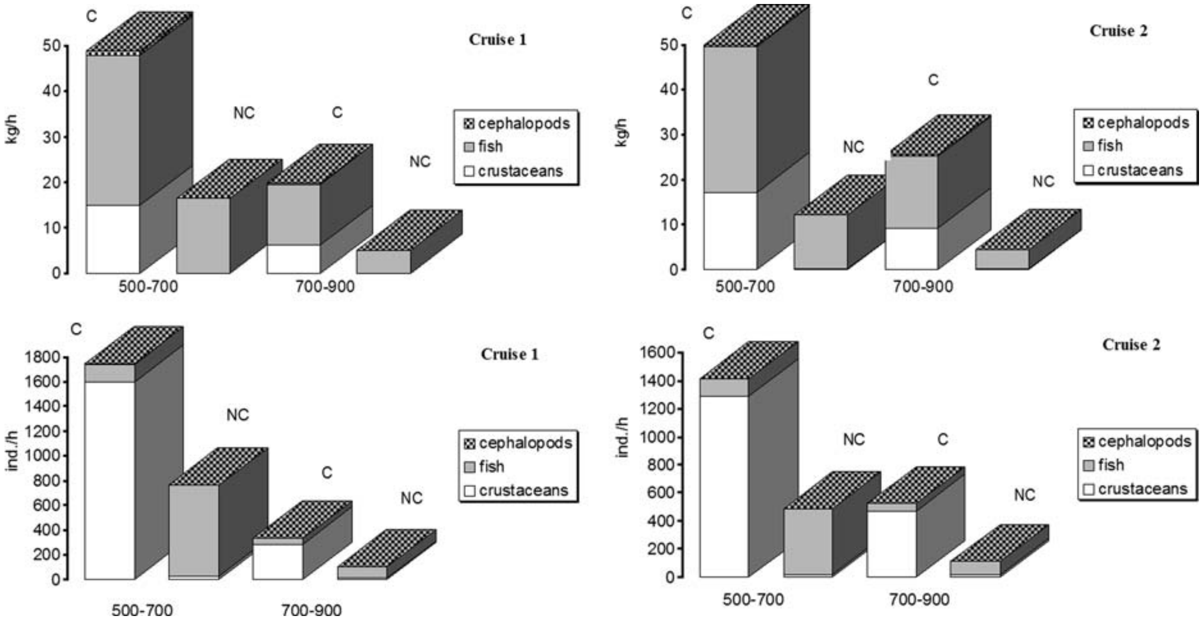

Figure 1. CPUE in terms of weight (kg/h) and numbers (ind./h) of commercial and non commercial crustaceans (white bar), fish (gray bar) and cephalopods (tiled bar) in the depth strata 500–700 and 700–900 m in the eastern Ionian Sea during September 2000 (cruise 1) and July–August 2001 (cruise 2).

commercial catch (<25 kg/h) have been mentioned in other Mediterranean areas (Tursi et al., 1992; Moranta et al., 2000; Ragonese et al., 2001; Sartor et al., 2001). Discards of non-commercial species constituted a relatively low proportion of the total catch (15–25%) in the study area, suggesting that the composition of the demersal deep-water resources in the southeastern Ionian is of high commercial value. Sartor et al. (2001) found a similar percentage of discards (20% of the total catch) in the deep-water trawl fishery of the northern Tyrrhenian Sea, but Moranta et al. (2000) estimated a higher percentage (42%) off the Balearic Islands. It should be noted, however, that the sampling techniques in the above-mentioned studies are not similar.

A decreasing tendency was obvious with depth in the CPUE by weight or numbers in the study area. The role of depth on the biodiversity, abundance or biomass in the Mediterranean deep waters has been sought by various researchers (e.g. Abelló et al., 1988; Cartes & Sardà, 1992; D'Onghia et al., 1998, 2003; Moranta et al., 2000; Kallianiotis et al., 2000; Politou et al., 2003, 2004; Mytilineou et al., 2004).

Considering the contribution percentages of crustaceans and fish in the total catch by weight, found in the present work, these are similar to values reported before for the Ionian Sea (Tursi et al., 1992; Politou et al., 2003) and the northern Tyrrhenian Sea (Sartor et al., 2001). On the contrary, lower values were found for the Balearic Islands (Moranta et al., 2000) and the Strait of Sicily (Ragonese et al., 2001). With respect to cephalopods, their low or negligible proportion in the deep waters, is in accordance to previous results (e.g. Tursi et al., 1992; Sartor et al., 2001; Politou et al., 2003; Lefkaditou et al., 2003).

Among the various commercially important species found in the eastern Ionian Sea during the present study, the highest CPUE values in terms of weight, in both depth strata, were obtained for *A. foliacea*. Similar CPUE values for *A. foliacea* have also been reported for the Strait of Sicily (Ragonese et al., 2001). The biomass index (kg/km^2) for this species in the Mediterranean was found to increase eastwards, the opposite occurring in *A. antennatus* (Cau et al., 2002). In fact, although the CPUE values of the latter species in the study area were low, higher values have been recorded in the Italian Ionian (Tursi et al., 1994; D'Onghia et al., 1998), the western Mediterranean (Sardà et al., 1997) and the adjacent Atlantic (Ribeiro-Cascalho, 1987). Regarding the importance of the other commercial species found in the study area, differences with other areas concern mainly the higher CPUE values of the commercial species *Squalus blainvillei* (Risso, 1827) found in the northern part of the eastern Ionian Sea (Politou et al., 2003) and *Nephrops norvegicus* (Linnaeus, 1758) found in the northern Ionian Sea (Vacchi et al., 1994), the Tyrrhenian Sea (Sartor et al., 2001), the Sicilian Channel (Ragonese et al., 2001), the Catalan Sea (Cartes & Sardà, 1992; Maynou et al., 1996) and the south coast of Portugal (Ribeiro-Cascalho, 1987).

The results of this study showed that important commercial fisheries resources exist in the southern part of the eastern Ionian Sea. In Greece, the commercial fishery is exercised mainly down to 400–500 m depth. This is the result of the narrow continental shelf, the lack of experience of the professional fishers to fish in deep waters their ignorance concerning the presence of commercially important deep-water resources, and the low commercial value of deep-water species in the Greek market until now. As a consequence, most of the shallow-water stocks are overexploited (Stergiou et al., 1997) and at the same time extended marine grounds remain unexploited. Taking into account the recommendations of the E.U. for reduction of the fishing effort and the low productivity of the Greek seas, the discovery of new fishing grounds and new resources could play an important role in the sustainability of all exploited Greek marine fishing resources. However, it should be taken into account that some of the deep-water resources are particularly sensitive to overexploitation. It is already known that sharks and rays, important representatives of the deep-water fauna, are vulnerable to the fishing pressure because of their k-selection strategy (Stevens et al., 2000; D'Onghia et al., 2003). *H. dactylopterus* is also vulnerable to the fishing activity (D'Onghia et al., 1996; Mytilineou et al., 2001), because of its low growth rate, low fecundity and the distribution of its young individuals in shallow waters (300–500 m of depth) where fishing activity is intensively exercised. *A. foliacea* is also sensitive to the fishing pressure (Matarrese et al., 1997; Mytilineou et al., 2001; D'Onghia et al., 2003), because of its lower reproductive potential, the shallower distribution of its young individuals and its higher presence on trawlable bottoms (comparing to *A. antennatus*) (Relini & Orsi-Relini, 1987). Taking into account this information and that *A. foliacea* is the most important deep-water fishing resource in the study area, particular attention should be paid on the management model that will be followed by the Greek deep-water fishery for the sustainability of this resource. Moreover, further comparison with the already exploited stocks in other Mediterranean areas could provide important information for improvement of their management in international level.

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