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Patterns and processes of variation of a rocky bottom cryptobenthic fish assemblage

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RESUMO

Esta tese analisa a dinâmica populacional dos peixes criptobênticos de substrato rochoso no Parque Marinho Luiz Saldanha. Foram estudadas as variações de composição e abundância durante a fase larvar e a fase juvenil/adulto, a duração da fase pelágica de algumas espécies criptobênticas, os habitats de assentamento e testou-se uma nova técnica de amostragem não destrutiva para peixes criptobênticos. No estudo da fase larvar foi amostrado o subtidal rochoso próximo da costa onde se capturam maioritariamente espécies criptobênticas a três profundidades distintas. As larvas mais desenvolvidas ocorreram em maior abundância a maior profundidade. Algumas destas espécies parecem permanecer junto à costa, por exemplo *Pomatoschistus pictus*, ao longo de toda a fase pelágica. A duração desta fase é variável mas há famílias como os Gobiesocidae que passam períodos curtos no plankton. Quando assentam alguns juvenis de *Lepadogaster lepadogaster* ocorrem em maiores abundâncias em habitats com elevada complexidade. A técnica de contagem visual com interferência demonstrou ser eficaz para contar peixes criptobentónicos e permitiu descrever a composição desta comunidade e analisar a sua variação espacio-temporal. Várias espécies usam um número restrito de habitats, (e.g. *Gobius xanthocephalus* e *Tripterygion delaisi*), e uma usa praticamente todos os habitats, *Parablennius pilicornis*. Adicionalmente, observou-se uma variação sazonal na utilização dos diferentes habitats. A diversidade e abundância dos peixes criptobênticos mantiveram-se estáveis ao longo de dois anos apresentando uma variação sazonal consistente. O pico de abundância coincidiu com a chegada de novos indivíduos no Outono mas subsequentemente registou-se um acentuado decréscimo. A dinâmica populacional dos peixes criptobênticos parece ser determinada por factores pós-assentamento e pode ser compreendida à luz das suas características ecológicas nas diferentes fases do seu ciclo de vida.

Palavras –chave: Peixes criptobênticos, ciclo de vida, dinâmica populacional

Title

Patterns and processes of variation of a rocky bottom cryptobenthic fish assemblage

Abstract

The population dynamics of criptobentic fishes in the Arrábida Marine Park was investigated. The fluctuations in composition and abundance, the duration of the pelagic phase and the choice of settlement habitats were assessed. A new non-destructive technique to count cryptobenthic fishes was tested. To study the larval phase we sampled the shallow rocky subtidal where we found mostly cryptobenthic species. Some of these species remain close to shore throughout the entire pelagic phase, e.g. *Pomatoschistus pictus*. The duration of the period spent in the plankton is variable within the cryptobenthic fishes but there are some, such as the Gobiesocidae, that spend very short periods as larvae. *Lepadogaster lepadogaster* settlers occur in higher abundances in high complexity habitats. An interference visual technique was described, tested and used to describe the composition of the cryptobenthic assemblage and assess its spatio-temporal variation. Several species use a restricted number of habitats (e.g. *Gobius xanthocephalus* and *Tripterygion delaisi*), whereas only one used all habitats available, *Parablennius pilicornis*. Moreover, the habitat utilization changed seasonally. Diversity and abundance of the cryptobenthic assemblage remained stable during two years and a consistent seasonal trend was observed. The peek in abundance coincided with the arrival of recruits, in the autumn, but there was a clear decrease in abundance in the subsequent seasons. The population dynamics of cryptobenthic fishes seems to be structured by post-settlement processes and can be understood by the particular ecological features displayed throughout their life-cycle.

Key-words: Cryptobenthic fishes, life-cycle, population dynamics

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Introduction

Population dynamics of coastal fish

Some of the most widely accepted paradigms that explain the dynamics of coastal fishes today have been developed in tropical regions. Early ecologists described fish communities as stable, equilibrium assemblages structured primarily by competitive interactions (Ehrlich 1975). In this view, communities were expected to have stable compositions, and populations were expected to have saturated densities that tracked the carrying capacities of benthic habitats. The number of pre-settlement larvae available was supposedly unlimited comparing to the carrying capacity of saturated benthic populations to encompass new individuals (Doherty 1981).

Working with tropical damselfishes Doherty (1981) concluded that the populations studied could not be at carrying capacity because they absorbed a variable number of recruits without detectable compensatory mortality. This idea was to become the "recruitment limitation hypothesis", which is arguably the most widely accepted demographic model of coral reef fish populations. Its principal tenet is that populations are limited by an under-supply of larval recruits, i.e., there is insufficient recruitment to increase the population beyond the environmental carrying capacity at which density-dependent population regulation occurs (Williams 1980, Victor 1986). Thus, if post-settlement processes do not modify recruitment patterns, the density of adult populations should reflect spatial and temporal variability in recruitment (Doherty 2002). The apparent randomness in distribution and abundance of fish species has reinforced the suggestion that stochastic processes structure reef fish communities, particularly recruitment variation (Sale & Dybdahl 1975; Talbot et al. 1978). Oceanographic variability coupled with high mortality of pelagic larvae causes considerable spatial and temporal variation in larval recruitment at multiple scales (Doherty & Williams 1988; Doherty 1991).

Non-equilibrial alternatives turned to replenishment and predation as major determinants of density variation within populations (Talbot et al. 1978). The non-equilibrial alternatives are nowadays supported by several studies that have documented a high degree of spatial and temporal variation in the structure of some fish assemblages (e.g. Sale & Steel 1986; 1989; Sale et al. 1994). Sale (1978; 1991) defined the “lottery hypothesis” where recruitment need not be limiting to structure fish assemblages. If recruitment rates are sufficient to saturate free space, but free space is limiting and becomes available unpredictably, and if there is little difference in competitive ability between species, then community structure would appear to be an unpredictable lottery.

In the present view there is an ongoing debate over the relative importance of recruitment and post-recruitment processes in structuring marine fish communities (Doherty & Williams 1988; Jones 1991; Forrester 1995). In fact, some studies have shown that post-settlement processes modify the patterns established by stochastic recruitment. Among these processes competition for limiting resources (e.g. Macpherson 1994; Forrester 1995; Booth 1995; Hixon & Carr 1997; Schmitt & Holbrook 1999), predation (Hixon 1991; Hixon & Beets 1993; Carr & Hixon 1995), and migration (Robertson 1988) are certainly the most studied. Even though tropical and temperate systems are different in many aspects such as species diversity and habitat complexity (Ebling & Hixon 1991) they also have strong similarities such as density dependent mortality and predation (reviewed by Hixon & Webster 2002). Hence the hypotheses that explain population dynamics in tropical areas should be tested in temperate areas.

Nowadays there are multiple examples of recruitment-limited populations (reviewed by Doherty 2002) For example Victor (1986) studied the recruitment of *Thalassoma bifasciatum* by back-calculating the age of adult fishes, and showed that the recruitment signal measured in the previous year was maintained in the adult population. He concluded that the population was limited by recruitment because the habitat carrying capacity had not been reached. Other examples showed that post-settlement processes drastically reshape the patterns set at recruitment (e.g. Forrester 1995; Forrester & Steele 2000). In 1997 Steele studied the influence of variable recruitment on the populations of two temperate gobies, the bluebanded goby (*Lythrypnus dalli*) and the blackeye goby

(*Coryphopterus nicholsii*). He manipulated the densities of fish in artificial reefs and verified that strong density-dependent mortality virtually eliminated any linear relationship between adult density and “recruit” density after a period of approximately 3 months. He noted that recruitment was above the densities observed in the field for each species. Therefore both hypotheses may be right depending on the spatial and temporal scales and subjects under study. In other words, patterns of variable abundance among open populations can be set simultaneously by input (recruitment), subsequent density dependent mortality and or density independent mortality (Warner & Hughes 1988). In this view, attention is shifting from identifying one or another type of model, towards studies that examine how pre- and post-dispersal processes and density-independent and density-dependent factors combine to determine reef fish population dynamics (Caley et al. 1996; Schmitt & Holbrook 1999; Schmitt et al. 1999; Shima 2001; Doherty 2002).

Spatial scales, temporal scales and study subjects

To investigate the population dynamics of a particular species or assemblage, the appropriate sampling scales need to be carefully chosen. Many of the studies that originated some of the previously mentioned hypothesis studied different species at different spatial and temporal scales.

The spatial scale selected in each study is fundamental for describing dynamics in a population community structure. On small units, such as coral heads occupying an area of 0.25 m^2, the community structure is often highly variable in time and space (Sale & Douglas 1984; Sale & Steel 1989). In contrast, larger patch reefs in the order of tens to hundreds of square meters in area have a relatively stable fish composition (Ogden & Ebersole 1981). Small scale studies such as the one performed by Forrester (1995), where 0.65m2 squares where used he showed a clear inverse relationship between density and the survival of *Coryphopterus glaucum*. Large-scale studies on the other hand support recruitment determination based on correlations between measures of recruitment and subsequent adult density (e.g. Doherty 1981). Doherty and Fowler (1994) performed a large-scale study within the Great Barrier Reef spanning for 1° in latitude. Based on nine-year surveys of recruitment they assessed the age structure of several populations through analysis of the otoliths and concluded that the strength of recruitment was preserved in the age structure of the *Pomacentrus moluccensis*. The population dynamics of this species at these scales can be almost entirely explained by the interaction of variable recruitment and density independent processes. This kind of knowledge justifies the choice of a relevant scale that encompasses the appropriate habitats in the sampling scale chosen.

The temporal scale selected in these studies is also extremely important. In temperate waters there is a marked seasonal and yearly variation in a number of abiotic factors, such as water temperature, that can affect both the diversity and abundance of fish communities (Magurran & Henderson, 2003). Other factors include stochastic phenomenons such as storms or more or less periodic phenomena such as upwelling events (Dixon et al. 1999; Davis 2000), which can have profound effects on fish community dynamics. Furthermore, not only can recruitment be tremendously variable over time (Doherty & Williams 1988), but post-settlement processes causing mortality may also operate on juvenile life stages of reef fishes, primarily during a brief period immediately after settlement (Macpherson 1994; Jones 1987; Forrester 1990; Caley et al. 1996; Schmitt & Holbrook 2000; Hixon & Webster 2002). Choosing the appropriate scale for each particular study depends on the specific objectives set and extrapolations need to be very cautious, taking into account the fact that different processes operate at very different time scales.

The ecological peculiarities of each species can largely affect the outcomes of a study. Depending on the species under study the importance of recruitment versus post-recruitment processes in the population dynamics is probably very variable. Whilst several studies on tropical bentho-pelagic species of Damselfishes and Labrids found recruitment to be the major driver of cohort strength (Doherty 1981; Warner & Hughes 1988; Caselle & Warner 1996; Caselle 1999; Schmitt et al. 1999; Shima 2001), in temperate areas ecologically different species such as cryptobenthic species provided contrasting results. In the case of temperate gobies predation played a major role among the post-settlement processes shaping the population dynamics (Forrester 1995; Steele 1997; Forrester & Steele 2000). Thus, the appeal to expand the taxonomic coverage of the study systems to test the validity of population dynamics hypothesis has been made (Hixon & Webster 2002).

This thesis analyses the population dynamics of a temperate cryptobenthic fish assemblage and addresses specific aspects of the life cycle of these species. The cryptobenthic guild, i.e. group of species with similar ecological requirements, has specific features that make them good models to study abundance and diversity fluctuations. Furthermore the ecological differences between cryptobenthic and other species may provide contrasting results as far as population dynamics is concerned.

Cryptobenthic fishes

Cryptobenthic fishes have been defined in slightly different ways. The root of the word cryptic derives from the Latin *crypticus*, from Greek *kryptikos*, from *kryptos*, meaning secret or concealed. Perhaps one of the first definitions of cryptobenthic fish is the one given by Miller (1979). He defined three groups of marine fishes according to the ecotopes occupied: necktonic, epibenthic and cryptobenthic. The latter category encompassed "small bodied fishes (<10 cm) that exploit restricted habitats where food and shelter are obtained in, or in relation to, conditions of substrate complexity and/or restricted living space, with a physical barrier likely to be interposed between the small fish and sympatric predators". Millers' (1979) categories are not mutually exclusive, i.e. some of the areas used by fish from different groups are superimposed. Both cryptobenthic and epibenthic species use cavities and surfaces of rock and sand. The main difference between these two categories is that cryptobenthic fishes do not swim freely in the water column. Epibenthic species are considered by many authors to be bentho-pelagic species such as wrasses and basses from the genus *Symphodus* and *Serranus* (e.g. Guidetti et al. 2004). More recently, Depczynski &

Bellwood (2003) defined (tropical) cryptobenthic fishes as ‘adult fishes of typically <5 cm that are visually and/or behaviourally cryptic, and maintain a close association with the benthos’. The difference in size considered in these definitions is probably derived from the fact that temperate cryptobenthic fishes are larger than tropical species (Illich & Kotrshal 1990).

Cryptobenthic fishes have a suite of ecological and biological features that make them an appealing model to study population dynamics. Among those features are their diversity, abundance, growth, turnover rates and habitat association.

Diversity and abundance of cryptobenthic fishes

Assemblages of temperate cryptobenthic fishes in coastal areas are diverse (Gibson 1969; Miller 1986; Allen et al. 1992; Macpherson 1994; Prochazka 1998; Patzner 1999; Willis 2001; Willis & Anderson 2003; La Mesa et al. 2004). In the Northeastern Atlantic and the Mediterranean, several studies have either described the ecology of particular cryptobenthic species (e.g. Costello 1992; Macpherson 1994; La Mesa & Vacchi 2005) or the composition of the whole cryptobenthic assemblages (Patzner 1999; La Mesa et al. 2004, 2006). While some of these studies used traditional visual census methods to count these fishes, others have used destructive methods to do an exhaustive description of the cryptobenthic assemblage. Overall these assemblages are characterized by a high number of Gobiidae, Blenniidae and sometimes other families such as Scorpaenidae or Tripterygiidae (e.g. Macpherson 1994; Kovačić 1997; Almada et al. 2001; La Mesa et al. 2004, 2006).

A number of cryptobenthic fishes were believed to be extremely rare (Miller 1986) but upon closer examination was found to be rather common (Gonçalves et al. 1998; Gonçalves et al. 2005). In temperate areas several cryptobenthic species have recently been described and their geographical ranges expanded (Ahnelt & Patzner 1995; Hofrichter & Patzner 1997; Van Tassell 2001; Gonçalves et al. 2005). This may be the result of increasing efforts done to study coastal fish communities; nonetheless a full evaluation of the diversity of cryptobenthic fishes is yet to be achieved.

Cryptobenthic fishes are often very abundant in nearshore habitats (Gibson 1969; Macpherson 1994; Kovačić 1997; Gonçalves et al. 1998; Patzner 1999; Hofrichter & Patzner 2000; La Mesa et al. 2004). In fact they can be four times as dense as conspicuous fishes (Allen et al. 1992). However the majority of studies concerning cryptobenthic fishes have only evaluated particular families or species groups (e.g. Illich & Kotrshal 1990; Gonçalves et al. 1998; Hofrichter & Patzner 2000; Almada et al. 2001; Kovačić 2003; Nieder et al. 2000; La Mesa & Vacchi 2005) or assemblages in a qualitative perspective (e.g. Patzner 1999).

From an ecological standpoint, the influence of these taxa in coastal areas must be significant; however this subset of the reef fish community has seldom been included in coastal community studies (Allen et al. 1992; Depczynski & Bellwood 2004; Stephens et al. 2006).

Ecological features of cryptobenthic fishes

Peculiar features of cryptobenthic fishes encompass several aspects of their biology and ecology such as weight and size, growth, turnover rates, trophic role, reproduction and behaviour. The majority of cryptobenthic species have small sizes and low weights. For example: *Lepadogaster lepadogaster* can weigh 2.8 g at 5 cm (following Froese & Pauly 2006); *Gobius paganellus* can weigh 23.1 g at 12 cm (following Azevedo & Simas 2000); and *Parablennius gattorugine* can weigh 54.5 g at 15 cm (following Koutrakis & Tsikliras 2003). Although by definition cryptobenthic fishes are primarily considered to be small species (e.g. Miller 1979), many recent studies have encompassed larger species that utilize similar habitats (e.g. La Mesa et al. 2004). In this study we also included relatively large species such as some Gobiidae (*Gobius cruentatus*), Blenniidae (*Parablennius gattorugine*) and Scorpaenids (e.g. *Scorpaena porcus*) that occur in the same habitats as the smaller species.

Many cryptobenthic species present rapid growth rates. A number of species were found to grow up to half their total size in the first year of life. For example: *Gobius paganellus* can grow up to 6 cm during the first year of life and attain a maximum length of 12 cm after 6 years (Miller 1986, Azevedo & Simas 2000), *Pomatoschistus pictus* can grow up to 4.3 cm in the first year of life and reach a maximum size of 6 cm in 2 years (Miller 1986; Arruda et al. 1993), *Gobius niger* reaches 7.6 cm in the first year and may reach up to 18 cm in 4 years (Arruda et al. 1993).

Another interesting feature of cryptobenthic fishes is their trophic role. The diets of small cryptobenthic fishes are mainly composed of micro-crustaceans (Gibson 1972; Zander 1982; Costa 1988; Azevedo & Simas 2000). Nonetheless this is not a resolved issue in the sense that a few studies on the diet of temperate cryptobenthic species showed large amounts of algae in the stomachs of *Gobius paganellus* ranging from 16 to 37% (Dunne 1978; Costa 1988; Azevedo & Simas 2000). If herbivory is a possible trophic pathway for cryptobenthic fishes (e.g. Stepien 1990) and given that it is so rare among temperate fishes (Harmelin-Vivien 2002), then the trophic role of these fishes may be substantially different from that presently assumed. Recently, the diets of several tropical cryptobenthic fishes were investigated and yet a new major trophic pathway was described. Detritus seem to be a major dietary component from several tropical cryptobenthic Blenniidae and Gobiidae (Wilson et al. 2003; Depczynski & Bellwood 2004). In tropical regions, cryptobenthic fishes may play a significant role in reef trophodynamics, with up to 25% of the energy flow by fishes passing through these taxa (Ackerman & Bellwood 2002).

Theory predicts that small fishes lay fewer eggs than larger fishes (Gibson 1969). Cryptobenthic species have several strategies and behaviours to improve the survival of the offspring. Most cryptobenthic species lay benthic eggs (e.g. Gibson 1969), defend territories (e.g. Gonçalves & Almada 1998) and hatch fairly developed larvae in relation to larger pelagic species (Gibson 1969). Recent studies on the development of larval abilities have shown that a large number of species possess very strong swimming abilities (e.g. Williams et al. 1996), sensory abilities and the development of those abilities may start early in development (for a review see Leis & McCormick 2002; Myrberg & Fuiman 2002).

The life cycle of cryptobenthic fishes

Cryptobenthic fishes, like many marine organisms, have complex life cycles (*sensu* Roughgarden et al. 1988) divided in two main phases: the larval phase generally spent in the plankton as larvae, and the juvenile/adult phase, which starts when larvae settle into benthic habitats. Between these phases there is a relatively short transition period from pelagic to benthic environments called settlement.

Larval phase

In the larval phase many cryptobenthic fish occur in the nearshore area (Kingsford & Choat 1989; Tilney et al. 1996; Sabatés et al. 2003; Vélez et al. 2005). There is evidence that, prior to settlement, fish larvae, including cryptobenthic fish, school over shallow rocky bottoms (Marliave 1986; Kingsford & Choat 1989; Breitburg et al. 1995). Even though the mechanisms that explain the recruitment of fishes are not completely understood, there have been a number of suggestions including passive drift mechanisms (Cowen 1985; Shanks 1988), active swimming of late stage larvae following perceptual cues (e.g. Leis & Carson-Ewart 1998) and also behavioural mechanisms that retard the drift process, keeping them in the general area for subsequent settlement (e.g. Marliave 1986).

Many fish larvae actively modify their position in the water column, which can result in retention in the vicinity of the reefs (Leis 1991; Jones et al. 1999; Swearer et al. 1999; Cowen 2002; Leis & McCormick 2002; Taylor & Hellberg 2003).

Recent studies have looked into the larval sensory abilities such as sound, odour and visual cues and also swimming capabilities of coastal fishes (Leis & Carson-Ewart 1998; Myrberg & Fuiman 2002; Lecchini et al. 2005). Hence larvae seem to be able to control their position in the water column. Studies on the larval abilities of some coastal cryptobenthic fish are still taking their first steps. However Tomilieri et al. (2000) showed that trypterigiidae larvae respond to sound cues in the marine environment.

At the end of the planktonic stage, larvae may be able to select specific habitats (Montgomery et al. 2001), resulting in non-random patterns of juvenile distribution (Danilowicz 1996; Ohman et al. 1998). There have been observations of schooling behaviour previous to settlement in a number of species (e.g. Breitburg 1989) but quantitative sampling over high relief coastal areas has been undermined by the use of classical methodological approaches. Most methods used to sample nearshore ichthyoplankton communities are inadequate for shallow high-relief bottoms as they miss those specimens that stay close to the bottom (Leis 1991; Olivar & Sabatés 1997). The composition and abundance of cryptobenthic late larval stages remains to be adequately assessed.

Settlement

Settlement is the period of time at the end of the larval phase, when fish do not exhibit the coloration or behavioral characteristics of well-established juveniles (Kaufman et al. 1992). Discontinuities in otolith-increment structure and changes in the opacity of otoliths have been associated with the settlement of pelagic stages of demersal species into their juvenile benthic habitat (Victor 1986; Sponaugle & Cowen 1994; Wilson & McCormick 1997, Wilson & McCormick 1999). Otoliths are calcareous accretions found within paired otolithic organs, that together with the semicircular canals, make up the inner year of teleost fishes (Thorold & Hare 2002) and are used for balance and hearing in teleost fishes (Campana 1999). There are three types of otoliths varying in size, shape and composition (Campana 1999): *sagittae*, *lapillae* and *asterici*. These structures have been used as time-markers to back-calculate larval durations and recruitment patterns (Wilson & McCormick 1999). The settlement-mark can be defined as the alteration or transition in otolith-increment structure (width, number and optical properties) associated with the settlement event (Wilson & McCormick 1999). The settlement marks seem to be species-specific but categorization into several settlement mark types has been proposed by Wilson & McCormick (1999). The intimate link between a fish's physiology and environment and the growth of its otolith suggests that the time period incorporated in the settlement-mark may be related to the degree of metamorphosis associated with settlement (Campana & Neilson 1985; Wilson & McCormick 1997). Apart from Raventos and Macpherson's (2001) study, where the pelagic larval durations of a number of Mediterranean coastal fishes including some cryptobenthic fishes is described, very little is known about the time these species spend in the plankton. At settlement, some species undergo habitat and species associations shifts (McCormick & Mackey 1997). Observational studies have shed some light into the occurrence of juvenile cryptobenthic fishes in some habitats (e.g. Patzner 1999; Hofrichter & Patzner 2000). Ontogenetic habitat shifts for several cryptobenthic species have also been addressed in a more quantitative manner (e.g. Gonçalves et al. 2002). The different habitats may enhance survival of particular developmental stages. In fact, settling into specific habitats, either cryptic habitats or highly complex ones, may reduce predation risk over recruits (e.g. Hixon 1991; McCormick & Mackey 1997; Patzner 1999), which can be a strong determinant of recruit density in some cryptobenthic fishes (Steele 1997; 1999; Carr 1991; Webster 2004).

Ontogenetic habitat shifts are probably related to particular habitat requirements such as shelter availability. Specifically for younger stages, the availability and size of refuges seems to be a good predictor of survival (e.g. Berhents 1987; Steele 1999).

Adult and juvenile phase

In the adult and juvenile phases, cryptobenthic fishes spend a long time keeping close to the substrate and have strong relationships to habitat features (La Mesa et al. 2004). Habitat requirements of cryptobenthic fishes may involve several environmental factors, which operate at different spatial scales. Microhabitat features of substratum, such as types of cover, complexity and heterogeneity, have a strong influence on the habitat choice of many small cryptobenthic fishes

(Connell & Jones 1991; Costello 1992; Wilkins & Myers 1992; Macpherson 1994; Syms 1995; Macpherson & Zika 1999; La Mesa et al. 2004; Malavasi et al. 2005).

One of the major constraints in studying cryptobenthic fishes has been the fact that unbiased data can only be retrieved through destructive sampling given that traditional visual census methods strongly underestimate the abundance of these species (e.g. Harmelin-Vivien et al. 1985; Kulbicki 1990; Ackerman & Bellwood 2000; Willis 2001; Edgar et al. 2004). The efficacy of each particular technique depends on several factors such as the habitat complexity and study object (Harmelin-Vivien et al. 1985). Due to the destructive nature of some methods (e.g. rotenone) or the strong bias that traditional visual census methods have in sampling cryptobenthic species, very few studies have adequately assessed the temporal variation in subtidal cryptobenthic fish assemblages. However intertidal cryptobenthic species have been studied for a number of years (Grossman 1986; Yoshiama et al.1986).

Growing evidence from different geographic regions suggest a consensusal view on the stability, long-term persistence and resilience of intertidal fish assemblages (Gibson & Yoshiama 1999; Faria & Almada 1999; Almada & Faria 2004). In contrast, very little is known about the density fluctuations of their subtidal counterparts. At a smaller temporal scale, seasonal changes can also occur among coastal cryptobenthic fishes. Davis (2000) observed a decline in abundance during the winter months in a guild of tidepool fishes. This decline may be related to stronger waves, which are in turn often correlated with lower number of species (Grossman 1982). Several habitat shifts have been described for shallow-water and intertidal cryptobenthic fishes. While there are some species that are strictly intertidal or subtidal, there are also those that utilize both areas (Allen & Pondela II 2006). The habitat shifts may be related to ontogeny (e.g. Faria & Almada 2001; Gonçalves et al. 2002), sex (e.g. Stepien 1987, Stepien & Rosenblatt 1991), reproduction microhabitats (e.g. Patzner 1999), biotic interactions or environmental factors (Horn & Martin 2006). Even though there are some studies on the habitat utilization of one or two individual species (e.g. Costello 1992; Wilkins & Myers 1995), the seasonal habitat shifts have not been addressed at the assemblage level.

Other studies on the temporal variation in coastal areas recorded the density fluctuations for the whole fish community (e.g. La Mesa & Vacchi 1999; Magill & Sayer 2002). Given the specific characteristics of cryptobenthic species when compared to pelagic species, and the differences between the intertidal and subtidal environments, the patterns of variation in density and diversity remain poorly understood.

A comprehensive approach to population dynamics

The spatial structure and temporal dynamics of fish populations depend, to varying extents, on pre and post settlement processes (Schmitt & Holbrook 1999). If recruitment is low and variable (relative to resource availability), it can limit and determine population size below levels at which resources are limiting (i.e. recruitment limitation Doherty 1981). If on the other hand recruitment is high, recruits will saturate resources and post-settlement density dependent process will contribute more to spatial and temporal variability in population size (Carr & Syms 2006). Recognition of this is reflected in the growing number of conceptual and analytical models that explore the relationships of these processes (Armsworth 2002; Doherty 2002; Hixon & Webster 2002; Osenberg et al. 2002).

Understanding the density fluctuations of any particular species or assemblage require a comprehensive approach, i.e. should ideally encompass all the life phases. First of all, knowing the fish larval composition and densities close to shore may bring further insight into understanding how larval composition and density translate into recruitment. Secondly, accompanying settlement and understanding the processes that affect the distribution of recruits, and ultimately their survival, will help to explain the diversity and abundance of the adults. A number of factors that influence the distribution of fishes vary at different scales. For example some larvae may be able to select a specific microhabitat prior to settlement (Montgomery et al. 2001). Higher settlement or settler survival into particular habitats (Connell & Jones 1991) will influence the population density. Ontogenetic habitat shits and settler migration have been identified for a number of species (e.g. Carr & Hixon 1995; Gonçalves et al. 2002) is bound to increase or decrease recruitment estimates depending on the spatial scale selected. Even in the juvenile and adult phases the ecological preferences, such as reproduction habitat versus general habitat may clearly influence density estimates. It becomes clear that it is crucial to investigate the different periods in the life cycle of fish in order to understand their population dynamics.

Aims and outline of this dissertation

In order to study the population dynamics of a temperate cryptobenthic fish assemblage we addressed the following questions within different periods of their life cycle:

In the larval phase and settlement

1.1What is the composition, abundance and structure of the larval assemblage in nearshore shallow rocky bottoms? Does it change across depths? Is there an ontogenetic distribution across depths, if so is it taxon-specific?

1.3 How long do cryptobenthic species spend in the plankton?

1.4 How does diversity and abundance of cryptobenthic fish change across three habitat types of different complexities? Are there ontogenetic habitat shifts?

In the adult phase

2.1 Describe an interference census technique. How does it perform comparatively to anaesthetic counts?

2.2 Describe the geographical range a new species in continental Portugal. Compare the meristic and morphometric characteristics of the specimens collected to those described in the reference paper.

2.3 What is the composition of the cryptobenthic assemblage in the no-take area of the Arrábida Marine Park?

2.4 What is the spatial distribution, diversity and abundance of the cryptobenthic assemblage across the main habitat types? Do these parameters change seasonally? Are there seasonal habitat association shifts among the most abundant species?

2.5 In the no-take area of the Arrábida Marine Park what is the yearly and seasonal dynamics of the cryptobenthic assemblage in terms of diversity, total abundance and structure? What is the size and density variation of those species to investigate specific yearly and seasonal trends?

The aims of this dissertation were pursued through seven studies that are published or have been submitted to various journals, and are presented in the next seven chapters:

1. Depth distribution of nearshore temperate fish larval assemblages near the rocky substrate. Beldade, R., Borges, R. and Gonçalves, E.J. In press *Journal of Plankton Research*

2. Pelagic larval duration of nine cryptobenthic species found in Portuguese waters
Beldade, R., Pedro, T. and Gonçalves, E.J. Submitted to the *Journal of Fish Biology*

3. Settlement habitats of temperate cryptobenthic fishes and the evaluation of complexity. Beldade, R. and Gonçalves, E.J. Submitted to *Marine Biology*

4. An interference visual census technique applied to cryptobenthic fish assemblages Beldade, R. and Gonçalves, E.J. In press *Vie Millieu*

5. First record of *Chromogobius britoi* (Teleostei: Gobiidae) on the mainland European coast. Beldade, R., Van Tassell, J. and Gonçalves, E.J. Published by the *Journal of Fish Biology* 68:608-612

6. Seasonality in the spatial distribution of a cryptobenthic fish assemblage. Beldade, R. and Gonçalves, E.J. Submitted to *Marine Ecology Progress Series*

7. Composition and temporal dynamics of a temperate rocky cryptobenthic fish assemblage. Beldade, R., Erzini, K. and Gonçalves, E.J. Published by the *Journal of the Marine Biological Association of the UK* 86:1221-1228

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Depth distribution of nearshore temperate fish larval assemblages near the rocky substrate

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Abstract

In this study we compare the composition, abundance and structure of a temperate fish larval assemblage at different depth intervals ([0-4] m, [4-8] m and [8-12] m) in the extreme nearshore. We used a plankton net attached to an underwater scooter to sample in close proximity to the rocky substrate (< 50 cm). A total of 868 larvae from 27 taxa in 13 families were caught. The majority of larvae belonged to benthic reef-associated species (Blenniidae, Gobiidae, Gobiesocidae, Tripterygiidae), the 4 most abundant comprising 76% of the total larvae caught. A non-metric MDS analysis showed that there was a single multispecific larval patch near the substrate in the extreme nearshore up to 12 m depth. Nonetheless, distinct larval abundances were found in this relatively small depth range, with the majority of species being more abundant at the deepest interval, particularly *Pomatoschistus pictus* and *Gobius xanthocephalus*. *Tripterygion delaisi* was an exception being more abundant at the shallowest depth as young larvae. The density of pre-flexion larvae was not significantly different across depth intervals, but post-flexion larval density increased with depth. The full size range (from hatching to settlement) of *P. pictus* was present at the extreme nearshore. In close proximity to the bottom, depth is an important factor influencing the distribution of several taxa and ontogenetic stages.

Keywords

Larval fish, temperate waters, nearshore, assemblage structure, depth influence

Introduction

The spatial distribution of larvae (both horizontal, from the coast to open water environments, and vertical, from the surface to the sea bottom) can be a major determinant of adult population sizes (Sinclair 1988). However, in temperate coastal areas studies on larval distribution have focused mainly on commercial fishes and at offshore waters (Nielsen & Perry 1990; Cushing 1995) whereas coastal rocky bottom species have received little attention (Leis & McCormick 2002).

Horizontal distributional studies have found that inshore larval assemblages have a distinct composition from that of offshore assemblages (Marliave 1986; Sabatés 1990; Gray 1993) and are also characterized by higher densities of shorefishes (Sabatés 1990; Gray 1993; Jenkins et al. 1999; Sabatés et al. 2003). Horizontal distribution can however be strongly influenced by the vertical position of larvae in the water column (Armsworth 2001). In coastal waters (less than 100m deep) vertical distribution patterns have been described for several taxa (Leis 1991a; Cowen 2002). In some of the few vertical distribution studies performed in inshore waters, higher abundances of larvae were found in the deeper water layer (Gray 1993) and there is growing evidence of vertical depth related distribution of larvae even at small spatial scales (Leis 1991a; 1991b). Taxon-specific vertical distribution patterns were described by several authors at small spatial scales, mainly in coral reefs (Leis 1991b; Hendricks et al. 2001) but also in temperate waters (Boehlert et al. 1985). A highly structured vertical distribution pattern of nearshore coral-reef fish larvae with several taxa (e.g. Labridae and Gobiidae) being more abundant in deeper waters during the day has been described (Leis 1991b). Hendricks and colleagues (2001) also found some gobies to be more abundant at deeper water.

Ontogenetic vertical distribution of larvae in coastal waters is also poorly understood. Little evidence of age related vertical distribution was found for several taxa on coral reefs (Leis 1991b). On the other hand, Cowen (2002) found pre-flexion larvae to occur shallower than post-flexion larvae to be the most common pattern, as is the case of the damselfish *Stegastes partitus* (Paris & Cowen 2004).

Most of these studies have assessed the vertical distribution of larvae in the water column and have traditionally relied on methods such as oblique tows (Boehlert et al. 1985; Leis 1991b; Paris & Cowen 2004); vertical hauls (Gray 1996); horizontal tows (Olivar & Sabatés 1997; Sabatés et al. 2003) and hand net collections (Marliave 1986). These methods are however inadequate to sample over high-relief bottoms at the extreme nearshore as they miss those specimens that stay close to the bottom (Leis 1991b; Olivar & Sabatés 1997). Even though the development of light traps allowed sampling in these environments (Milicich et al. 1992; Hendricks et al. 2001), this method attracts only photopositive larvae from varying distances and possibly from all directions, making a clear indication of the exact position of the larvae caught impossible. Therefore, there is a sampling gap in ichthyoplankton studies of nearshore assemblages because the water layer close to the bottom is not sampled most of the times. Late-stage larvae in particular are known to school at close proximity to the bottom in coastal areas (Breitburg 1989; 1991; Leis 1986; Steffe 1990) where they can profit from particular current regimes (Marliave 1986) that ultimately enable them to remain nearshore.

Even though there is evidence for the presence of some larval stages near the bottom at the nearshore, the epibenthic water layer remains to be adequately sampled and the effect of depth over the distribution of larvae near the bottom is not known. In this study we propose to fill this sampling gap in nearshore larval distribution studies using a diver steered sampling method. The following questions are addressed: How do larval assemblage composition, abundance and structure change with depth close to the bottom? Are there taxon-specific vertical distribution patterns? Are there differences between ontogenetic stages in their vertical distribution?

Methods

Sampling location and period

This study was carried out at the Arrábida Marine Park (west coast of Portugal – 38º 27’ 03’’N, 009º 01’ 24’’W) in July 2002, during the spawning season of most of the resident species (unpublished data). The extreme nearshore were selected for sampling in the sector of the Park with the highest biodiversity (Gonçalves et al. 2003). Underwater rocky habitats extend to around 13 m depth and are highly heterogeneous resulting from the disintegration of the calcareous cliffs that border the coastline. This area faces south and is therefore highly protected from the prevailing north and northwest winds and waves.

Sampling methodology

A plankton net (mouth diameter 30 cm; mesh size 350 µm; diameter/length ratio 1:3) attached to an underwater scooter was used to sample in close proximity to the substrate (closer than 50 cm) (Fig. 1). A Hydrobios flowmeter attached to the mouth opening measured the volume of filtered water (mean volume = 8.51m-3 SD = 2.70m-3). Trawling speed was approximately 1.3 knots (SD = 0.21, N = 10). In spite of the low speed, advanced stage larvae of several species were caught. Therefore, net avoidance by larvae due to low trawling speed does not seem to be a problem in this study.

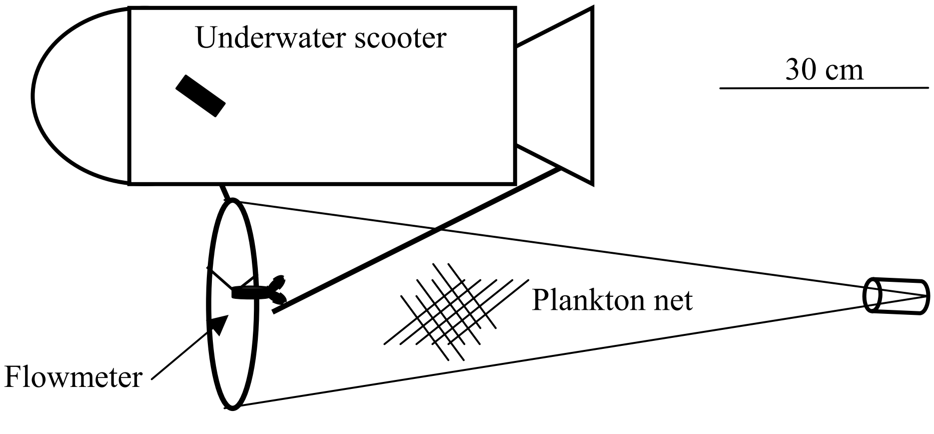


Fig. 1. Diagram of the scooter-plankton net apparatus used for sampling.

Sampling was performed in the morning in good sea and weather conditions. Each sample consisted of a 5min trawl parallel to the shoreline. After reaching the bottom, the diver opened the net and begun the trawl following a direction parallel to the shoreline. Three depth intervals were chosen: [0-4] m, [4-8] m and [8-12] m. These depth intervals were chosen according to the ability to accompany the bottom relief in an approximately straight line, avoiding large obstacles when necessary, without leaving the chosen depth strata with the aid of a diving computer attached to the scooter. A total of 27 samples were taken at each depth, three days a week collecting 3 samples a day for 3 weeks, totalizing 81 samples. The trawls were performed around the middle depth value in each depth interval selected. For each sample the difference between the maximum and minimum sampling depths was on average 2.22 m (SD = 0.54).

Larvae were preserved in 4% buffered formalin for at least one month and identified to the lowest taxonomic level possible (6.8% of the larvae could not be identified). Larvae were assigned a developmental stage according to the flexion stage of the urostyle, following Leis and Carson-Ewart (Leis & Carson-Ewart 2000) but considering only two categories: ‘pre-flexion’ and ‘post-flexion’ (after initiation of the flexion process). Larval length is defined as body length (BL) and corresponds to notochord length in pre-flexion larvae or to the standard length (SL) in post-flexion larvae. Measurements were taken to the nearest 0.1mm using a micrometer scale under a stereo-microscope (3.3% of the larvae were damaged and therefore were not measured).

Statistical Analysis

Two diversity indexes were calculated for each sample. The Shannon Diversity Index (H’) calculated from the proportional abundances p_i of each species (abundance of the species / total abundances, noted here as $p_i = n_i / N$) using the natural logarithm in its formulation. This index reflects diversity based on the number of species and relative abundance of each species (Zar, 1996).

$$H' = - \sum_{i=1}^{s} p_i \ln(p_i)$$

The Average Taxonomic Distinctness Index (Δ^*) where X_i ($i=1,\ldots, s$) denotes de abundance of the i^{th} species, n ($= \Sigma_j X_i$) is the total number of individuals in the sample and ω_{ij} is the "distinctness weight" given to the path length linking species i and j in the hierarchical classification. The double summations are overall pairs of species i and j (with $i < j$). For the calculation of Δ^*, equal step-lengths were assumed between these taxonomic levels: Family, Genera and Species. This index reflects the taxonomic spread of species among samples (Clarke & Warwick 1999).

$$\Delta^* = [\Sigma\Sigma_{i<j}\omega_{ij}X_iX_j]/[\Sigma\Sigma_{i<j}X_iX_j]$$

Differences in these indexes across depths were tested using a One-way ANOVA for the H'and a Kruskal-Wallis test for the Δ^* given that normality assumptions were not met in the second case (Zar 1996).

Differences in total densities and densities of pre-flexion and post-flexion larvae per sample among depths were tested using the Kruskal-Wallis test given that data did not conform to normality or homogeneity of variances. The post-hoc Dunn's test was used to identify where differences lay.

The assemblage structure analysis was performed with the multivariate statistical package Primer-E (Clark & Warwick 2001). A Bray-Curtis similarity index for log(x+1) transformed data was applied to the sample matrix (abundance of each species per sample) to reduce the contribution from numerically dominant species. A non-metric multidimensional scaling (MDS) was applied to the similarity matrix to visualize the relationships among samples. In this plot, samples that are closer together are less distinct and the stress coefficient measures the extent to which the plot displays the relationships among samples (Clarke & Warwick 2001). The analysis of similarities test (ANOSIM) was used to investigate differences in the structure of the assemblage between depth intervals (999 permutations). This test is analogous to a univariate analysis of variance and identifies whether differences between the MDS groupings are significant.

Differences in size (BL) at each depth interval were compared among all the specimens of the four most abundant species. Given that data did not conform to normality and variances were not homogeneous even after transformation, Kruskal-Wallis test and post-hoc Dunn's tests, or a Mann-Whitney U test, were used to identify differences between the sizes of larvae across depths.

Results

Assemblage composition

The 868 larvae captured belonged to 27 taxa in 13 families (Table I). The majority of larvae (74%) belonged to benthic reef-associated species of the Families Gobiidae, Blenniidae, Tripterygiidae and Gobiesocidae, with 52% of the species common to all depth intervals. The four most abundant ones (*Pomatoschistus pictus*, *Gobius xanthocephalus*, *Tripterygion delaisi* and *Symphodus melops*) comprised 76% of the total larvae caught. Several species occurred in the deeper interval but were absent or very scarce in the shallowest interval: *Lepadogaster* sp., *Lepadogaster candolii*, *Ctenolabrus rupestris* and *Symphodus bailloni*, Sparidae sp1 and Sparidae spp. Some species presented an inverse pattern, as they were captured at the shallowest interval, in low densities, and were absent from the deeper interval: *Coryphoblennius galerita*, *Trachurus trachurus*, *Gobiusculus flavescens*, *Serranus* spp. In terms of total diversity, both the Shannon Diversity Index and the Average Taxonomic Distinctness Index were not significantly different across depths (Table II).

Table I. Mean density ± SD for each species in each depth interval given in specimens/1000m^3. No id = unidentified larvae.

Family	**Genus**	**Species**	**[0-4] m**		**[4-8] m**		**[8-12] m**	
			Mean	±SD	Mean	±SD	Mean	±SD
Blenniidae	*Parablennius*	*Parablennius pilicornis*	12.06	±42.66	4.68	±24.33	6.49	±33.74
	Coryphoblennius	*Coryphoblennius galerita*	3.50	±16.81	0		0	
Bothidae	*Arnoglossus*	*Arnoglossus thori*	4.72	±22.66	30.88	±137.36	8.09	±42.02
Callionymidae	*Callionymus*	*Callionymus* spp.	10.90	±28.94	33.13	±78.23	24.50	±76.24
Carangidae	*Trachurus*	*Trachurus trachurus*	8.20	±27.17	5.53	±28.72	0	
Clupeidae	*Sardina*	*Sardina pilchardus*	15.51	±34.64	71.41	±194.08	67.88	±144.57
Engraulidae	*Engraulis*	*Engraulis enchrasicolus*	0		8.73	±45.38	0	
Gobiesocidae	*Lepadogaster*	*Lepadogaster candolii*	0		0		16.78	±69.98
	Lepadogaster	*Lepadogaster lepadogaster*	0		0		4.73	±24.59
Gobiidae	no id	Gobiidae spp.	22.16	±38.40	13.06	±67.85	8.09	±42.02
	Gobius	*Gobius xanthocephalus*	71.03	±224.14	72.39	±120.27	415.92	±777.74
	Gobiusculus	*Gobiusculus flavescens*	2.96	±14.21	7.78	±28.12	0	
	Pomatoschistus	*Pomatoschistus microps*	0		0		8.36	±43.42
	Pomatoschistus	*Pomatoschistus pictus*	97.26	±163.67	412.85	±512.68	1808.64	±2397.32
Labridae	*Centrolabrus*	*Centrolabrus exoletus*	3.60	±17.25	0		72.65	±143.31
	Coris	*Coris julis*	0		13.78	±51.53	0	
	Ctenolabrus	*Ctenolabrus rupestris*	0		4.17	±21.66	28.01	±78.38
	Symphodus	*Symphodus bailloni*	0		31.66	±76.54	17.26	±50.00
	Symphodus	*Symphodus melops*	4.13	±19.82	118.35	±185.49	93.57	±131.27
	Symphodus	*Symphodus* spp.	4.10	±19.67	34.95	±88.23	67.42	±97.75
	Symphodus	*Symphodus roissali*	19.29	±37.65	34.73	±101.05	6.49	±33.74
no id	no id	no id	20.72	±48.74	26.64	±59.79	42.00	±90.53
Serranidae	*Serranus*	*Serranus* spp.	9.10	±30.56	21.08	±62.75	0	
Soleidae	no id	Soleidae spp.	4.10	±19.67	14.38	±59.57	8.09	±42.02
Sparidae	*Boops*	*Boops boops*	4.10	±19.67	31.73	±80.34	111.72	±188.45
	no id	Sparidae sp1	0		5.03	±26.14	62.96	±162.92
	no id	*Sparidae* spp.	0		5.53	±28.72	53.71	±173.03
Tripterygiidae	*Tripterygion*	*Tripterygion delaisi*	110.33	±130.39	67.92	±99.28	50.87	±103.95
		TOTAL	427.78	±956.71	1070.39	±2178.11	2990.69	±4980.71

Table II. Shannon diversity index (H') and average taxonomic distinctness index (Δ*) in each depth interval. F = value of One-Way ANOVA; H = value of Kruskal-Walis test.

Depth	**n**	**Average H'**	**SD H'**	**Test**	**p**	**Average Δ***	**SD Δ***	**Test**	**p**
[0-4] m	27	0.84	0.54	H = 4.26	0.127	77.67	38.97	F = 1.28	0.284
[4-8] m	27	0.95	0.55			74.67	33.49		
[8-12] m	27	1.07	0.39			91.29	8.09		

Species abundances

There were significant differences across depths in total larval densities (Kruskal-Wallis test: H (2, n = 81) = 38.14; P < 0.001). All depth intervals were significantly different from each other, with higher densities registered at the deepest interval (Fig. 2). In each depth interval, the abundance rank for the different species changed. In the shallowest interval, the most abundant species was *T. delaisi*, followed by *P. pictus* and *G. xanthocephalus*. In the [4-8] m interval, *P. pictus* dominated followed by *S. melops* and *G. xanthocephalus*. Finally, in the deeper interval, *P. pictus* was again the dominant species followed by *G. xanthocephalus* and *Boops boops* (Table I). Overall, *P. pictus* was the most abundant species. At the species level, the only significant differences in densities across depth strata were recorded for *P. pictus* (H (2, n = 81) = 33.161; P < 0.001) and *G. xanthocephalus* (H (2, n = 81) = 7.599; P < 0.05). The highest differences were registered between extreme depth intervals for *P. pictus* (Dunn's test: [0-4] m and [4-8] m, p < 0.05; [0-4] m and [8-12] m, p < 0.001; [4-8] m and [8-12] m, P < 0.01) and *G. xanthocephalus* (Dunn's test: [0-4] m and [4-8] m, n.s.; [0-4] m and [8-12] m, P < 0.05; [4-8] m and [8-12] m, n.s.).

Assemblage structure

The MDS did not show a clear segregation across depth intervals (Fig. 3). The stress coefficient obtained was 0.16 which is inferior to the value of 0.2 considered the limit to adequately represent similarity or dissimilarity between samples in the MDS plot (Clark & Warwick 2001). The ANOSIM analysis revealed significant differences between depths but the global R value was low (global R = 0.16, p = 0.001). This means that the differences in assemblage structure observed across depth intervals were not very strong (Clark & Warwick 2001). Pair-wise comparisons between depth intervals yielded low R values ([0-4] m vs. [4-8] m: R = 0.12; P = 0.007; [4-8] m vs. [8-12] m: R = 0.053; P = 0.015; [0-4] m vs. [8-12] m: R = 0.341; P = 0.001). The small difference in structure observed between the extreme depth intervals is likely the result of two factors: i) the smaller number of taxa shared between the extreme depth intervals (n=14) than between any other pair of depth intervals ([0-4] m vs. [4-8] m, n=17; [4-8] m vs. [8-12] m, n=18) as well as ii) the great difference in average densities of the most abundant species between extreme depths (e.g. *P. pictus* varied two orders of magnitude and *G. xanthocephalus* varied one order of magnitude).

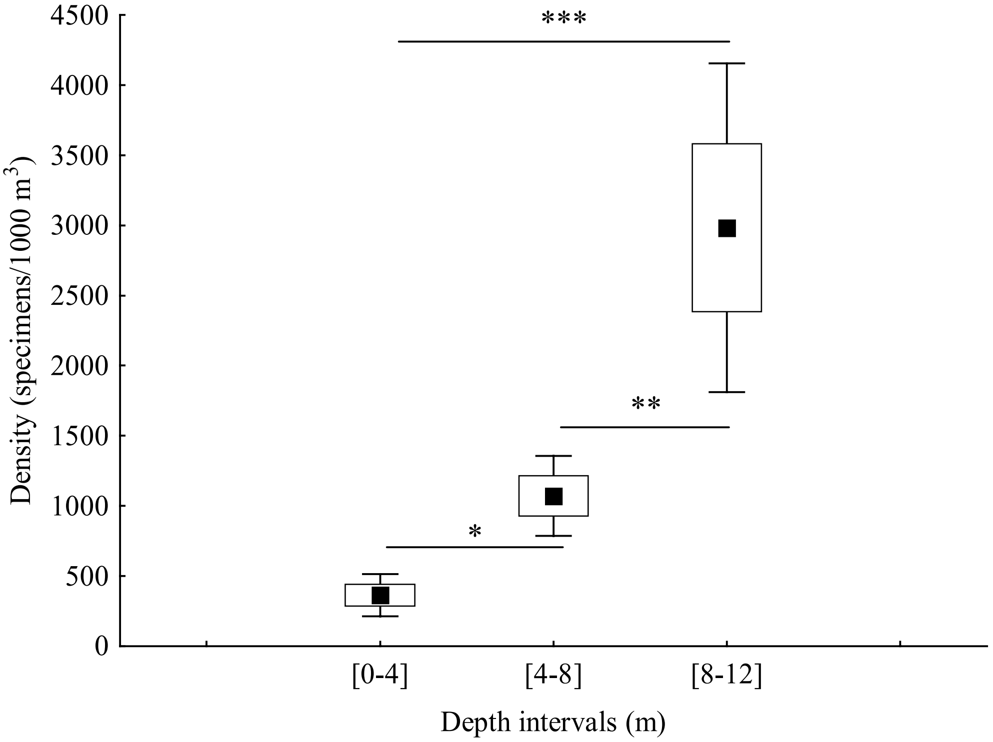


Fig. 2. Larval density variation across depth intervals. Legend: Mean density (black square), mean ± SE (boxes), and mean ± 1.96*SE (whiskers). Post-hoc test results represented by * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

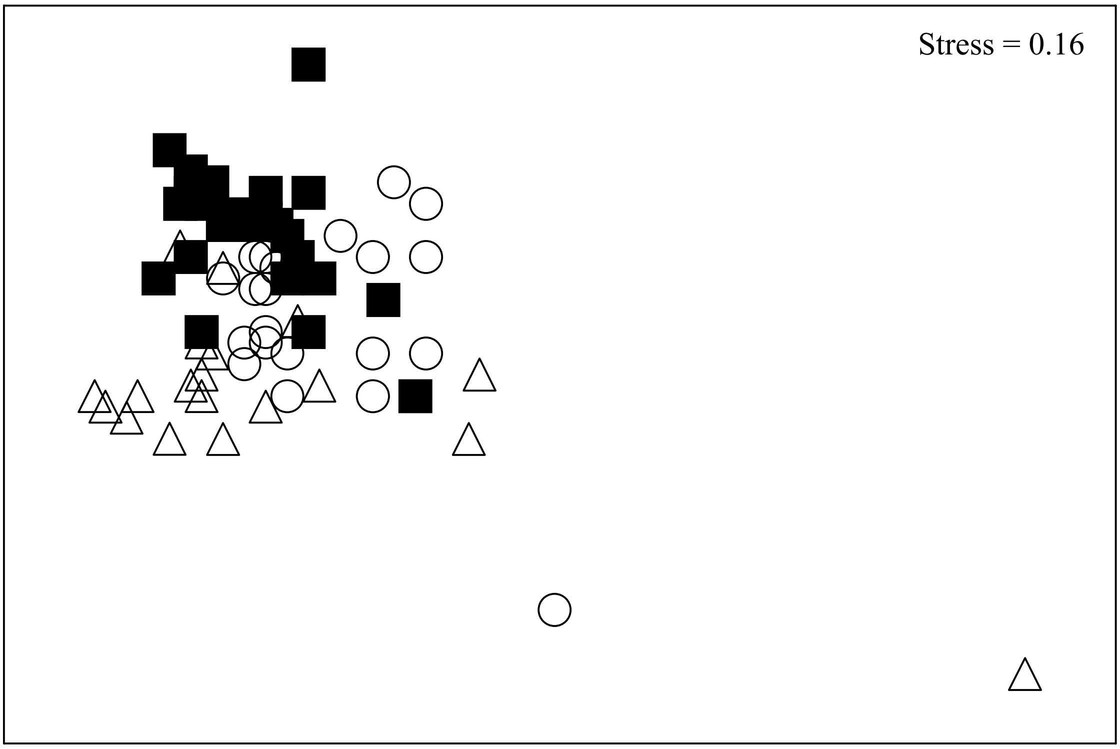


Fig. 3. Non-metric MDS based on the similarity matrix of samples by species. [0-4]m = Δ; [4-8]m = O; [8-12]m = ■.

Ontogenetic vertical distribution

There were no significant differences in the density of pre-flexion larvae across depths (H (2, N=81) = 1.74, P = 0.42)) (Fig. 4a). On the contrary, post-flexion larvae were significantly more abundant at the two deepest intervals (H (2, N= 81) = 34.30, P < 0.001; post-hoc tests only found differences between [0-4] and [4-8] m, P < 0.01 and [0-4] and [8-12] m, P < 0.001) (Fig. 4b).

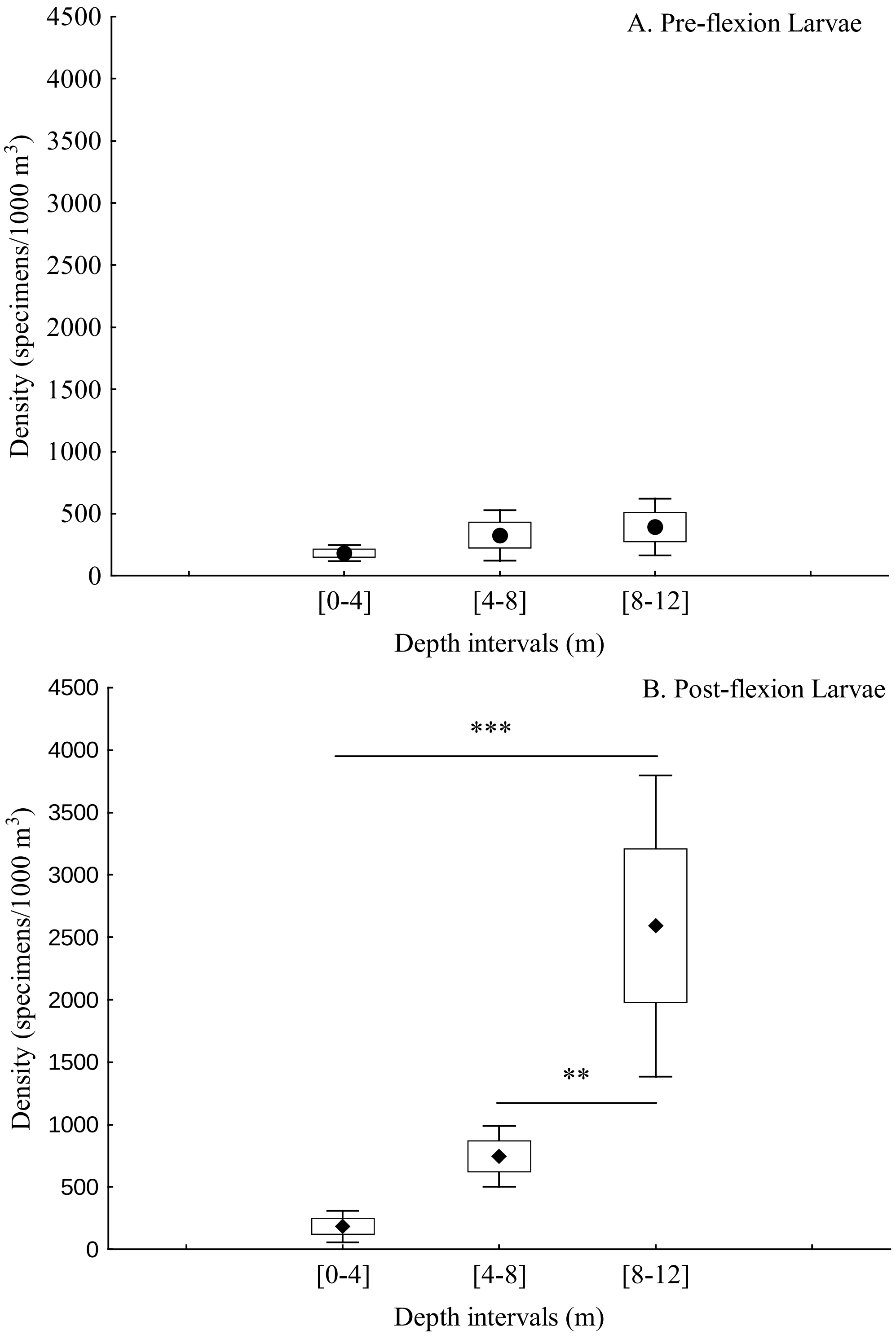
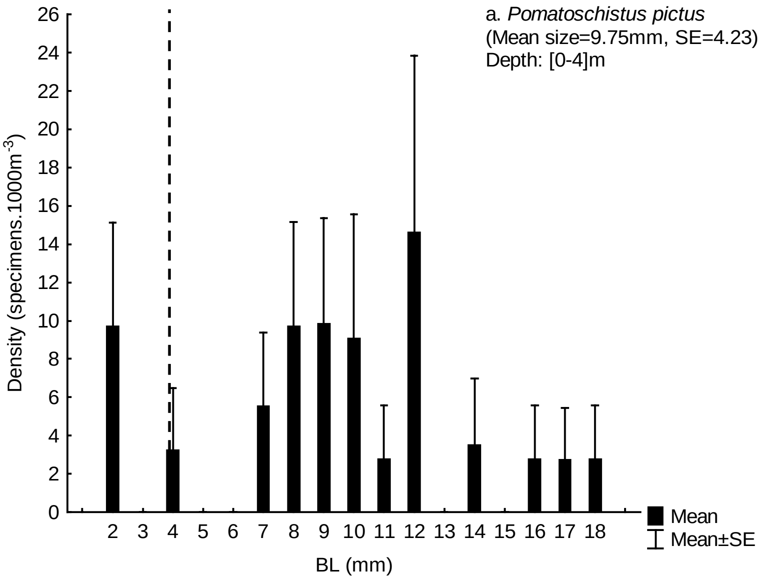
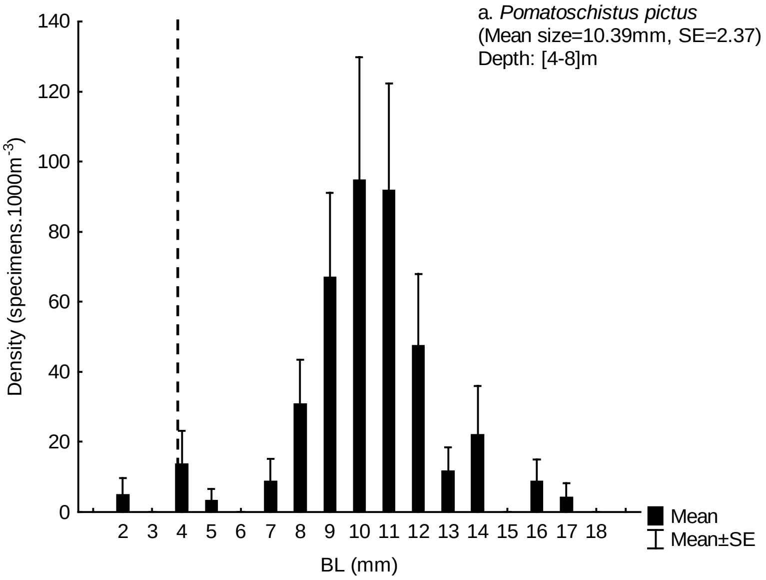
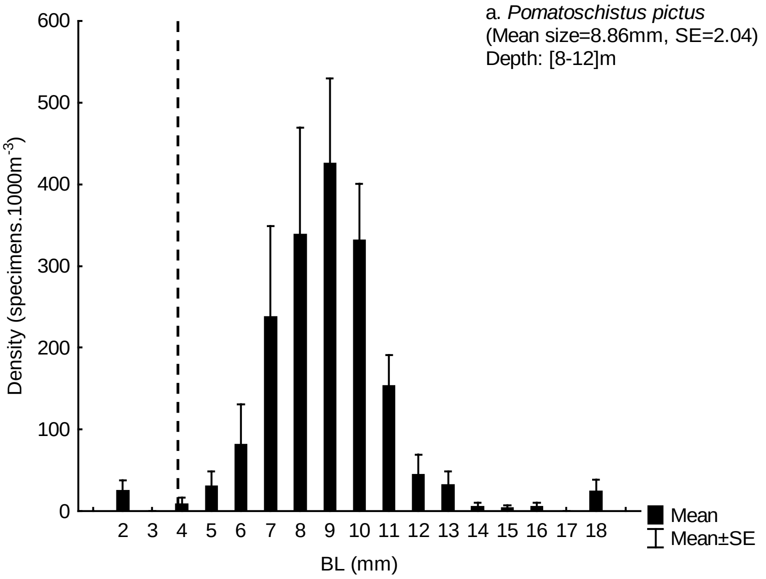


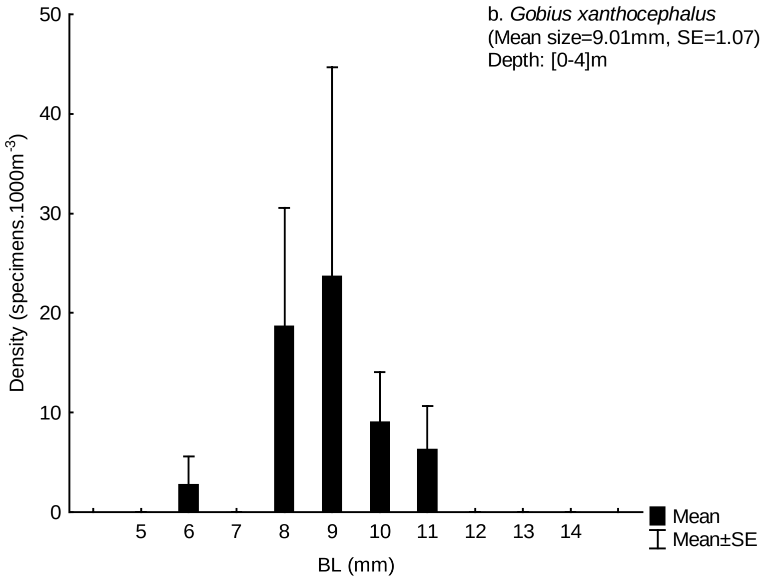
Fig. 4. Larval density variation across depth intervals for (a) pre-flexion and (b) post-flexion larvae. Legend: Mean density of (a) pre-flexion (black circle) and post-flexion larvae (black diamond), mean ± SE (boxes), and mean ± 1.96*SE (whiskers). Post-hoc test results represented by ** = $P < 0.01$; *** = $P < 0.001$.

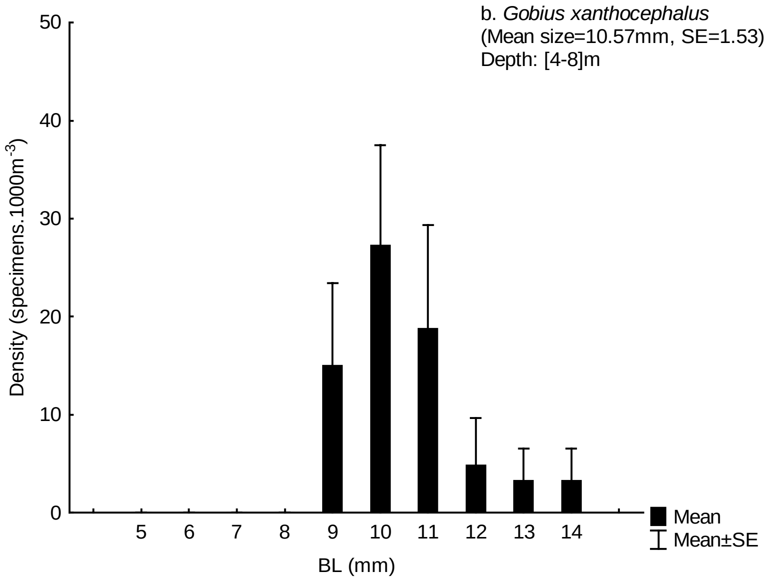
The size of the four most abundant species changed with depth. All size classes of *P. pictus* larvae, from hatching to recruitment and varying between 1.6-18 mm BL, were present near the bottom, (Fig. 5a). In the deepest interval, mean larval sizes were significantly smaller than in the intermediate depth but larvae caught at the shallowest depth were not significantly different from others (H (2, N= 429) =68.28, $P < 0.001$; post-hoc tests only found differences between [4-8] and [8-12] m, $P < 0.001$). Nevertheless, *P. pictus* larvae were present in the whole size range at all depths. *Gobius xanthocephalus* larvae ranged between 5.5-14 mm BL (Fig. 5b). Significant differences in size were found across depth intervals (H (2, N = 117) = 49.37 $P < 0.001$), with larvae caught at [8-12] m being significantly smaller than at both [0-4] m ($P < 0.001$) and [4-8] m ($P < 0.001$). In fact the pre-flexion larvae of *G. xanthocephalus* were not collected in any depth interval. *Tripterygion delaisi* larvae captured were between 3-6 mm BL (Fig. 5c) and were more abundant in the shallowest depth interval (Table I). There were, however, no significant differences between the sizes of these larvae across the whole depth range (H (2, N= 49) = 2.11 P = 0.348). *Symphodus melops* larvae ranged from 5.5-8.4 mm BL (Fig. 5d) and were significantly larger at the deepest interval (Mann-Whitney U test; N [4-8] m = 22 and N [8-12] m = 17; Z = -2.22; $P < 0.05$). However pre-flexion individuals were not captured at any depth interval. The only specimen of *S. melops* captured at the shallowest depth was 6.9 mm SL and was not considered in this analysis.

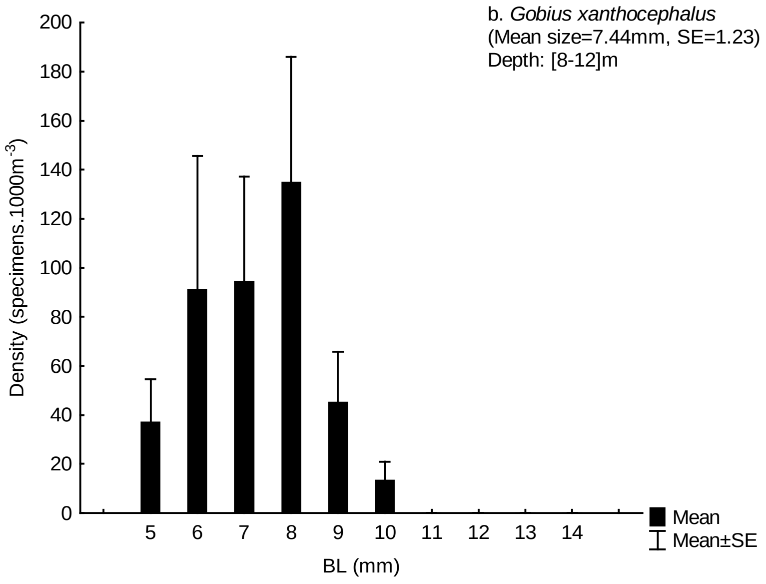

a. *Pomatoschistus pictus*
(Mean size=9.75mm, SE=4.23)
Depth: [0-4]m
Density (specimens.1000m^{-3})
0
2
4
6
8
10
12
14
16
18
20
24
22
26
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
BL (mm)
■ Mean
Mean±SE

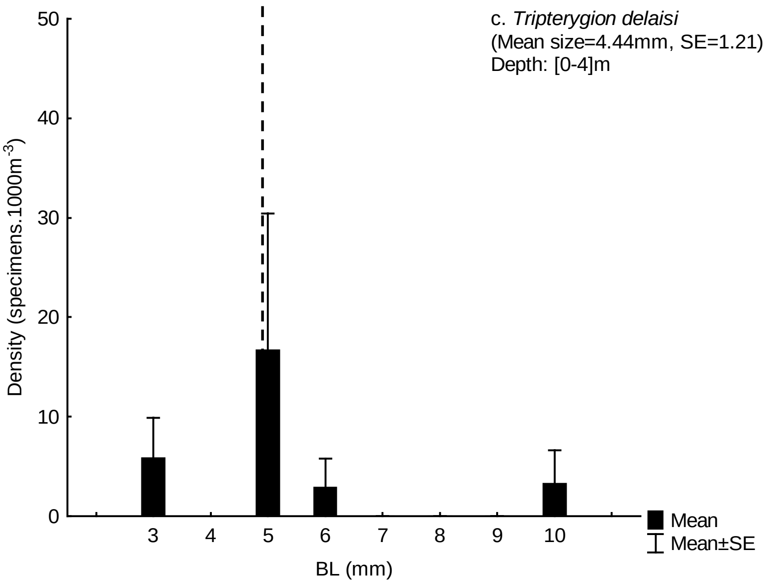

a. *Pomatoschistus pictus*
(Mean size=10.39mm, SE=2.37)
Depth: [4-8]m
Density (specimens.1000m^{-3})
0
20
40
60
80
100
120
140
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
BL (mm)
■ Mean
Mean±SE

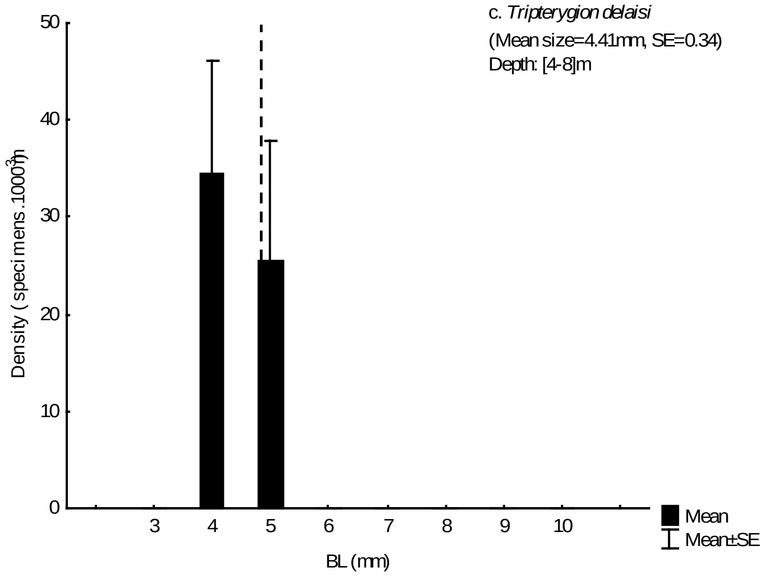

a. *Pomatoschistus pictus*
(Mean size=8.86mm, SE=2.04)
Depth: [8-12]m
Density (specimens.1000m^{-3})
0
100
200
300
400
500
600
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
BL (mm)
■ Mean
Mean±SE

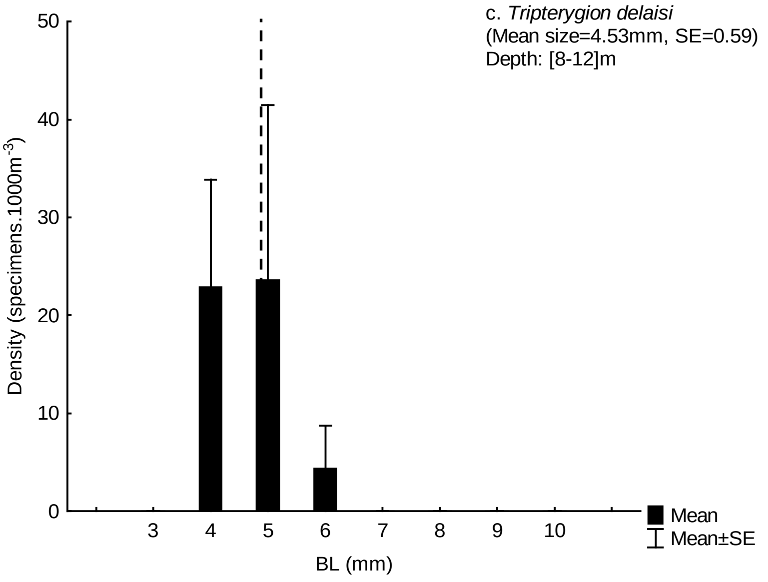

b. *Gobius xanthocephalus*
(Mean size=9.01mm, SE=1.07)
Depth: [0-4]m
Density (specimens.1000m-3)
0
10
20
30
40
50
5
6
7
8
9
10
11
12
13
14
BL (mm)
■ Mean
⌶ Mean±SE


b. *Gobius xanthocephalus*
(Mean size=10.57mm, SE=1.53)
Depth: [4-8]m
Density (specimens.1000m-3)
0
10
20
30
40
50
5
6
7
8
9
10
11
12
13
14
BL (mm)
■ Mean
⌶ Mean±SE


b. *Gobius xanthocephalus*
(Mean size=7.44mm, SE=1.23)
Depth: [8-12]m
Density (specimens.1000m-3)
0
20
40
60
80
100
120
140
160
180
200
5
6
7
8
9
10
11
12
13
14
BL (mm)
■ Mean
⌶ Mean±SE


c. *Tripterygion delaisi*
(Mean size=4.44mm, SE=1.21)
Depth: [0-4]m
Density (specimens.1000m-3)
0
10
20
30
40
50
3
4
5
6
7
8
9
10
BL (mm)
Mean
Mean±SE


c. *Tripterygion delaisi*
(Mean size=4.41mm, SE=0.34)
Depth: [4-8]m
Density (specimens.1000m-3)
0
10
20
30
40
50
3
4
5
6
7
8
9
10
BL (mm)
Mean
Mean±SE


c. *Tripterygion delaisi*
(Mean size=4.53mm, SE=0.59)
Depth: [8-12]m
Density (specimens.1000m-3)
0
10
20
30
40
50
3
4
5
6
7
8
9
10
BL (mm)
Mean
Mean±SE

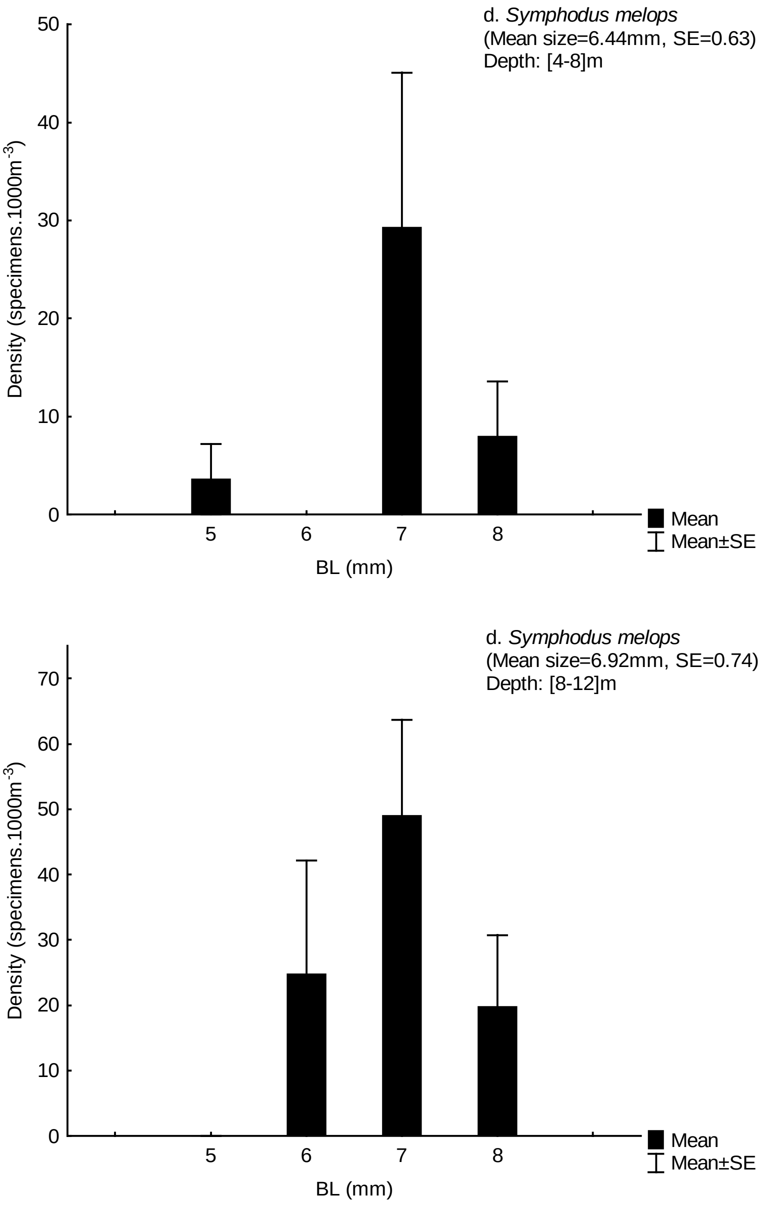


Fig. 5. Body length (BL) range for the four most abundant larval species in each depth interval presented as the mean density (columns) and SE (whiskers). Mean BL and SE are presented between brackets. a. *Pomatoschistus pictus*; b. *Gobius xanthocephalus*; c. *Tripterygion delaisi*; and d. *Symphodus melops*. Vertical dashed lines separate the pre-flexion larvae from post-flexion larvae, except for *G. xanthocephalus* and *S. melops* that were only captured in post-flexion stage.

Discussion

In this study larvae were sampled across depths using a method that enables a fine resolution of vertical distributions near the bottom. Distinct depth strata were characterized and compared in terms of taxonomic composition and diversity, total density, assemblage structure and ontogenetic composition. Clear differences were found across depths for taxonomic, ontogenetic composition and total larval density, but not in diversity or assemblage structure.

The very nearshore larval assemblage described here was mainly composed of shore fishes. Occurrence of larvae from the families Gobiidae, Labridae and Tripterygiidae has also been described in other nearshore studies performed both in temperate (Gray 1993; Gray & Miskiewicz 2000; Sabatés et al. 2003) and tropical regions (Leis 1986; Leis 1991a; Thorrold & Williams 1996). In this study, *Pomatoschistus pictus* larvae represented over 50% of all larvae caught. Larvae from other spring-summer spawners, which are very abundant in the study area as adults, in particular *Lepadogaster* sp. and *Parablennius pilicornis* (Henriques et al. 1999; Gonçalves et al. 2003), were almost absent from our nearshore captures. The few specimens caught were invariably locally produced newly hatched larvae. These species may be present at other depths in the water column, they may disperse offshore or somehow avoid capture by the method we used. *Lepadogaster* sp. and newly hatched *P. pilicornis* larvae have been captured in high abundances at night at the same site (unpublished data). Older stages of *P. pilicornis* have not been captured close to shore, these larvae may disperse offshore such as described by Olivar (1986) in other regions.

Even though there were several species unique to one or two depth intervals, there were no significant differences for any of the diversity indexes calculated across depth intervals. In the few studies that have analysed larval diversity at different depth strata nearshore, Sponaugle and colleagues (2003) found no differences in diversity at different depths, while Leis (1986) found higher diversity in deeper water. In the first study, sampling was performed at 1-5m depth over an 8m deep bottom and in the second case sampling was performed at 0-6m over 10-15m bottoms. In both these studies however, the water layer near the bottom was not sampled, hence the effect of depth on diversity in this layer was not ascertained. The present study showed that for the depth range sampled, depth had no effect on diversity close to the bottom and that assemblage structure did not change with depth. Therefore, in our study area, a single larval patch seems to exist close to the bottom in the extreme nearshore. However, there was a slight difference between the extreme depth strata, which is probably related to the lower number of common taxa between these strata and also to the higher densities of the most abundant species (*P. pictus*), which were 18 times higher in the deepest interval.

In spite of the above-described results regarding diversity and structure, overall larval densities close to the bottom were significantly different among relatively narrow depth intervals. Higher densities were registered in the deepest interval whereas lower densities were recorded for the shallowest interval. Higher larval abundances in deeper waters were also found by Leis (1986) and Gray (1993) although the near bottom layer was not sampled in those studies. The present study showed that depth might influence small scale larval distributions with taxon specific patterns in close proximity to the bottom. While *P. pictus* and *G. xanthocephalus* were significantly more abundant in the deepest interval, *T. delaisi* was more abundant at the shallowest interval and *S. melops* at the intermediate depth strata. Some of the reasons why larvae were more abundant at particular depth intervals may be related to water movement, specific behaviours, or the interaction of these factors. The small and micro-scale water circulation at the study area is not known, however this is a factor that can strongly influence the distribution of larvae (Marliave 1986; Leis 1991a; Reiss et al. 2000; Paris & Cowen 2004). Also, different species can respond to different sensorial cues (Myrberg & Fuiman 2002) and strong swimming abilities have been described for some shore fish larvae (Fisher 2005), allowing them to control their position in the water column. Furthermore, Breitburg and colleagues (Breitburg et al. 1995) suggested that larvae might respond to particular current regimes associated to bottom topography. A better understanding of the larval behaviour of the different species and the microscale patterns of oceanographic features at the study site might help explain the differences observed across depths.

Another interesting result of this study is the variation across depths of the overall pattern of distribution between developmental stages. We found significantly higher densities of post-flexion larvae at the two deepest strata but found no differences for the pre-flexion larvae. The increase of post-flexion larvae with depth has also been described for several species (Cowen 2002; Paris & Cowen 2004). However, those studies did not sample the epibenthic water layer. We found that post-flexion larvae also occur near the bottom even at shallow depths. Proximity to the substrate is an important factor influencing the distribution of larvae, therefore the epibenthic water layer should be sampled in vertical distribution studies. Moreover, depth is also an important factor influencing the distribution of post-flexion larvae near the bottom at the very nearshore given that there was a clear increase in the number of post-flexion larvae in the deeper intervals. At the species level, *T. delaisi* presented no distinct ontogenetic vertical distribution pattern. For *S. melops*, larvae were larger at greater depths. On the contrary, *G. xanthocephalus* larvae were smaller at the greatest depth with the smallest larvae (5-8mm BL) being present only at the deepest interval. Intermediate size larvae (8-10mm BL) of this species were present at all depths, and the largest larvae were only found at the intermediate depth interval. The hatching and settlement sizes are not known for *G. xanthocephalus* larvae, nonetheless specimens ranging from 5 to 14mm BL were captured. In the case of *P. pictus*, larvae were present in the full range of sizes (and development stages) at all depth strata from hatching, which is 2.8mm (Lebour 1920), to settlement which is 17-18mm (Petersen 1919), but were smaller at the deeper strata than at the intermediate depth. These data indicate that *P. pictus* and *G. xanthocephalus* may be spending their entire pelagic phase in close proximity to the reefs. Other studies have also shown that the whole range of larval developmental stages in gobies may occur near shore. In the Dutch delta, the full range of larval sizes of *Pomatoschistus* spp. (both *P. minutus* and *P. lozanoi*) was present near the substrate (Beyst et al. 1999). In the French Polynesia, several taxa, including Gobiidae, were present in different lagoons in the full larval size range (Leis et al. 2003). This is also the case of other species found in inshore waters such as *Oligocottus maculosus* (Marliave 1986) and *Callionymus simplicicornis* (Leis et al. 1998). Thus the presence of larval stages near shore may be a common phenomenon among certain coastal fish species. Remaining close to shore may have several advantages among which finding a suitable settlement habitat at the end of the larval stage (Hickford & Schiel 2003) and growing in a more productive environment.

Small scale studies of larval fish distribution, like the present one, provide important evidence on the distribution and abundance of fish larvae at nearshore waters and could greatly benefit from the integration with behavioural studies and characterization of the oceanographic features of each study site. One central aspect for the interpretation of the patterns found in this and other studies which remains to be fully acknowledged is the role of larval behaviour and its interaction with small scale physical features of the nearshore environments at different geographic areas and oceanographic conditions. This is probably a fruitful direction for future studies of nearshore fish larval distributions.

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Pelagic larval duration of nine temperate cryptobenthic fishes

(Submitted to the *Journal of Fish Biology*)

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Abstract

We investigated the pelagic larval duration (PLD) for nine temperate cryptobenthic species belonging to three families: Gobiidae, Gobiesocidae and Blenniidae. Overall the Gobiesocidae presented short PLDs varying between 11 and 17 days, the Gobiidae had an average of 20 days and the Blenniidae had an average of 29 days. These results are discussed in light of the ecological features of the larval and adult stages of these species.

Keywords

Cryptobenthic fishes; Gobiidae, Gobiesocidae: Blenniidae; settlement mark type; PLD

Introduction

Many marine organisms, including cryptobenthic, fishes have complex life cycles (sensu Roughgarden et al. 1988) divided in two main phases: the larval stage, which is generally spent in the plankton as gametes and/or larvae, and the juvenile/adult phase, which starts when the larvae settle into benthic habitats. Between these phases there is a transition period, i.e. settlement, which is characterized by more or less abrupt morphological and physiological changes (Leis 1991). Settlement from the plankton to the benthos may be marked by a rapid change in width of daily increments in fish otoliths (Wilson & McCormick 1997). These transition zones or settlement marks allow the duration of planktonic life and the timing of settlement to be estimated (e.g., Wellington & Victor 1989).

Given that the population dynamics of fishes can depend to a great extent on pre-recruitment processes (Doherty 2002), the duration of life in the plankton is particularly important because it provides information about the duration of the period in which mortality is high (Doherty & Williams 1988). Knowing how long a particular species spends in the plankton, as larvae, might also provide a rough estimate of its dispersion potential (e.g. Victor 1986; Shanks et al. 2003).

In coastal areas, cryptobenthic species can be very abundant (e.g. La Mesa et al. 2004) and their importance in coastal processes has only recently been assessed (Depczynski & Bellwood 2003). These fishes have very strong associations with their habitats (e.g. Patzner 1999), as other coastal fishes they hatch from benthic eggs and typically present functional eyes, fins and guts, and have better swimming abilities than pelagic species (Thresher 1984; Hickford & Schiel 2003). Many cryptobenthic fishes show particular behavioural characteristics such as defending eggs (e.g. Gonçalves & Almada 1998), which together with data on the presence of all larval stages close to shore in some species (Beldade et al. unpublished data), suggest they may be able to remain nearshore.

Recently, a study on Mediterranean coastal species, including a few cryptobenthic species, has shed some light on the PLD of these fishes. Nonetheless, the PLDs of such species are still largely unknown. The objective of this study was to identify settlement marks and back calculate the pelagic larval duration for a number of cryptobenthic fishes.

Methods

Newly settled juveniles from small cryptobenthic fishes were sampled in shallow rocky substrates at two Portuguese marine protected areas: Arrábida Marine Park and the Natural Park of the Ria Formosa. With few exceptions, collections were performed during the settlement season for the majority of cryptobenthic fishes at the Arrábida Marine Park (Beldade et al. in press). These fishes were collected using the anesthetic Quinaldine dissolved in alcohol at 15.1 and a small hand net. The specimens were stored in 70% ethanol prior to otolith extraction. Specimens were measured (TL in mm) to the nearest 0.01 mm under a binocular microscope.

Lapilli and *sagittae* were extracted from each fish and processed following Secor et al. (1992). After observation of both otolith types we performed counts and measurements on the otoliths that had increments of higher clarity. Otoliths were mounted in thermoplastic cement Crystal BondTM (Aremco Products®), and polished in the sagital plane using 12 to 0.3 mm grit lapping film (3M products) to obtain a sagital section through the nucleus. Otoliths were then viewed under immersion oil with transmitted light, using a compound microscope (Olympus BX50 light microscope) at 1000x magnification. The number of daily increments was determined from three replicate counts from two different observers, and the mean value was taken. Counts from different observers that did not deviate by more than 3 increments were accepted. Increment widths were measured following the longest or clearest axis of the otolith.

Settlement marks were identified through measurements of increment width transitions. We also observed the density changes in the otoliths that matched the width transitions. Pelagic larval duration (PLD) was back calculated based on the increments occurring from the nucleus (beginning at the first well defined increment for each species) to the settlement mark. The daily nature of the increments for the species was assumed based on several previous reports (Sponaugle & Cowen 1994; Iglesias et al. 1997; Shafer 2000). Nonetheless, given that we do not know the exact time of initiation of increment deposition in our species, our counts can slightly over or underestimate the PLD.

Results

The otoliths' increment width and density in nine cryptobenthic species were analyzed. The most common settlement mark type was type Ia characterized by a sharp decrease in increment width across the settlement-mark completed within a few increments (Wilson & McCormick 1999). A total of 50 specimens belonging to nine species, four Gobiidae, four Gobiesocidae and one Blenniidae were examined.

A summary of results is presented in Table I for the three families: (i) Gobiidae. *Gobius xanthocephalus* Heymer & Zander and *Gobius paganellus* Linnaeus had an average PLD of 20 days. Two different subtypes of settlement marks were observed in each of these species corresponding to a single transition increment or a few transition increments. *Chromogobius britoi* Van Tassell, had a shorter PLD than the other gobies, averaging 19 days. We also collected *Pomatoschistus pictus* Malm that showed no clear settlement mark within the first 76 increments (maximum count), neither in width nor in increment density for all specimens analysed. (ii) Gobiesocidae. Gobiesocidae had lower PLDs than the gobies, but PLD values varied among clingfishes. While *Lepadogaster lepadogaster* Bonnaterre and *Opeatogenys gracilis* Canestrini had 13 and 11 days PLD, *Lepadogaster candolii* Risso and *Apletodon dentatus* Facciolà had 15 and 16 days PLD, respectively. For the latter two species, we were able to collect newly settled individuals, i.e. with very few increments following the settlement mark. Our results show that *L. candolii* and *A. dentatus* may settle in sizes smaller under 1cm. (iii) Blenniidae. *Parablennius pilicornis* Cuvier displayed a type II settlement mark, characterized by a wide transition zone void of increments and post-settlement increments that are wider than the immediate pre-settlement increments (Wilson & McCormick 1999). Given that differences in increment width were not as evident as in other species, the identification of the settlement mark and back calculation of the PLDs was based on the observation of the zone void of increments. This zone was easily identified in all specimens. This species presented the longest PLD among all the species studied here.

Table I. Mean, standard deviation (SD) and range (in parentheses) for: total length (TL in cm) and the pelagic larval duration (PLD in days). Settlement mark type (SM, following Wilson & McCormick 1999) and otolith used.

Family	**Species**	***n***	**TL** Mean ± SD (Range)	**PLD** Mean ± SD (Range)	**SM**	**Otolith**
Gobiidae						
	Gobius xanthocephalus	7	2.34 ± 0.22 (1.91-2.53)	20 ± 4 (14 – 26)	Ia/Ib	*Lapilli*
	Gobius paganellus	8	2.48 ± 0.44 (1.67-2.96)	20 ± 3 (15 – 23)	Ia/Ib	*Lapilli*
	Chromogobius britoi	3	2.02 ± 0.24 (1.80-2.27)	19 ± 2 (17 - 20)	Ia	*Lapilli*
Gobiesocidae						
	Lepadogaster lepadogaster	10	1.71 ± 0.30 (1.32-2.15)	13 ± 2 (10 - 15)	Ia	*Lapilli*
	Apletodon dentatus	9	0.83 ± 0.11 (0.62-0.97)	17 ± 2 (15 - 19)	Ia	*Lapilli*
	Lepadogaster candolii	4	1.19 ± 0.42 (0.75-1.56)	16 ± 1 (15 - 17)	Ia	*Lapilli*
	Opeatogenys gracilis	5	1.78 ± 0.19 (1.49-1.94)	11 ± 2 (9 - 15)	Ia	*Sagittae*
Blenniidae						
	Parablennius pilicornis	3	2.95 ± 0.09 (2.87-3.05)	32 ± 1 (31 - 33)	II	*Sagittae*

Discussion

In this study we present the PLD for a number of cryptobenthic species based on density and increment widths in otoliths. Type Ia settlement mark, i.e. a sharp decrease in increment width across the settlement mark, was identified in every species except for one. This is the most common type found among bottom-dwelling species including all Blenniidae, Gobiidae and Gobiesocidae analyzed by Raventós & Macpherson (2001). The settlement mark found on *P. pilicornis* was type II which differs from the type observed in two congeneric blennies *Parablennius incognitus* Bath and *Parablennius sanguinolentus* Pallas which displayed a type Ia settlement mark (Raventós & Macpherson 2001). This type of settlement mark was previously found in temperate labrids with relatively long PLDs (Raventós & Macpherson 2001). Similarly *P. pilicornis* had one of the longest PLDs among the species analyzed in this study. *G. xanthocephalus* and *G. paganellus* presented two settlement mark subtypes a and b. These subtypes differ in the number of rings occurring across the settlement mark. Type Ia is characterized by a single increment transition while in type Ib there is a multi-increment transition. In Wilson & McCormick (1999) each species had one type of settlement mark, which varied only at the genus level in some cases. This is, therefore, the first time that two subtypes of settlement marks are found within the same species.

The Gobiidae studied here presented relatively short PLDs averaging 20 days. *G. xanthocephalus* and *G. paganellus* presented a PLD similar to other Gobiidae in nearby geographic areas, such as *Gobius bucchichi* Steindachner 10 days (Raventós & Macpherson 2001). Both these species presented the highest variation in the PLD ranges. Some larvae are able to expand their PLD considerably by delaying metamorphosis (Victor 1986). The increase in the duration of the competence period has been pointed out as a mechanism to maximize chances of returning to coastal areas or colonizing new geographical areas (McCormick, 1994). Nonetheless, evidence from larval distribution patterns suggests that *G. xanthocephalus* may remain in the nearshore (Beldade *et al.*, unpublished results). *P. pictus* newly settled individuals and adults can be considered epibenthic species because they live on the sand surface but also swim up to the water column remaining close to the bottom (personal observations). As late stage larvae they school in the vicinity of reefs keeping close to the bottom (Beyst 1999). Considering that they may not undergo an abrupt habitat change during settlement, the lack of a clear mark is not very surprising. Two specimens belonging to another sand dwelling goby analyzed in this study, *G. xanthocephalus*, had a maximum PLD of 26 days.

The only Blenniidae captured in this study, *P. pilicornis*, had the longest PLD among the species analyzed here, but similar to what has been described for *P. sanguinolentus* (Raventós & Macpherson 2001). A long PLD is consistent with the idea that this species may disperse offshore, in fact only newly hatched larvae have been captured close to shore (Olivar 1986).

This study describes the pelagic larval durations of several temperate cryptobenthic species. The variability of the PLD duration observed for some species may be related to the dispersal capabilities of those species as well as their behavioural capabilities (Wellington & Victor 2000). Growing knowledge on the distribution of larval stages and the genetic differentiation among coastal populations should integrate the information provided in studies such as the present one.

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The effect of habitat complexity on the density and habitat shifts in a temperate cryptobenthic assemblage

(Submitted to *Marine Biology*)

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Abstract

During the recruitment season of the majority of cryptobenthic species at the Arrábida Marine Park, diversity, abundance and size of species were compared across three habitat types. Three complexity measures, relief, number and size of items, were taken in each habitat type. A total of 618 specimens, belonging to 11 species in 4 families were recorded in all habitats. Richness and abundance were highest in the habitat with intermediate complexity. For the most abundant species, *Lepadogaster lepadogaster*, recruits used the gravel habitat whereas the adults occurred almost strictly in cobbles. The habitat used by recruits had the lowest relief, measured with the chain and tape method, the highest number and smaller size of items. We discuss these results in light of the importance of refuges for different ontogenetic stages and highlight the importance of encompassing several measures of complexity to accurately assess habitat features.

Introduction

The structural complexity of a habitat is a major determinant of local abundance and diversity (Bell et al. 1991). Positive relationships between fish abundance and habitat complexity, or high relief, suggest that complexity has an important role in structuring fish assemblages (e.g. Hixon & Beets 1993; Caley & St John 1996; McCormick 1994; Macpherson 1994; Tupper & Boutilier 1997; Willis & Andersen 2003; Stephens et al. 2006). There maybe several different mechanisms underlying such effects for example because habitat complexity influences the outcomes of competition and predation (Hixon & Menge 1991; Almany 2003).

Cryptobenthic fishes (cf. Miller 1979) are closely associated with the substrate and often dominant in nearshore habitats (Gibson 1969; Miller 1979; La Mesa et al. 2004). The relationships between fish and habitat features are quite strong in some small cryptobenthic species (Costello 1992; Wilkins & Myers 1992; Macpherson 1994; Macpherson & Zika 1999; La Mesa et al. 2004). In fact the diversity and abundance of cryptobenthic fish assemblages seem to be positively correlated to habitat complexity (Willis & Andersen 2003; La Mesa et al. 2004).

Many fishes, including cryptobenthic species, have complex life cycles that involve a change between a pelagic stage, as larvae, and the benthic stage. Settlement can be defined as the period of time at the end of the larval phase when fish do not exhibit the coloration or behavioural characteristics of well established juveniles (Kaufman et al. 1992). In this transition phase some species undergo rapid metamorphoses in body form and physiology (Leis 1991) and also habitat shifts and species associations (McCormick & Mackey 1997). Substratum type would appear to exert an effect right after settlement (Connell & Jones 1991; Macphersen 1994; Caley & St John 1996). Settling into cryptic habitats may reduce predation risk and seems to be a common strategy among benthic species (e.g. Hixon 1991; McCormick & Mackey 1997; Patzner 1999; Gonçalves et al. 2002). Different habitats can provide a different number of shelters. It has been shown that the abundance of newly settled individuals may increase with the number of shelter holes (Berhents 1987; Steele 1999).

Several different studies described associations between cryptobenthic fish and particular habitats, but few have considered the development stage of the fishes. For example: *Lepadogaster candolii* inhabits stones, clefts, over-hangs while the sub-adults live in cavities and the juveniles under sea-urchins and empty bivalve shells (Patzner 1999; Hofrichter & Patzner 2000); *Apletodon dentatus* settles into several algae species and latter move to sea-urchins and cobble (Hofrichter & Patzner 2000; Gonçalves et al. 2002). Very few studies quantified the availability of these particular habitats. Details of the settlement stage like the habitat transitions are likely to be species-specific, with unique ramifications for the survival and abundance patterns of latter life-stages (Connell & Jones 1991; McCormick & Mackey 1997). The environmental requirements for each of the development stages may not be exactly the same, i.e. recruits may benefit from exploring smaller refuges than the adults. In fact, the size distribution of fishes may be influenced by the size of available shelter holes (Hixon & Beets 1993).

In this study we aimed at comparing: three measures of complexity across habitats; compare richness and abundance across those habitats; and identify the settlement habitat and possible ontogenetic habitat shifts for the cryptobenthic species.

Methods

This study was performed in Arrábida Marine Park during the recruitment season for the majority of cryptobenthic fishes (Almada et al. 1999) between May to August 2003. The coastal habitats present in the study area result from the disintegration of the calcareous cliffs found on the shoreline. At the sampling location Risco (38º 27' 03''N, 009º 01' 24''W), habitats are diverse and occur in very different abundances (Beldade et al. unpublished data).

Habitat characterization

We selected three of the less abundant habitats: gravel, cobble and small rocks (<30cm). To characterize these habitats we sampled eight 50x50cm quadrates per habitat. In each quadrate we measured rugosity (i.e. contoured vs linear distance); counted the number of items (gravels, cobbles or small rocks) in the diagonal of each quadrate; and measured the size of each item (size of each item). Rugosity, measured in the diagonal of the quadrate, was calculated as the ratio of the length of chain moulded to the surface and the linear distance between its start and end point. This method is also known as the chain and tape (Luckhurst & Luckhurst 1978). Note that in the gravel habitat only the first 50 items were sampled.

Fish counts

Two divers collected all fishes inside 50x50cm quadrates using the anaesthetic Quinaldine diluted in alcohol 1:15 (following Patzner 1999). While one diver pumped the anaesthetic under the microhabitat items, from the border to the center of the quadrate to avoid fishes from escaping the observers, the other caught the fish. All fish were identified, measured over a ruler designed on the writing plate or with a plastic calliper in the case of the specimens smaller than 2cm. The position of each fish, under or over the habitat items was recorded. During 6 fortnights we randomly placed eight quadrates in each habitat type, therefore a total of 48 quadrates were sampled by habitat. All fishes were returned to their respective quadrates after measurement.

Statistical analysis

We looked for differences in rugosity, number of items across habitats using one-way ANOVAs. The number of items was transformed using the square root function. To find out which habitats differed significantly from each other we used Tukey HSD tests (Zar 1986). The size of items was compared across habitats using a non-parametric ANOVA given that parametric assumptions could not be meet. Dunn's post-hoc tests were used to identify where differences lay.

We compared the number of species per quadrate, i.e. richness, and the number of specimens per quadrate transformed according to the function $y=\log(x+1)$, i.e. abundance, across habitats using one-way ANOVAs. To find out where differences lay we used Tukey HSD tests (Zar 1986). For the most abundant species we analyzed the differences in TL (total length in mm) across habitats using t-tests.

Results

Habitat characteristics

There were significant differences in rugosity ($F(2, 21)= 1662.1$, $P < 0.001$) and number of items ($F(2, 21)= 1768.7$, $P < 0.001$). Rugosity decreased significantly across habitats (Tukey test $p<0.001$ between all habitats), from the lowest values measured in gravel to the highest in small rocks (Table I). All habitats had significant differences in the number of items (Tukey test $P < 0.001$ between all habitats) with a decreasing number of items from the gravel to small rocks (Table I). The size of the items found in each habitat type were significantly different ($H(2,N= 555) = 302.29$ $P < 0.001$), with the small rocks and cobble being larger than gravel (Dunn's test: $P < 0.001$) but not each other.

Table I. Mean and SD for three physical variables (relief, number of items and average size of items) measured and number of characterization quadrates sampled in each habitat: gravel, cobble and small rocks.

	Gravel	Cobble	Small rocks
Relief	1.12 ± 0.02	1.42 ± 0.09	1.97 ± 0.12
Number of items	84.5 ± 5.01	10.4 ± 1.06	5.6 ± 1.41
Size of items (cm)	4.0 ± 0.8	12.7 ± 4.3	16.1 ± 4.6

Richness and abundance across habitats

A total of 618 specimens, belonging to 11 species in 4 families were recorded in all habitats (Table II). Small rocks hosted the highest number of species, 8 species, followed by gravel and cobble with 6 and 5 species respectively. In each habitat and overall, there was a number of rare species, e.g. *Chromogobius britoi*, *Scorpaena porcus*, *Parablennius gattorugine* and *Lepadogaster lepadogaster* in small rocks (Table II).

Table II. Total number of specimens caught in each habitat: gravel, cobble and small rocks.

Family	Species	Gravel	Cobble	Small rocks
Blenniidae	*Parablennius gattorugine*			1
	Parablennius pilicornis		23	6
Gobiesocidae	*Lepadogaster candolii*		20	26
	Lepadogaster lepadogaster	68	323	2
	Lepadogaster purpurea	1	37	
Gobiidae	*Chromogobius britoi*			2
	Gobius cruentatus	1		
	Gobiu paganellus	1	3	25
	Gobius xanthocephalus	8		31
	Pomatoschistus pictus	38		
Scorpaenidae	*Scorpaena porcus*			2

Richness was significantly different across habitats (F(2, 141)= 15.883, $P < 0.001$). Cobble had the highest richness (Tukey test $P < 0.001$), whereas small rocks and gravel did not differ significantly between each other (Fig. 1). There were significantly different abundances across habitats (F(2, 141) = 93.148, $P < 0.001$). Cobbles had significantly higher abundances than small rocks or gravel (Tukey test $p<0.001$), which did not differ significantly between each other (Fig. 1).

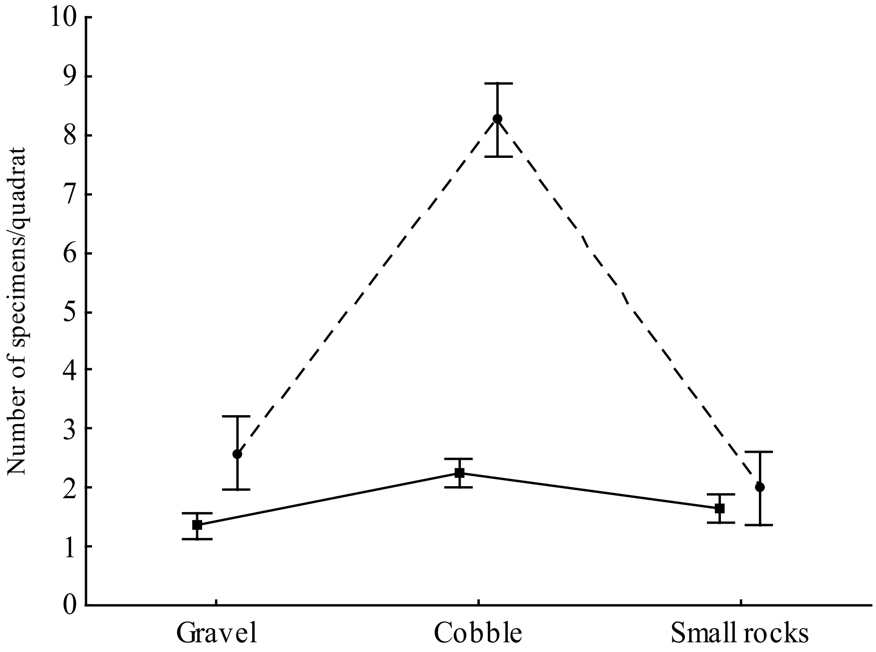

Fig. 1. Mean richness (square) and abundance (circle) across habitat types: gravel, cobble and small rocks. Vertical bars denote 95% confidence intervals.

Ontogenetic habitat shifts

There were some intriguing differences in the sizes of specimens across habitats (Table III), which suggested that there could be ontogenetic habitat shifts for *Lepadogaster lepadogaster*. In fact, while the smaller specimens used the underside of gravel, the larger specimens were caught almost exclusively under cobbles (Fig. 2; Cobble N=323, Gravel N=68; t=15.19 P < 0.001).

Table III. Average size (TL in cm) and SD for each species in each habitat: gravel, cobble and small rocks.

Family	Species	Gravel	Cobble	Small rocks
Blenniidae	*Parablennius gattorugine*			4.4
	Parablennius pilicornis		7.9 ± 2.85	6.7 ± 3.51
Gobiesocidae	*Lepadogaster candolii*		4.5 ± 1.29	4.4 ± 1.00
	Lepadogaster lepadogaster	1.7 ± 1.11	3.8 ± 1.02	3.7 ± 0.71
	Lepadogaster purpurea	3.2	4.1 ± 0.82	
Gobiidae	*Chromogobius britoi*			3.6 ± 0.71
	Gobius cruentatus	14.5		
	Gobiu paganellus	6.0	2.5 ± 0.13	6.5 ± 3.03
	Gobius xanthocephalus	4.9 ± 1.97		4.7 ± 1.64
	Pomatoschistus pictus	4.2 ± 1.27		
Scorpaenidae	*Scorpaena porcus*			16.5 ± 0.71

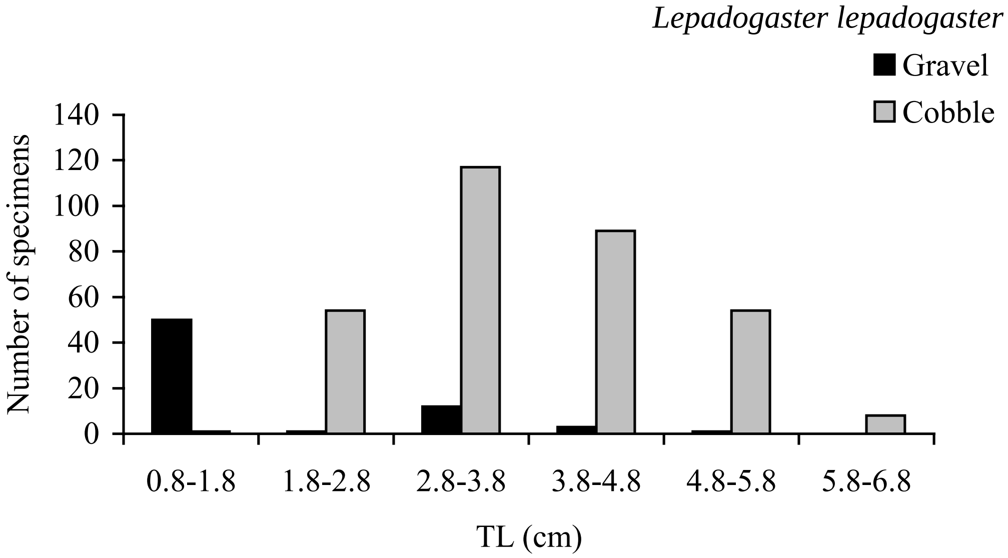

Fig. 2. Number of *Lepadogaster lepadogaster* specimens per size class in the gravel (grey bars) and cobble (black bars).

Discussion

Habitat types displayed clear differences in richness and abundance. The species lists for each habitat revealed an overall higher number of species in small rocks and gravel than in cobble. However the number of species per quadrate, i.e. richness was significantly higher in cobble. These results show that the quadrates sampled on cobble had consistently higher number of species per quadrate than any other habitat. Abundance was also higher in the cobble habitat due especially to *Lepadogaster lepadogaster*. Large differences in abundance across habitats for few species show that there is a high degree of habitat partioning. However, in this study we selected only three of the main habitat types at the Arrábida Marine Park and therefore we do not have a full picture of the species distribution. Nonetheless, as in previous studies encompassing the whole range of habitat types, some species only occur in the habitats sampled here (Beldade et al. unpublished data).

A decrease in relief, as was found from small rocks to gravel does not necessarily mean a decrease in overall complexity. The number of items increased inversely to relief giving the contrary notion of complexity variation. Relief or rugosity, traditionally used as proxies for complexity, estimated through the ‘chain and tape method’ (e.g. Willis & Andersen 2003; La Mesa et al. 2004) may not provide a complete assessment of complexity. In particular, when studying small cryptobenthic fishes that utilize specific microhabitats, such as very small holes, cracks, etc, one should encompass several measurements of complexity to fully ascertain its value. Furthermore depending on the habitats under study there may be specific ways to quantify complexity. In our study all habitat types were composed by relatively small measurable items, which allowed us to include the size of the items as another complexity measure. Specific habitats types need to be characterized using several measures.

Even though this study was performed during the recruitment season for the majority of the cryptobenthic species in this study (Almada et al. 1999), few recruits of several species were captured. These recruits may have occurred in other habitats not sampled here or in the same habitat as the adults. In this second case we would still be able to observe recruits in the same habitat as the adults, but that was not the case. One of the most abundant cryptobenthic fishes in this area, *Lepadogaster lepadogaster* was captured mainly on cobble as adults where other authors had already found them (Gonçalves et al. 1998; Patzner 1999; Hofrichter & Patzner 2000) but also in gravel as recruits. This study reveals an association between the number and size of items and the sizes of *L. lepadogaster*. Setran & Behrens (1993) found that soon after settlement (17-22mm TL), *Xiphister mucosus* and *Chebidichthys violaceus* prefer gravel and cobble, then at 30-36mm preferred cobble to gravel, apparently because the latter provided insufficient interstitial space for larger juveniles. Similarly, our results indicate that the interstitial spaces created by gravel seem to be extremely important for the initial benthic stages of this species. Smaller interstitial spaces may mean less predator access and therefore bigger protection for *L. lepadogaster* recruits. While in the cobble and small rock habitat predators were sometimes seen, e.g. *Muraena helena* and *Scorpaena porcus* (Beldade person obs) the other species observed in gravel were mostly sand-dwelling gobies. Independently of the predator pressure the increase in shelter availability may increase survivorship in small cryptobenthic fishes (Steele 1999). The habitat partitioning that was found between the recruits and the adults may also result in decreased competition (for space or food). The presence of conspecifics can facilitate settlement (Booth 1992; Steele

1997), have no effect (Steele et al. 1998) or reduce settlement (Wilson & Osenberg 2002). Other clingfishes select different habitats than the adults (Stepien 1990) perhaps because larger fish tend to win intraspecific contests (Richkus 1981). However, in this case there was a clear segregation between the habitats used by the adults and the recruits of *L. lepadogaster*. Either high mortality of newly settle juveniles in lower complexity habitats, such as was found in a temperate blenny *Forsterygion varium* (Connell & Jones 1991) or selection of habitats by pre-settlement individuals (Montgomery et al. 2001) may have caused the observed patterns.

Highly complex and cryptic habitats are used by a number of settlement stage fish species, probably because they provide shelter during the settlement phase (McCormick 1994). In this study we used three measures of complexity that varied in opposite ways to characterize three habitat types. Both richness and abundance were highest in the habitat that was neither the most complex nor the least complex according to the complexity measures used. Ontogenetic habitat use was found for the most abundant species *Lepadogaster lepadogaster*. It was also striking the importance that the least abundant habitats, such as those studied here, may have a disproportional importance for some species. In fact, *L. lepadogaster* is one of the most abundant species in this area (Beldade et al.unpublished results) and settled into gravel. In a biodiversity management perspective, the importance of the least studied component of coastal assemblages (cryptobenthic fishes) is growing given that biodiversity is one of the main criteria to choose a marine protected area. These species use habitats whose features should be evaluated at a fine level, taking into consideration the results presented here.

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An interference visual census technique applied to cryptobenthic fish assemblages

(in press in *Vie Milleu*)

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Abstract

Here we compare the accuracy of an interference visual census technique (IVC), in which dismantling of the habitat is performed, to traditional underwater visual census (VC) and anaesthetic census. We compare the performance of these techniques applied to a temperate cryptobenthic fish assemblage using two strategies: sampling over the whole depth extent of the rocky bottom, and stratified sampling over the main microhabitats present at the study site. The number of species encountered was lower using the traditional VC for both strategies. Fish density estimates were significantly lower using the traditional VC technique then using the anaesthetic counts; however the IVC counts were not significantly different from the anaesthetic collections, in the transect strategy. These differences were larger for clingfishes and some gobies, which occurred preferably under cobble and small rocks. No differences were found when comparing the IVC and anaesthetic census in the habitat strategy, for each microhabitat considered. We conclude that dismantling the habitat increases the performance of the visual census technique and is therefore a valuable approach when applied to temperate cryptobenthic fish assemblages.

Key words

Cryptobenthic fish assemblages; visual census methods

Introduction

Sampling marine habitats with minimal lasting interference effects is fundamental for studies in ecology. Underwater visual census (VC), firstly used by Brock (1954) in a pioneering study of Hawaiian fishes, are nowadays applied to different types of fish ecology studies (Edgar et al. 2004), including those on assemblage structure (Prochazka 1998), ecological processes (Nanami & Nishihira 2003) and biogeographic patterns (Gasparini & Floeter 2001). Biases in sampling introduced by visual census are however recognized by most authors and there have been a number of suggestions on how to reduce them (Luckhurst & Luckhurst 1978; Sale & Sharp 1983; Bellwood & Alcala 1988; Lincoln Smith 1988; Kulbicki 1990; Samoilys & Carlos 2000). In particular, when dealing with cryptobenthic species, the use of small areas and minimum fish sizes (e.g. only fishes larger than 5cm are recorded) have been appointed as possible solutions to reduce bias (Harmelin-Vivien et al. 1985). However, traditional VC biases remain to be fully tested (Edgar et al. 2004). Miller (1979) defined cryptobenthic fish as “small bodied fishes (< 10 cm) that exploit restricted habitats where food and shelter are obtained in, or in relation to, conditions of substrate complexity and/or restricted living space, with a physical barrier likely to be interposed the small fish and sympatric predators”. As suggested by different authors, habitat complexity can greatly influence the observed distribution patterns of cryptobenthic fish assemblages (Harmelin-Vivien et al. 1985; Connell & Jones 1991).

Traditional visual census methods have been frequently used to count benthic and nektobenthic fishes but it is generally accepted that they cannot correctly sample cryptic fish species (Sale & Douglas 1981; Brock 1982; Willis 2001). However, many of the studies that tried to assess biases in counting fish have mainly dealt with tropical species (Sale & Douglas, 1981; Brock, 1982; Fowler, 1987; Lincoln Smith, 1988; Bortone et al. 1989; Kulbicki 1990; Samoilys & Carlos 2000) and visual in situ evaluation methods of fish populations were essentially developed on tropical environments. Coral reefs in particular are amongst the most diverse marine habitats where numerous species can typically be found in a relatively small area (Ackerman & Bellwood 2000). It is thus conceivable that the use of the same techniques in temperate regions may offer different results. Given that some microhabitats are composed of small movable items that create interstitial spaces where many of the cryptobenthic fish hide (Gonçalves et al. 2002) it may be worthwhile to include a more thorough sampling of particular microhabitat types in the visual census techniques.

In this paper we had two main goals. Firstly compare the performance of a traditional censusing technique and a modified visual technique to anaesthetic sampling by randomly sampling the rocky bottom. Secondly, compare the performance of the modified technique and the quantitative (anaesthetic) sampling across microhabitat types.

Material and Methods

This study was performed during January and February 2004 in the Arrábida Marine Park (Portugal) at two stations, Risco (38º27’03’’N, 9º01’24’’W) and Cozinhadouro (38º26’54’’N, 9º02’12’’W), which were characterized by the highest diversity of coastal fish species (Gonçalves et al. 2003). The highly heterogeneous underwater habitats result from the disintegration of calcareous cliffs that border the shoreline. Different microhabitats: sand, gravel, cobble, small rocks (<30cm) and large rocks (>30cm), were patchily distributed on this area. Fish sampling was performed in the morning with good sea-weather conditions. The local cryptobenthic fish species were easily identified according to distinct morphological and colouration characteristics, except for the gobiesocids *Lepadogaster lepadogaster* and *L. purpurea*. Since it is very difficult to distinguish between these species in the field (Henriques et al. 2002), they were generally indicated as *Lepadogaster* sp. Data on the cryptobenthic fish assemblage was collected using three techniques.

Visual Census (VC)

This technique has been used by several authors (Harmelin-Vivien et al. 1985, Willis 2001; La Mesa et al. 2004; La Mesa & Vacchi 2005). In a 0.25 m^2 quadrat the observer recorded all fish, taking note of the microhabitat where they were firstly seen. The use of a flashlight allowed the observer to look for fish inside clefts and small holes but no habitat manipulation was performed.

Interference Visual Census (IVC)

This technique was applied to the same quadrats as the VC. After counting all visible fish over the substrate (VC) we systematically looked for fish hidden under rocks and cobbles, buried in gravel or sand. This technique was therefore not strictly a “visual” technique since it involved lifting all microhabitat items (smaller than 30cm in maximum length). In each quadrat all fish were identified and their position recorded. After displacement, the microhabitat items were put back in their place. This procedure could have attracted fish from nearby areas, but given the small quadrat area used we are convinced that this cases (less than 3% of the occasions) were spotted and excluded from the census.

Anaesthetic Census

Quinaldine (2-methyloquinolina) diluted in alcohol at 15:1 (Patzner 1999) was used to count all fish present in each 0.25 m^2 quadrat, by squirting it into cavities, clefts and under all microhabitat items present. Approximately 125ml of the anaesthetic was slowly applied per quadrat from the boundaries to the centre. The search for fish started immediately after this procedure. Although we used open stations, the relatively small quadrat size allowed us to record all fishes before they escaped. We also controlled the potential influence of the anaesthetics on fish outside the quadrat by searching from the boundaries to the centre of the sampling point and therefore detecting any anaesthetised fish that entered the quadrat. The searching effort and method was similar to the one applied in the IVC.

We applied these techniques in two sampling strategies: sampling over the whole depth extent of the rocky bottom, and stratified sampling over the main microhabitats present at both stations. While the first strategy aimed at sampling each microhabitat proportionally to its occurrence (random sampling), the second strategy aimed at balancing the sampling effort among the main microhabitats present (Table I). This later strategy allowed us to evaluate bias in sampling the different microhabitat types since by sampling all microhabitats equally we could ascertain that our results would be consistent in all microhabitats.

Table I. Microhabitat area sampled in the transect and habitat strategies using underwater visual census (VC), interference visual census (IVC) and anaesthetic census (see text for details)

	Transect strategy (m^2)		Habitat strategy (m^2)	
	VC/IVC	Anaesthetic	VC/IVC	Anaesthetic
Sand	1.10	0.52	1	1
Gravel	0.35	0.45	1	1
Cobble	0.73	1.08	1	1
Small rocks	0.71	0.30	1	1
Large rocks	9.36	9.90	1	1

Strategy 1: Sampling over the rocky bottom

Eight parallel transects were established five meters apart over the subtidal rocky bottom, from the deeper sandy area (depth range 8.9 m to 11.2 m, average = 10.3, S.E. = 0.3) to the infralittoral (depth range 1.3 m to 2.3 m, average = 2.0, S.E. = 0.2). Four transects were sampled with the visual techniques (VC and IVC) whilst the other four were sampled with anaesthetic census. Transect length varied according to the extent of rocky bottom (range = 55m to 70m, average = 61.25, S.E. = 3.15). On each transect, a 0.25 m^2 quadrat was sampled every 5 m. The quadrat area chosen was smaller than in previous studies (*e.g.* Willis 2001). The choice for such an area was a compromise between the time necessary to sample each quadrat before the anaesthetic dispersed, especially in the more complex microhabitats, and the size of the microhabitat patches sampled in Strategy 2 (see below). A total of 98 quadrats were sampled, half using the visual techniques and the other half anaesthetic census. The sampling procedure began by examining the first quadrat on the visual transect after which the diver swam to the parallel transect and sampled the first quadrat on the anaesthetic transect. This procedure was repeated until the infralittoral area was reached. Cover percentage of each microhabitat present in each quadrat was visually estimated.

Strategy 2: Stratified sampling over the main microhabitats

Five microhabitats were sampled using 0.25 m^2 quadrats: sand, gravel, cobble, small rocks (<30 cm maximum length) and large rocks (>30 cm maximum length). At each microhabitat patch, eight quadrats were randomly deployed, half of which were sampled with the visual techniques (VC and IVC) while the other half were examined using anaesthetic census. Sampling was performed in narrow depth intervals (1m depth range) to avoid confounding depth effects in data.

Data analysis

To evaluate the efficiency of the visual techniques we used two one-way ANOVA's and test for differences between VC and anaesthetic and between IVC and anaesthetic in the transect (random) strategy. To compare both visual techniques, a visibility index was calculated based on the percentage of specimens recorded by the IVC but missed by the VC in both strategies. To compare the efficiency of the IVC to quantitative census (quinaldine) in the different microhabitats (strategy 2), we used a two-way ANOVA and post-hoc Student-Newman-Keuls tests to find out where differences lay. All data was transformed following a squareroot + 1 transformation to meet homoscedasticity assumptions.

Results

A total of 15 species belonging to 8 families were observed in our study site (Table II). The overall densities obtained returned an average value of 2.37 individuals/m^2

(S.E. = 0.38, range 0-12) for the VC, 4.46 individuals/m^2 (S.E. = 0.54, range 0-16) for the IVC and 6.20 individuals/m^2 (S.E. = 0.65, range 0-28) for anaesthetic census. The total number of species encountered using each technique was: VC = 7, IVC = 11, anaesthetic census = 12 (Table II).

For strategy 1 (random sampling) the VC recorded significantly less fish than the anaesthetic census (ANOVA: F = 11.2, $P < 0.001$), whereas no significant differences were found between the IVC and anaesthetic census (ANOVA: F = 1.61, $p > 0.05$) (Fig. 1).

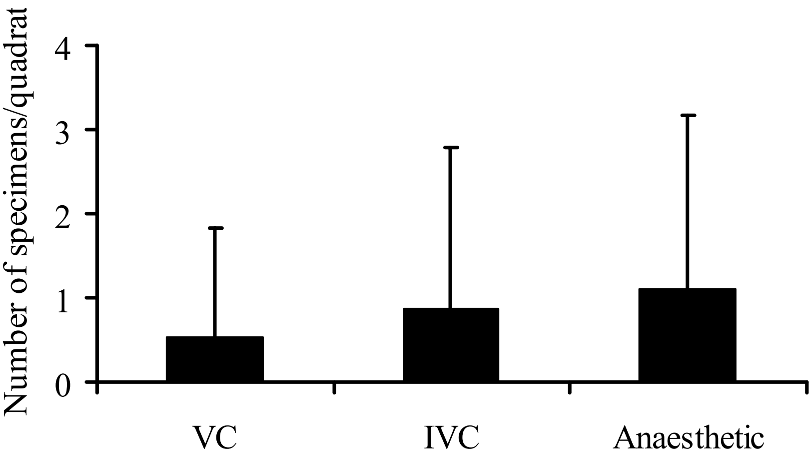

Fig. 1. Mean density of cryptobenthic fishes (number of specimens per quadrat ± 95% confidence limits) recorded in the transect strategy (random) by visual census (VC), interference visual census (IVC) and anaesthetic census (ANA).

Table II. Number of specimens of each species recorded by visual census (VC), interference visual census (IVC) and anaesthetic census. * *Lepadogaster* sp. was used to refer to two co-occurring species, *L. lepadogaster* and *L. purpurea* which are very difficult to distinguish in the field (Henriques et al. 2002)

Family	Species	VC	IVC	Anaesthetic
Blenniidae	*Parablennius gattorugine* (Brünnich, 1768)			2
	Parablennius pilicornis (Cuvier, 1829)	8	8	11
Callionymidae	*Callionymus reticulatus* Valenciennes, 1837			1
Gobiesocidae	*Apletodon dentatus* (Facciola, 1887)			1
	Diplecogaster bimaculata (Bonnaterre, 1788)		1	
	Lepadogaster candolii Risso, 1810		1	9
	Lepadogaster sp.* (Bonnaterre, 1788)		17	35
Gobiidae	*Gobius cruentatus* Gmelin, 1789	5	5	1
	Gobius paganellus Linnaeus, 1758	1	5	5
	Gobius xanthocephalus Heymer and Zander, 1992	9	19	17
	Pomatoschistus pictus (Malm, 1865)	8	9	10
Muraenidae	*Muraena helena* Linnaeus, 1758		1	
Scorpaenidae	*Scorpaena porcus* Linnaeus, 1758	1	1	
Syngnathidae	*Nerophis lumbriciformis* (Jenyns, 1835)			1
Trypterigiidae	*Tripterygion delaisi* Cadenat and Blache, 1971	9	10	15
	Total	42	77	108

Using data from both strategies we calculated the percentage of fish counted with the IVC that was missed by the VC, and ascribed a visibility index to each species (Fig. 2). Three distinct groups can be identified. One composed by the gobiesocids *Lepadogaster* sp. which were completely missed by the VC; a second group composed by the gobies *Gobius paganellus* and *Gobius xanthocephalus* which were partially missed without interference; and a third group with *Tripterygion delaisi*, *Pomatoschistus pictus*, *Parablennius pilicornis* and *Gobius cruentatus* which were mostly recorded prior to interference. Therefore, without habitat dismantling during the visual census, the first two groups of species would have been partially or completely missed.

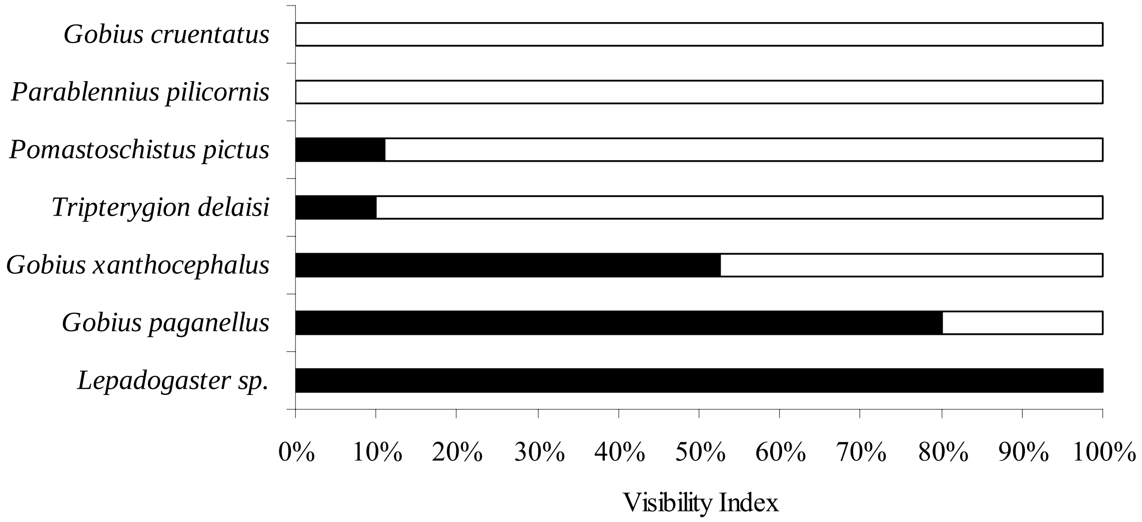

Fig 2. Percent of the number of specimens counted using visual census techniques, the interference visual census (IVC) (black) and the underwater visual census (VC) (white), for species with over five individuals.

The factorial ANOVA comparing the IVC and anaesthetic census data collected in the habitat strategy revealed significant differences between techniques and habitats but there was no interaction between these factors (Fig. 3, Table III). Post-hoc tests revealed that there were no differences between techniques in each of the microhabitats sampled. The only observed differences occurred between different habitats: gravel and all the other microhabitats (sand: $P < 0.05$; cobble: $P < 0.001$; small rocks: $P < 0.05$); large rocks with cobble ($P < 0.05$).

Table III. Factorial ANOVA results for the comparison between the interference visual census (IVC) and anaesthetic census (ANA) in the different microhabitats recorded in the habitat strategy

	df	MS	F	P
IVC - ANA	1	0.78	9.12	0.005
Microhabitat	4	0.68	7.95	0.000
IVC – ANA vs. Microhabitat	4	0.02	0.19	0.943
Error	30	0.09		

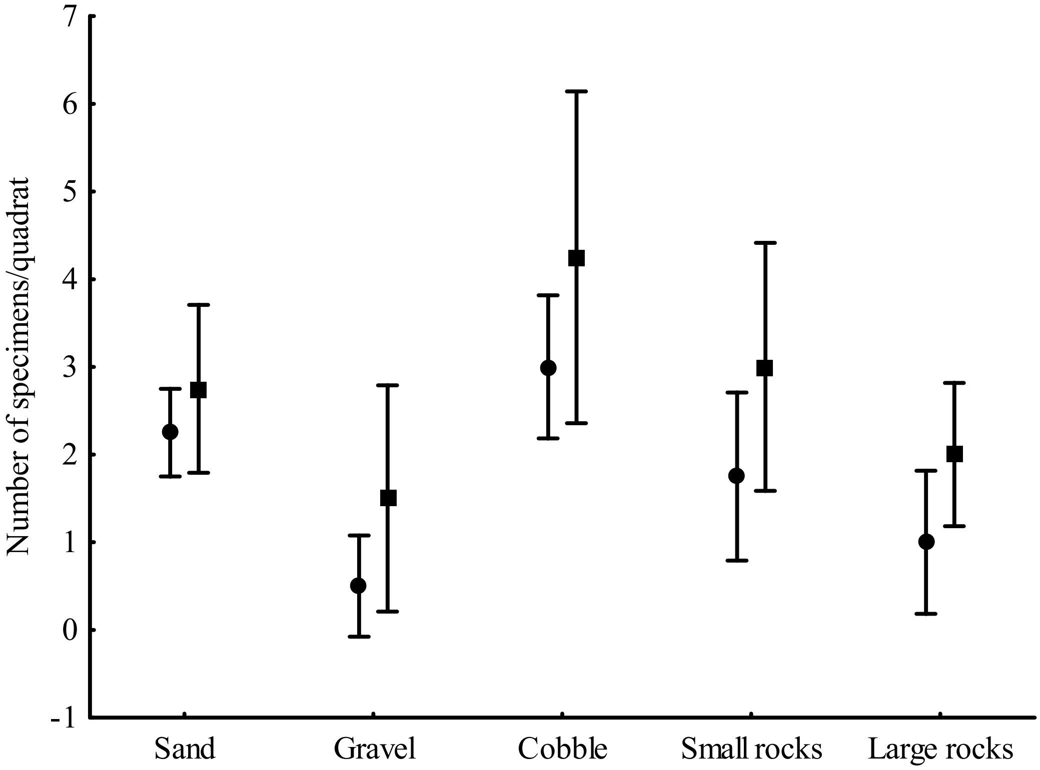

Fig. 3. Mean density of cryptobenthic fish (number of specimens per quadrat ± 95% confidence limits) recorded in the habitat strategy by interference visual census (IVC) (circle) and anaesthetic census (square) in each microhabitat.

Discussion

Cryptobenthic fish diversity observed in this study was lower than that reported in other studies of temperate fish assemblages: *e.g.*, 39 species from 9 families in South Africa (Prochaska 1998), 33 species from 17 families in New Zealand (Willis 2001), and 20 species from 5 families in Italy (La Mesa et al. 2004). This relatively low diversity is probably due to the smaller sampling size used in our test of the IVC. However, overall average densities obtained in our study with both the anaesthetic census and the visual census are comparable to those described by Prochazka (1998) and Willis (2001) using rotenone sampling: 3.41 specimens/m^2 and 3.61 specimens/m^2, respectively. Using a VC technique applied to northern Adriatic blennioids, Ilich & Kotrshall (1990) reported an average density of 4 specimens/m^2. In the Ciclopi Islands, Central Mediterranean Sea, La Mesa et al. (2004) using the same technique found an average density of between 0.60 and 0.67 specimens/m^2.

The observed differences between the traditional VC and the IVC were revealed by the visibility index. At the species level, major differences were related to the clingfishes *Lepadogaster* sp. which occur almost exclusively under stones (Henriques et al. 2002) and were completely missed by the VC. The gobies *G. xanthocephalus* and *G. paganellus*, use the space under small microhabitat items and were also underestimated by the traditional visual technique. Other benthic species such as *G. cruentatus*, *P. pilicornis*, *T. delaisi* and *P. pictus* present a less cryptic behaviour and were equally detected by both visual techniques. Therefore, traditional VC techniques underestimate different species in different degrees. In particular, species with cryptic habits are the most affected. By dismantling the substrate, a significant increase in the number of specimens detected is achieved.

In order to explore this result, a comparison of both visual techniques with a quantitative survey (anaesthetic counts) was performed. While differences were large between VC and anaesthetic counts, when interference was applied and specimens under microhabitat items were recorded (IVC) there were no significant differences to the quantitative survey. Moreover, when microhabitats were sampled proportionately (habitat strategy), no differences between the IVC and anaesthetic counts were found for each microhabitat type.

Most studies that quantitatively sampled these fish assemblages used visual census techniques that did not involve (or do not mention) disturbing the bottom by lifting items where fish could be hiding (Sale & Douglas 1981; Bortone et al. 1989; Willis 2001). Sampling other groups of marine animals, such as some invertebrates (e.g. Chapman 2002) is frequently done with interference techniques. In low complexity microhabitats such as sand interference has been used to improve censusing of cryptic fishes (Forrester 1995) but this has not been tested in other microhabitats. In this study we conclude that lifting small microhabitat items where fish could be hiding significantly increases the performance of the underwater visual census technique.

The interference visual census technique may render better abundance estimates, closer to those obtained with anaesthetics, depending on the specific behaviour of some species and the ability to sample some microhabitats. In the future, this IVC technique should be tested in different temperate cryptobenthic fish assemblages and its efficacy should be evaluated for other microhabitats.

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First record of *Chromogobius britoi* (Gobiidae) on the mainland European coast

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Abstract

Chromogobius britoi is a recently described and poorly studied goby, which was only known to occur in the Macaronesian islands of the Madeira and Canaries archipelagos. This species was captured for the first time in the mainland European coast (Portugal). The habitat, depth preferences and morphological characterization of the specimens captured in Portugal are compared with some specimens from the Canary Islands (Spain).

Key words

Chromogobius britoi, distribution, new record, Portugal

The *Chromogobius* genus is composed of three species: *C. quadrivittatus* (Steindachner), *C.zebratus* (Kolombatovic) and *C. britoi* Van Tassell. The first two species occur inside the Mediterranean, with the exception of two specimens of *C. zebratus* found in the Gulf of Cadiz by Alberto & Nieto (1993). The distribution of *C. zebratus* extends to Israel (Miller 1971), and the Adriatic Sea (Ahnelt 1990). *C. quadrivittatus* occurs from Catalunia (Spain) (Froese & Pauly 2005) to Israel (Golani & Ben Tuvia 1986) and also in the Black Sea (Miller 1986) and the Adriatic (Ahnelt 1990). *C. britoi* is a poorly studied goby which was recently described for the Macaronesian archipelagos of Madeira and the Canaries (Van Tassell 2001). In this paper, the occurrence of *C. britoi* in the mainland European coast (Portugal) is described and the morphological characterisation of the specimens is presented and compared with the specimens described by Van Tassell (2001) for the Macaronesian islands. Some new specimens from the Canary Islands are also included in this comparison.

Four females, three juveniles and one male of *C. britoi* were captured between June and August 2003 at the Arrábida Marine Park (38º27’03’’N, 9º01’24’’W), on the Western Coast of Portugal. The presence of this species at this study site was first detected while studying the cryptobenthic fish communities using underwater visual census (unpublished data). One female, one male and one juvenile were captured in the Canary Islands, at Tenerife (Los Abrigos) and Lanzarote (Puerto de Carmen), between July 1988 and August 1999. All specimens were captured using the anaesthetic quinaldine diluted in alcohol (1:15), fixed in 4% formaldehyde solution and preserved in 70% alcohol. Meristic characters and morphometric measurements (to the nearest 0.01mm) were taken under a binocular microscope. The specimens from Portugal were captured between 5 and 9m depth under boulders covered with algae and under small rocks on the sand. At the study site, the extension of the rocky bottom is relatively narrow (up to 100m long and 15m depth) and there are no tidepools in the area. In the Canary Islands two specimens were collected at 10m depth under boulders and one specimen in a 37cm deep tidepool.

The morphometric data is presented in Table I as body proportions of standard length, caudal peduncule length, head length and in eye diameter, following Miller (1988).

The meristic counts (number of individuals in parenthesis; new counts in bold) are the following. Fin-rays formulae: D1 - VI (11), D2 - I+9(1)/I+10(9)/I+11(1), A - I+9(11), P - 17(11), C (branched rays) **13**(2), 14(5), 15(3), **17**(1); total number of scales in the lateral line: 32(1), 33(1), 34(4), 35(2); 36(3). Number of sensory papillae in vertical row 1: 5(2), 6(4), 7(5); row 2: **3**(1), 4(4), 5(6); row 3: **3**(1), **4**(4), 5(4), 6(2); row 4: **3**(2), 4(1), 5(5), 6(3); row 5: **2**(1), 3(4), 4(5), **6**(1); row y: **0**(5), 1(6); row m: 0(8); 1(3).

The body proportions and meristic counts presented in our work are similar to the ones described by Van Tassell (2001), with a few exceptions, which constitute new values for the species but are only marginally different from the ones already described. There is however a correction that needs to be done. In Van Tassell (2001), the body proportions in caudal peduncle depth, head length and eye diameter were erroneously presented as proportions in standard length.

Table I. Minimum, maximum, mean and S.D. values for body proportions (following Miller, 1988) of eleven specimens of *Chromogobius britoi*.

New ranges are in bold

Sex	Juvenile				Female				Male				Total			
Size-range of fishes (LS in mm)	16.2-20.2				20.35-31.2				26.0-28.6				16.2-31.2			
Number of specimens	4				5				2				11			
	min	max	Mean	SD	min	max	Mean	SD	min	max	Mean	SD	min	max	Mean	SD
In standard length																
Head length	28.23	29.24	28.60	0.47	27.93	31.21	28.98	1.36	27.75	27.92	27.83	0.12	27.75	31.21	28.82	1.06
Head width	15.55	17.32	16.66	0.78	14.27	16.25	15.37	1.01	13.77	14.89	14.33	0.79	12.90	**17.32**	15.34	1.39
Snout to first dorsal fin origin	36.44	39.17	38.19	1.22	34.41	37.87	36.59	1.31	36.85	38.24	37.54	0.98	**34.41**	39.17	37.27	1.38
Snout to second dorsal fin origin	54.36	61.32	56.96	3.21	54.18	58.91	55.61	2.00	57.32	58.54	57.93	0.86	54.18	61.32	56.49	2.53
Snout to anus	53.70	56.53	54.83	1.21	52.68	58.56	55.49	2.40	52.85	53.80	53.33	0.67	52.68	58.56	55.30	1.80
Snout to anal fin origin	58.76	64.34	60.53	2.58	58.32	66.97	61.03	3.60	58.44	58.54	58.49	0.07	58.06	**66.97**	60.13	2.79
Snout to pelvic disc origin	25.35	30.84	28.88	2.55	27.70	31.06	29.16	1.39	28.29	28.42	28.36	0.10	**25.35**	32.72	29.30	2.13
Origin of pelvic spine to anus	21.89	26.35	24.31	1.89	22.79	27.60	24.71	2.03	24.19	25.04	24.62	0.60	**21.89**	26.35	24.20	1.51
Caudal peduncule length	17.55	22.52	19.71	2.41	18.51	21.45	19.93	1.10	20.30	21.59	20.95	0.91	**17.86**	22.52	20.13	1.45
First dorsal fin base	11.95	14.37	12.93	1.14	11.66	12.97	12.45	0.58	12.66	13.20	12.93	0.38	11.52	14.37	12.60	0.90
First to second dorsal fin space	6.34	7.67	6.74	0.63	5.26	7.47	6.47	0.94	6.77	7.07	6.92	0.22	5.26	7.67	6.47	0.73
Second dorsal fin base	23.04	26.35	24.45	1.40	25.21	26.76	25.67	0.63	24.36	26.43	25.39	1.46	23.04	26.76	24.92	1.31
Anal fin base	20.31	22.04	20.96	0.75	20.78	21.98	21.48	0.52	19.35	20.30	19.83	0.67	17.97	22.04	20.89	1.29

Table I (continued) Minimum, maximum, mean and S.D. values for body proportions (following Miller, 1988) of eleven specimens of *Chromogobius britoi*. New ranges are in bold

Sex	Juvenile				Female				Male				Total			
	min	max	Mean	SD	min	max	Mean	SD	min	max	Mean	SD	min	max	Mean	SD
Caudal fin length	19.67	25.92	23.78	2.80	22.73	25.81	24.00	1.21	22.33	24.94	23.64	1.84	19.82	25.92	23.88	1.92
Pectoral fin length	19.71	26.59	23.11	2.84	24.03	25.81	24.66	0.73	23.45	24.02	23.74	0.41	19.71	25.81	23.58	1.75
Pelvic disc length	20.21	23.00	22.13	1.30	19.54	23.14	21.70	1.49	19.73	22.67	21.20	2.08	19.54	23.14	22.16	1.21
Body depth at pelvic disc origin	14.89	15.81	15.30	0.44	13.92	15.51	14.87	0.69	11.91	16.24	14.08	3.06	13.92	16.24	15.16	0.73
Body depth at anal fin origin	13.29	15.55	14.48	1.13	12.99	15.77	14.71	1.19	11.54	13.53	12.54	1.41	13.53	**15.77**	14.78	0.89
Body width at anal fin origin	8.36	10.94	9.51	1.23	8.12	11.48	10.34	1.30	10.79	11.17	10.98	0.26	7.37	**11.48**	9.68	1.54
In caudal peduncule length																
Caudal peduncule depth	53.19	67.74	59.67	6.17	53.03	65.85	59.07	4.59	41.38	53.33	47.36	8.45	52.50	67.74	57.74	5.67
In head length																
Snout length	20.00	25.42	22.18	2.39	16.28	23.08	20.61	2.67	19.51	20.00	19.76	0.34	19.51	25.42	21.70	1.83
Eye diameter	23.73	29.09	27.12	2.41	20.22	24.42	22.31	1.56	23.17	25.33	24.25	1.53	20.22	28.57	23.51	2.68
Postorbital length	53.06	56.36	55.40	1.57	51.28	55.93	52.57	1.91	50.67	58.54	54.60	5.56	51.28	58.54	53.99	2.70
Cheek depth	16.33	22.03	18.76	2.74	17.44	29.67	21.35	5.32	16.00	24.39	20.20	5.93	15.63	29.67	20.49	4.75
Mouth width	27.99	48.98	39.65	9.67	38.20	43.96	40.24	2.22	31.71	40.89	36.30	6.49	27.99	48.98	37.03	7.02
In eye diameter																
Interorbital width	7.53	18.45	12.56	5.56	12.30	21.27	17.85	3.70	19.04	19.04	19.04	0.00	7.53	21.27	16.50	5.14

C. britoi has been described to occur mainly in small cracks and crevices in the vertical face of rock walls (Van Tassell 2001). All our specimens were captured under boulders covered with algae and under small rocks on the sand, except one specimen captured at a tidepool. These habitats are similar to the ones described for the other two species which have been described to occur in caves, under boulders, rocks on rock or sandy substrate and tide-pools (Miller 1971; Mercader 1994; Kovačić 1997).

Depth preferences also vary between species. *C. quadrivittatus* has been captured mainly in the shallow subtidal or intertidal areas (e.g. Ahnelt 1990) whereas *C. zebratus* has been captured both in the intertidal and subtidal areas to 10m depth (e.g. Bouchereau & Tomasini 1989). Although *C. britoi* has also been found in the intertidal and shallow subtidal, it can occur at greater depths (up to 65m) (Van Tassell 2001).

In the present work, the presence of *C. britoi* in the mainland European coast is recorded for the first time. The presence of this species at other sites should be investigated since it is a very cryptic and rare species, which can only be sampled adequately with anaesthetics or ichthyocides and can easily be overlooked.

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Spatial structure and seasonality of a cryptobenthic fish assemblage

(Submitted to *Marine Ecology Progress Series*)

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Abstract

A recently described visual sampling technique was used to study the cryptobenthic fish assemblage in a temperate marine reserve at the Arrábida Marine Park (Portugal). Seasonal variability in the spatial structure of the cryptobenthic assemblage was investigated at two spatial scales (sampling site and habitat type). A total of 5089 specimens belonging to 29 species in 11 families were sampled. Results showed higher abundances when compared to other studies in nearby geographical areas. At the assemblage level, no seasonal differences between species richness and abundance were found. Within seasons, clear differences in the abundances and richness across habitats arose. Among the nine most abundant species, habitat utilization was variable but we identified a large group of specialist species (defined as those with significantly higher abundances in one or two habitats). Seasonal abundance shifts in habitat utilization for a number of species are described. Our results indicate considerable spatial partitioning across habitat types and possible explanations for these patterns are discussed.

Keywords

Cryptobenthic fishes, Composition, Distribution, Seasonality, Community ecology.

Introduction

An accurate description of the association patterns between species and habitats is essential to understand the processes affecting communities. Until today it remains one of the major challenges facing ecologists. Different factors should be considered simultaneously for a complete overview of community structure, including breadth in taxonomic, spatial and temporal scales. The analysis of the structure of coastal cryptobenthic fish communities, in particular, has rarely considered both physical (area, depth, etc) and temporal (yearly, seasonal, etc) scales simultaneously.

Studies in coastal communities have typically focused on large pelagic and bentho-pelagic fishes and disregarded other species, including cryptobenthic fishes. These can be defined as "…small bodied fishes (<10 cm) that exploit restricted habitats where food and shelter are obtained in, or in relation to, conditions of substrate complexity and/or restricted living space, with a physical barrier likely to be interposed between the small fish and sympatric predators…" (cf. Miller 1979). The role of cryptobenthic fishes as trophic links between lower and higher order predators (Depczynski & Bellwood 2003) and the high seasonal fluctuations in their abundance (Beldade et al. unpublished data) suggest that they play a fundamental role as energy mediators in coastal ecosystems. In spite of this, and due to methodological limitations of traditional visual methods to sample these species (e.g. Harmelin-Vivien et al. 1985, Willis 2001), several coastal community studies have either excluded cryptobenthic fishes (e.g. García-Charton et al. 2004) or sampled them using traditional visual sampling methods (e.g. La Mesa & Vacchi 2005).

Cryptobenthic fishes are by definition, and by several habitat description studies (e.g. Patzner 1999) more reliant on their environments than their larger counterparts (Depczynski & Bellwood 2004). These species have numerous specializations in shape, colour and even body structures (Miller 1979) suggesting they are good candidates for habitat specialists. Specific feeding behaviours such as the sit-and-wait strategy described by Depczyncky & Bellwood (2004) together with nest guarding behaviours (e.g. Gonçalves & Almada 1998), small home-ranges and activity areas (Lukhurst & Luckhurst 1978, Wilkins & Myers 1995) and short-term site fidelity (Gonçalves et al. 1998, Depczynski & Bellwood 2004) further strengthen the idea of these being fairly sedentary fishes. Nonetheless, several studies on cryptobenthic fish found that many species had high abundances in more than one habitat type (Macpherson 1994, La Mesa et al. 2004, Depczynski & Bellwood 2004, La Mesa & Vacchi 2005, Malavasi et al. 2005). On the other hand, even though the same species can be present in several habitats, in some cases there may be a strong spatial segregation across habitats (e.g. Malavasi et al. 2005).

Temporal dynamics of fish populations has broadly been related to the input of recruits (e.g. Doherty & Williams 1988, Cushing 1995) and to post-settlement mortality processes that may reshape initial settlement patterns (e.g. Forrester 1995, Macpherson & Zika 1999). As survivorship of fish cohorts may increase with increasing habitat complexity (Connell & Jones 1991), or in specific habitat patches (Schmitt & Holbrook 1984), habitat shifts may be a common phenomenon. Habitat utilization shifts may occur at a seasonal scale (Davis 2000) due to a number of processes. The choice of nesting habitats (e.g. *Lipophrys pholis* uses nesting habitats during a short-period in the year after which they move to other habitats, Faria et al. 1999), settlement habitats (e.g. *Apletodon dentatus* settles into red algae stands, Gonçalves et al. 2002) and latter ontogenetic habitats shifts, which have been described for a number of species (e.g. *Gobius cobitis* moves from intertidal pools to other habitats as it grows, Faria & Almada 2001). The role of substratum type or habitat on the structure of the community can be felt immediately at the time of settlement (Macpherson 1994). Clearly, seasonality may play an important role in the dynamics of cryptobenthic fishes and it is thus important to consider a temporal scale in studies of habitat association.

In this study we will analyse the spatial and temporal variation in a cryptobenthic fish assemblage in a temperate coastal environment in the Atlantic (the Arrábida Marine Park). Specifically, we address the following questions: (1) What is the composition of the target cryptobenthic fish assemblage? (2) Are there seasonal differences in richness and abundance of the cryptobenthic fish assemblage in the Marine Reserve? (3) Within each season, do the main habitats vary in richness and abundance? (4) Are there habitat specialists and generalists among the most abundant species? (5) Are there ontogenetic habitat shifts among the most abundant species?

Methods

Sampling location and period

This study was performed in the marine reserve at the Arrábida natural park (Portugal) in the sector identified as having the highest biodiversity (Gonçalves et al. 2002). We investigated the seasonal dynamics of the cryptobenthic fish assemblage in 2003, at two spatial scales: sites (Risco - 38º27’03’’N, 9º01’24’’W and Derrocada - 38º26’54’’N, 9º02’12’’W) and habitats. The underwater rocky habitats at the sampling locations are heterogeneous and patchily distributed resulting in part from the disintegration of the calcareous cliffs that border the coastline. These habitats were grouped into five categories according to substratum type and rock size (Table I). Three sampling periods were selected: winter (January-February), spring (April-May) and autumn (October-November). During the summer months sampling of the cryptobenthic assemblage was impossible due to the high density of perennial macroalgae (mainly *Cystoseira* spp.).

Table I. General description of habitat types found in the rocky bottom at the Arrábida Marine Park and area analysed (in %).

Habitat	General description	Area
Sand	Flat sand and gravel patches, usually smaller than $2m^2$	11.5
Cobbles	Rocks larger than 5 cm and smaller than 25 cm	4.4
Small rocks	Rocks smaller than 30 cm	6.7
Large rocks	Rocks larger than 30 cm	60.3
Bedrock	Flat rock surfaces usually covered with red incrusting algae	17.1

Sampling procedure

During each season we sampled eight transects at each location, adding up to a total of 48 counts. Each transect consisted of a 1m wide corridor of variable length (mean length: 64.40 m; Stdev: 9.94) extending from the deepest part of the rocky substrate (mean depth: 9.53 m; Stdev: 1.55) until the intertidal (mean depth: 2.39 m; Stdev: 0.66). In each transect the area occupied by each habitat patch was visually estimated in 16 underwater counts covering a total area of $1038m^2$.

Sampling technique

An "all-occurrence" sampling procedure adapted to cryptobenthic fish assemblages was used (cf. Syms 1995). All census were performed by the same diver. In each census we registered each fish observed, identified it, estimated its size and recorded the habitat type in which it was observed. The visual census technique used was an interference technique (cf. Beldade & Gonçalves in press), which involved disturbing certain habitats like sand or gravel and dismantling others like small rocks or cobbles, to look for fish in accessible hideouts. This non-destructive technique has been shown to perform better than traditional visual techniques in counting cryptobenthic fishes. With training almost all species can be easily identified based on distinct morphological and coloration characteristics. Exceptions are two locally occurring clingfishes, *Lepadogaster lepadogaster* and *L. purpurea* (Henriques et al. 2002), which were grouped under the designation *Lepadogaster* spp. Given that the vast majority of fishes in this assemblage were under 10cm total length we estimated the total length of each specimen to nearest 10 mm (Edgar et al. 2004). In this study, prior to each sampling season the diver was trained to visually estimate fish lengths underwater following Bell et al. (1985), and the correlations between estimated and real values were always higher than 0.90 before sampling began and length was visually overestimated on average by approximately 10%.

Data analysis

Species richness and total density were calculated for each of the five habitat types in each of the 48 transects corresponding to a total of 240 samples. In the strategy used, habitats that did not have any specimens were not used to calculate densities. We assessed differences in the species richness (number of species) and total density (number of individuals per square meter) across seasons using a One-Way ANOVA after log (x+1) transforming the density data to meet parametric assumptions. We analysed the species richness and the log (x+1) total density across habitats with ANOVAs for data within each season. Scheffé's post-hoc tests were used to identify which habitats presented significant differences in species richness and density we used. Specific density variations were analysed across habitats in each season by focusing on the nine most abundant species that together made up 96% of the total number of observations (Table II). We used MANOVAS on log transformed data (as above) and Scheffé's post-hoc tests to identify the habitat types that had significantly different densities for each of the selected species in each season. Canonical discriminant function analyses (CDAs) were used to graphically display the relationships between species and habitats to the discriminant functions within each season. The square root of species densities multiplied by a constant was used to display relative abundances (cf. Depczynski & Bellwood 2004). The total length (TL in cm) of the nine most abundant species was compared across habitats. T-tests and one-way ANOVA followed by unequal N HSD tests, a modification of the Tukey HSD test, were used to identify size differences among specimens in different habitats.

Results

A total of 5089 specimens belonging to 29 species in 11 families were sampled (Table II). Total mean density across all habitats and seasons was 1.62 specimens per m^2 (5089 specimens in 3114 per m^2). The nine most abundant species, highlighted in Table II, make up for 95.8% of all specimens observed, with the family Gobiidae being the most abundant and having the highest number of species represented.

Table II. Families, species and density in specimens/100m^2 (mean and standard deviation) across seasons and total number of observations per species. The 8 most abundant species (in **bold**) make up for 95.8% of all specimens observed.

Family	Species	Winter		Spring		Autumn		n
		mean	SD	mean	SD	mean	SD	
Blenniidae	***Parablennius pilicornis***	57.85	104.06	66.98	181.35	46.53	65.61	**911**
	Parablennius gattorugine	7.42	19.06	11.09	34.31	5.43	17.86	83
	Lipophrys pholis	0	0	1.34	12.02	0	0	1
	Parablennius ruber	0	0	0	0	0.02	0.2	1
	Parablennius sanguinolentus	0	0	0.04	0.38	0	0	1
Callyonimidae	*Callionymus reticulatus*	1.03	6.63	1.52	10.81	1.8	11.32	14
Congridae	*Conger conger*	0.28	2.5	0.03	0.27	0	0	2
Gadidae	*Gaidropsarus mediterraneus*	0.33	2.66	4.43	30.39	0.61	4.05	11
	Gaidropsarus vulgaris	0.37	3.3	0.04	0.35	0.61	3.16	5
Gobiesocidae	***Lepadogaster* spp.**	70.95	210.66	98.34	267.31	50.45	146.74	**369**
	Lepadogaster candolii	42.77	127.03	32.67	105.8	20.7	53.14	**206**
	Apletodon dentatus	0	0	0.58	3	2.38	17.47	8
	Diplecogaster bimaculata	0	0	0	0	0.29	2.35	2
Gobiidae	***Gobius xanthocephalus***	77.43	186.69	60.71	103.69	189.21	272.58	**1563**
	Gobius paganellus	31.18	66.43	28.9	46.4	31.91	63.79	**311**
	Pomatoschistus pictus	2.53	11.06	17.42	115.77	21.35	71.51	**161**
	Gobius cruentatus	7.72	21.51	11.47	28.55	10.95	24.3	**144**
	Chromogobius britoi	0	0	0.4	3.35	0.24	1.58	2
	Thorogobius ephippiatus	0	0	0	0	0.51	3.22	6

Table II. (Continued) Families, species and density in specimens/100m^2 (mean and standard deviation) across seasons and total number of observations per species. The 8 most abundant species (in **bold**) make up for 95.8% of all specimens observed.

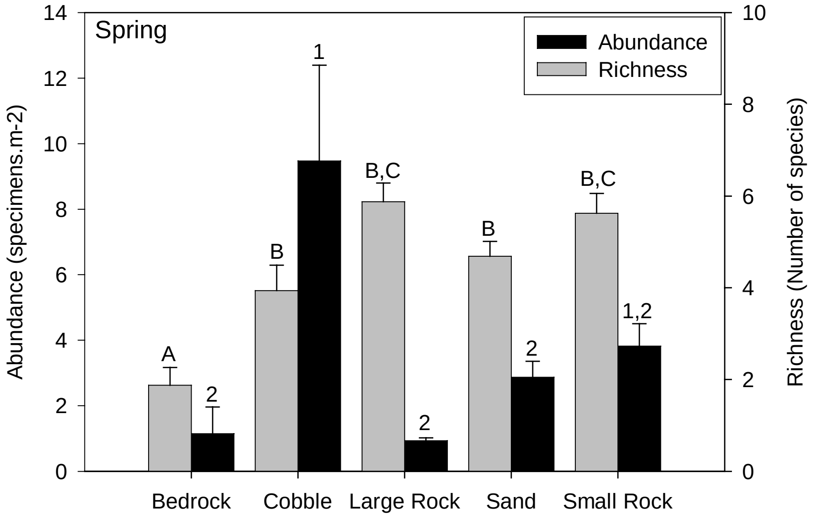
Family	Species	Winter		Spring		Autumn		n
		mean	SD	mean	SD	mean	SD	
Muraenidae	*Muraena helena*	0.93	4.75	1.42	12.42	1.6	11.4	11
Scophthalmidae	*Zeugopterus punctatus*	0	0	0.04	0.36	0	0	1
	Phrynorhombus regius	0.03	0.24	0.15	1.03	0	0	3
Scorpaeniidae	*Scorpaena notata*	0.81	5.31	1.28	10.36	0.4	2.99	11
	Scorpaena porcus	0.37	2.53	0.24	1.13	1.68	11.04	11
Syngnathidae	*Syngnathus acus*	0.99	4.79	1.02	7.6	1.15	4.42	21
	Nerophis lumbriciformis	2.48	10.1	2.09	9.23	0.54	2.96	16
	Entelurus aequoreus	0	0	0.04	0.32	0	0	1
Tripterygiidae	***Tripterygion delaisi***	17.53	31.29	22.61	39.56	39.27	85.12	**1213**
	Total global	323.0	470.5	364.8	616.5	427.6	506.5	5089

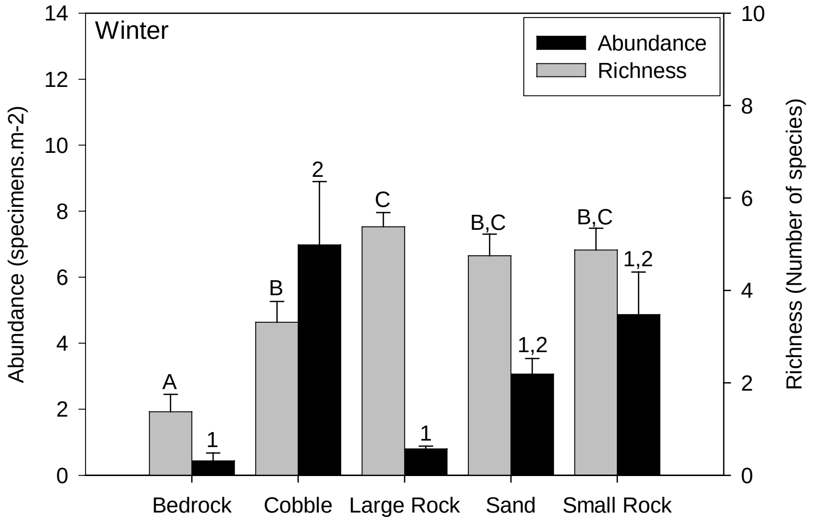
Seasonal assemblage dynamics

There were no significant differences across seasons, neither in species richness (F(2, 237) = 2.202, P = 0.113) nor in density (F(2, 237) = 0.776, P = 0.461). The increase in the total number of species across seasons in the spring (winter: 19, spring: 25, autumn: 22) is due in part to the accidental observation of two intertidal species (*Lipophrys pholis* and *Parablennius sanguinolentus*) and two other very rare species that have their southern distribution limit in the area (*Entelurus aequoreus* and *Zeugopterus punctatus*) (Henriques et al. 1999).

Habitat variation for each season

For each season, species richness and abundance varied across habitat types (Fig. 1). There were significant differences in species richness and density across habitats but not between sites in every season (Table III). Post-hoc tests showed that the differences in richness and abundance across habitats were not consistent from season to season (Fig. 1). Bedrock habitat displayed the lowest richness in all seasons and together with large rocks had the lowest abundance in all seasons. Cobble had a striking decrease in abundance in the autumn relatively to the other seasons, otherwise it displayed very high abundance relatively to other habitats. In the autumn the abundance in the sand and small rock habitats increased being higher than in any other habitats.





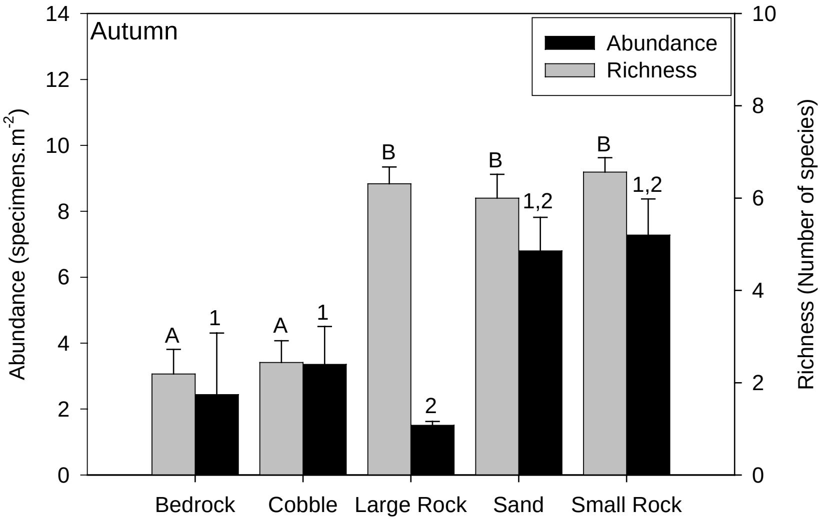


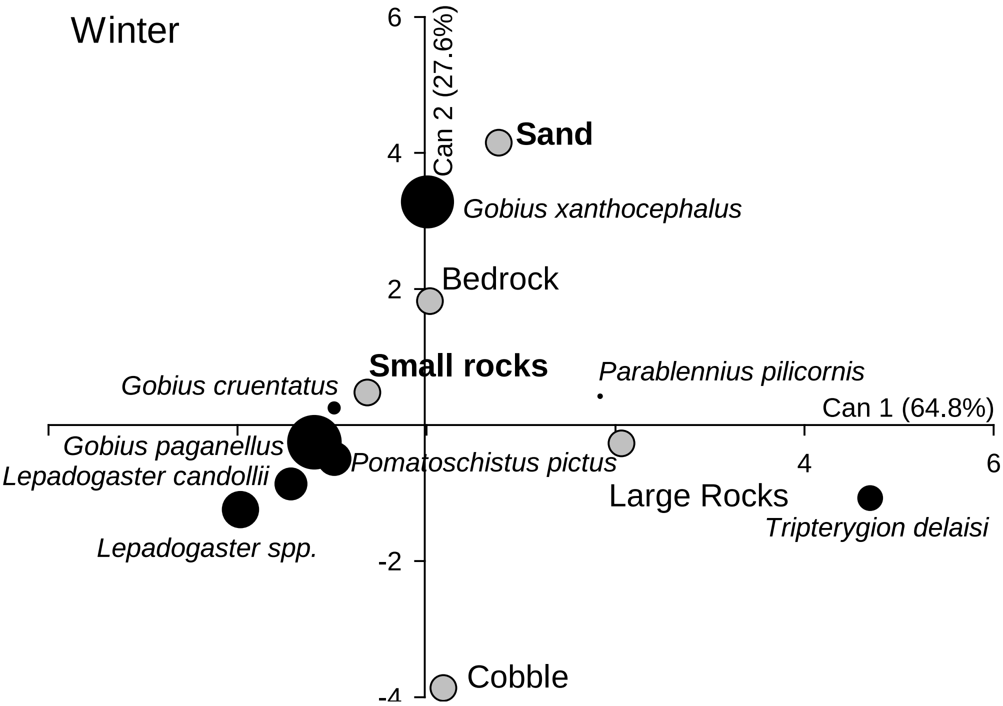
Fig. 1. Species richness (mean + SE) and abundance (mean + SE) variation across habitats within each season. Letters indicate Scheffé's post-hoc test results for richness between habitats and numbers indicate Scheffé's post-hoc test results for abundance.

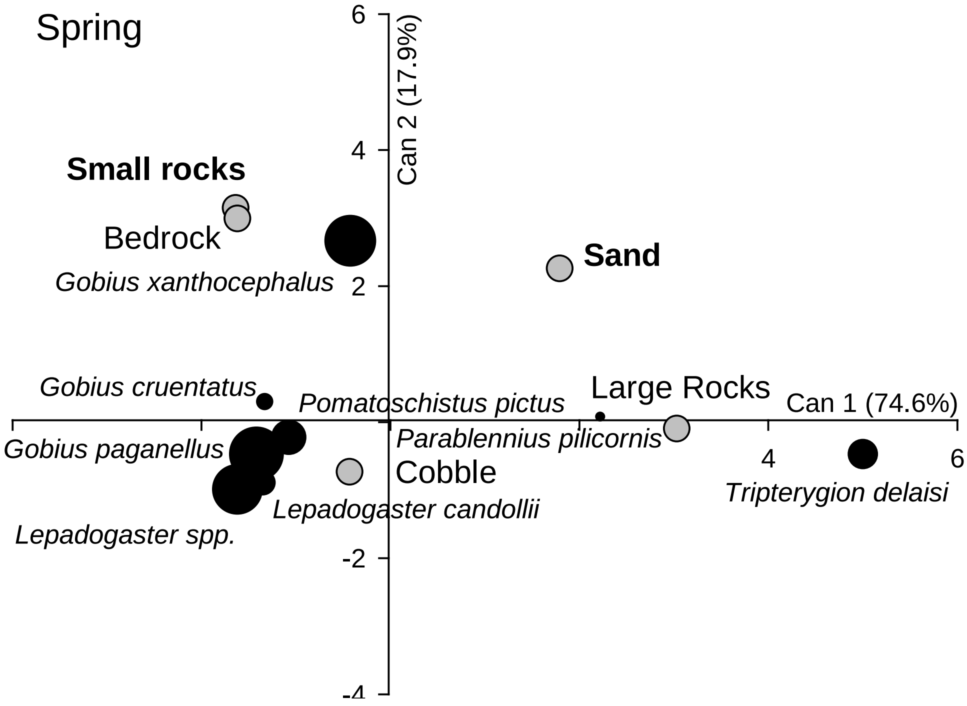
Table III. Two-way factorial ANOVA results for species richness and abundance (log(x+1)) within seasons between sites and habitats. Significant results displayed in **bold**.

	Species Richness				Abundance			
	df	MS	F	P	df	MS	F	P
Winter								
Site	1	6.61	2.52	0.116	1	56.10	3.44	0.067
Habitat	4	42.25	16.15	**<0.001**	4	121.92	7.47	**<0.001**
Site x Habitat	4	1.98	0.75	0.555	4	15.74	0.96	0.432
Error	70	2.616			70	16.32		
Spring								
Site	1	9.80	4.035	0.58	1	93.73	3.51	0.065
Habitat	4	41.39	17.04	**<0.001**	4	192.52	7.20	**<0.001**
Site x Habitat	4	6.45	2.658	**0.039**	4	67.06	2.51	**0.049**
Error	70	2.42			70	26.73		
Autumn								
Site	1	0.050	0.187	0.891	1	0.988	0.0045	0.833
Habitat	4	76.763	28.658	**<0.001**	4	109.11	4.970	**<0.001**
Site x Habitat	4	9.550	3.565	**0.011**	4	13.20	0.601	0.663
Error	70	2.679			70	21.95		

Habitat distribution patterns

We analysed the density variation by habitat within each season for the nine most abundant species (highlighted in table I). Significant differences were found between habitats for all of the species considered in each of the seasons: winter, Pillai's trace = 1.734; F(32, 284) = 6.789, P < 0.001; spring, Pillai's trace = 1.728; F(32, 284) = 6.747, P < 0.001; and autumn, Pillai's trace = 2.119; F(32, 284) = 9.997, P<0.001. Through canonical discriminant analyses, each species and habitat are plotted in a bi-dimensional space displaying the associations between species and the habitats significantly correlated to the discriminant functions (Fig. 2). *Gobius xanthocephalus* is clearly associated to the sand habitat in the winter and autumn, but in the spring its association to the small rocks becomes stronger than to any other habitat (Fig. 2). *Tripterygion delaisi* is associated to large rocks especially during the autumn (Fig. 2). A group of species including, *G. paganellus*, *G. cruentatus*, *Lepadogaster* spp., *L. candolii* and *Pomatoschistus pictus* seems to be associated to small rocks in the winter and to cobble in the other seasons (Fig. 2). Post-hoc tests revealed the significant differences in density (per m^2) for each species in each habitat within each season (Fig. 4). Following these results, we can group species into two distinct categories according to the number of habitats that they use: 1. a specialist group of species that occur in significantly higher abundances in one or two habitats, with *G. xanthocephalus*, *G. cruentatus*, *P. pictus*, *G. paganellus*, *L.* spp., *L. candolii* and *T. delaisi*; and 2. a generalist species that occur in similar abundances in more than two habitats, *Parablennius pilicornis* (Fig. 4). Among the specialists, *G. xanthocephalus*, *G. cruentatus* and *P. pictus* occur in significantly higher abundances in the sand habitat with the exception of *P. pictus* in the spring. *G. paganellus*, *Lepadogaster* spp. and *L. candolii* occurred in significantly higher abundances in the small rocks and cobble. The remaining specialist, *T. delaisi* occurred in significantly higher abundance in the large rock habitat. *P. pilicornis* the only clearly generalist species occurred in similar abundances in several habitats.


Winter
6
4
2
-2
-4
Can 2 (27.6%)
Can 1 (64.8%)
4
6
Sand
Gobius xanthocephalus
Bedrock
Small rocks
Parablennius pilicornis
Gobius cruentatus
Gobius paganellus
Lepadogaster candollii
Pomatoschistus pictus
Large Rocks
Tripterygion delaisi
Lepadogaster spp.
Cobble


Spring
6
4
2
-2
-4
Can 2 (17.9%)
Can 1 (74.6%)
4
6
Small rocks
Bedrock
Gobius xanthocephalus
Sand
Gobius cruentatus
Pomatoschistus pictus
Large Rocks
Parablennius pilicornis
Gobius paganellus
Cobble
Tripterygion delaisi
Lepadogaster candollii
Lepadogaster spp.

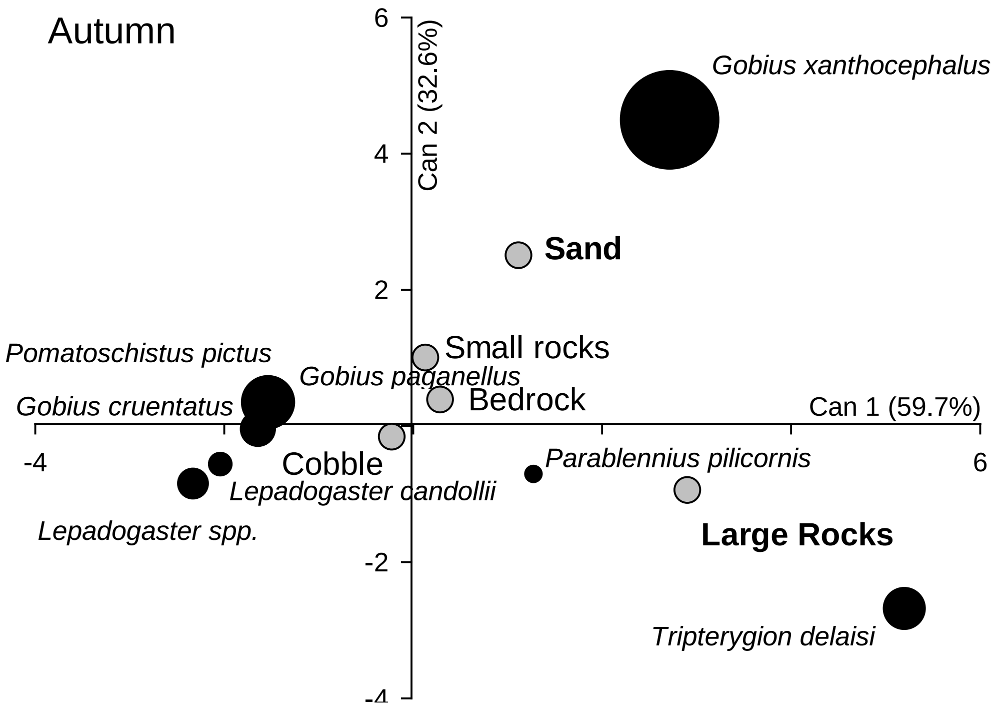
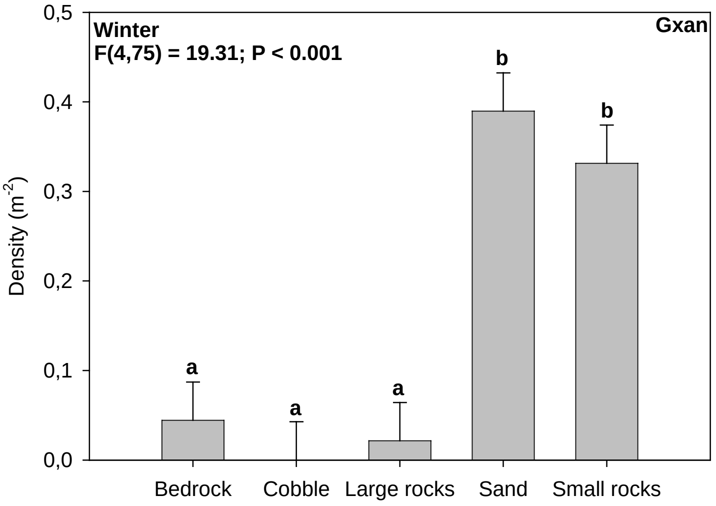


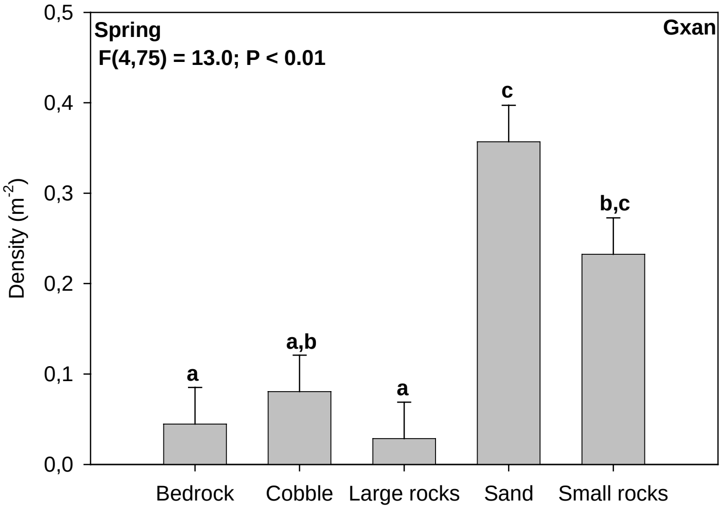
Fig. 2. Canonical discriminant analyses displaying the relationship between the nine most abundant species and the habitat types in the (a) winter, (b) spring and (c) autumn. The habitats that were significantly correlated to the discriminant functions are highlighted in bold. Species bubble sizes are proportional to the species abundance in each season (square root transformation of the densities).

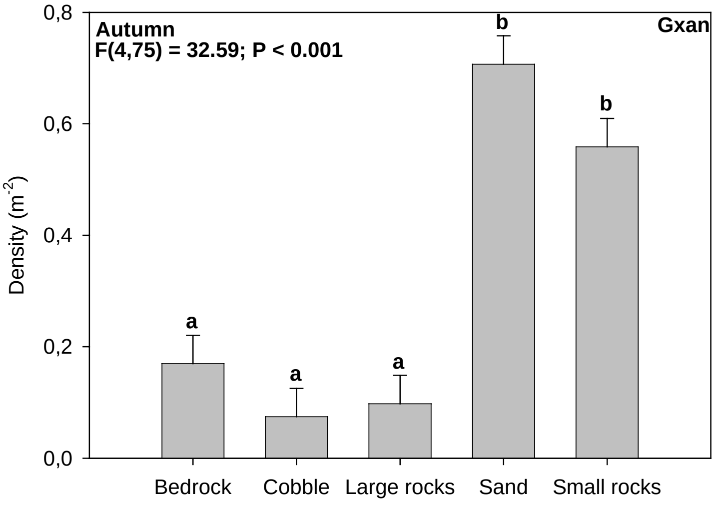
Seasonal habitat shifts and recruitment

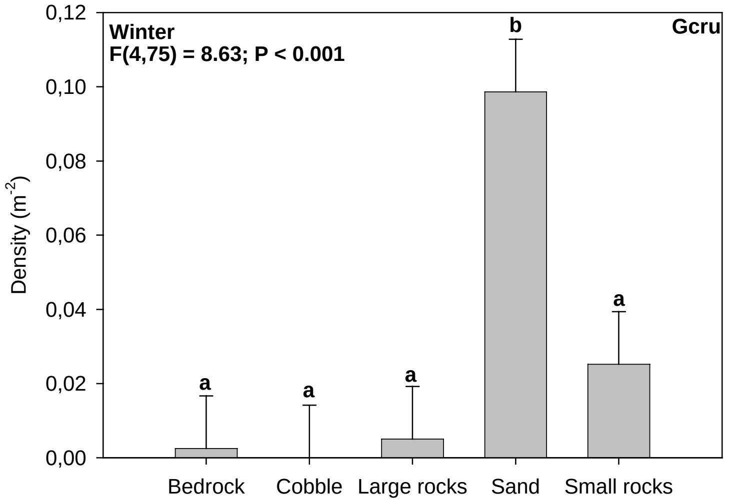
The largest seasonal density shifts by habitat were observed for *Gobius cruentatus*, *Gobius paganellus Lepadogaster* spp. and *Lepadogaster candolii* (Fig. 4). In the case of *G. paganellus* and *L. candolii* there was a clear relative decrease in abundance in the cobble habitat in the autumn. *G. cruentatus* is probably a winter spawning goby, which had an increase in abundance in the spring. Since the *Lepadogaster* spp. designation encompasses two species and we cannot be certain as to which species, the observed relative abundance increase in the small rocks habitat in the autumn is related to.

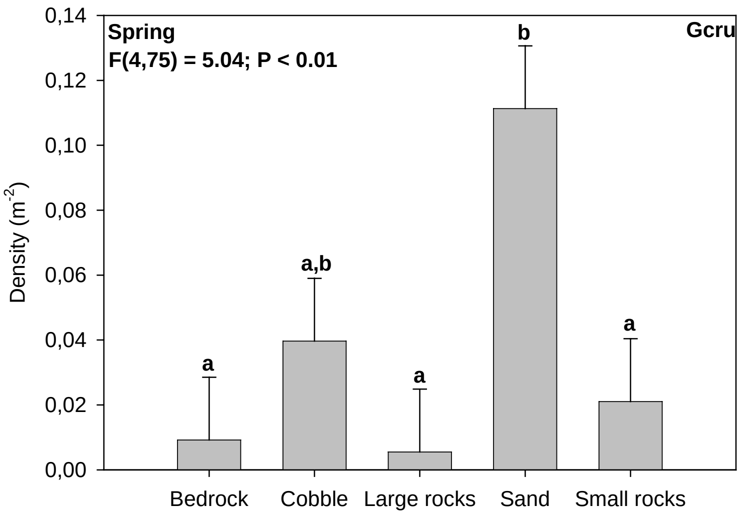
Seasonal changes in habitat use by a number of species may be due to several factors among which the arrival of recruits. In order to ascertain if seasonal habitat utilization was related to recruitment, we analysed the size (TL) variation for a group of species. We selected the species with clearer seasonal differences in habitat use and at the same time had 10 or more observations in each of the habitats considered (*Gobius xanthocephalus*, *G. paganellus* and *L. candolii*). For each of these we compared the sizes of specimens across habitats where higher abundance shifts were observed. No differences in length of *L. candolii* between the cobble and small rock habitats during the winter (mean size in cobble: 3.83cm; mean size in small rocks: 3.83cm; T = -0.004, df = 62, P = 0.997) or the spring (mean size in cobble: 4.05cm; mean size in small rocks: 3.79cm; T = 0.84, df = 38, P = 0.405) sampling. *G. paganellus* specimens also did not differ in mean size across cobble, large rocks, sand or small rocks in the winter period (ANOVA: F(3, 90) = 2.113, P = 0.104) nor in the spring (F(3, 87) = 1.695, P = 0.174). *G. xanthocephalus* however presented significant differences in size of specimens across habitats counted during the recruitment period (autumn) (F(3, 996) = 15.553, P < 0.001). Post-hoc tests showed that there was a significant lower size of specimens in large rocks (mean size: 4.82 cm) than in sand (mean size: 5.78 cm) (P < 0.001) and also between large rocks than in small rocks (mean size: 5.61 cm) (P < 0.001). Cobble was excluded from the analysis because it had less than 10 specimens. In the case of *G. xanthocephalus* the seasonal habitat shifts were driven by the habitat utilization of different ontogenetic stages.

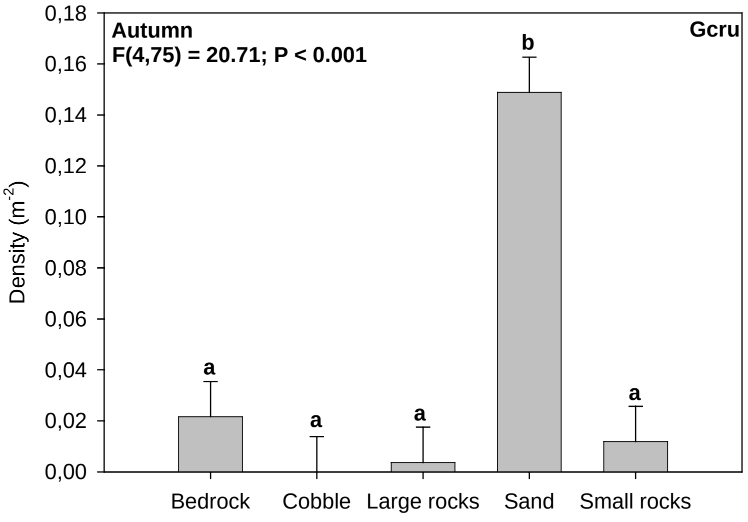

Winter
F(4,75) = 19.31; P < 0.001
Gxan
Density (m-2)
0,5
0,4
0,3
0,2
0,1
0,0
a
a
a
b
b
Bedrock
Cobble
Large rocks
Sand
Small rocks

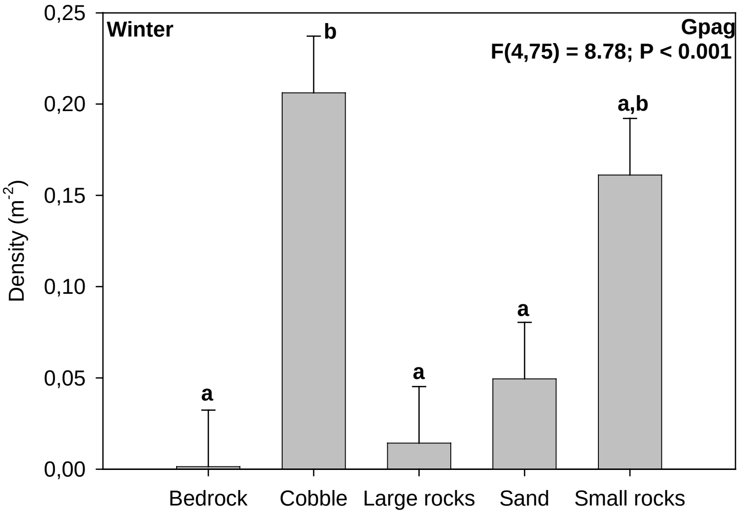

Spring
F(4,75) = 13.0; P < 0.01
Gxan
Density (m-2)
0,5
0,4
0,3
0,2
0,1
0,0
a
a,b
a
c
b,c
Bedrock
Cobble
Large rocks
Sand
Small rocks

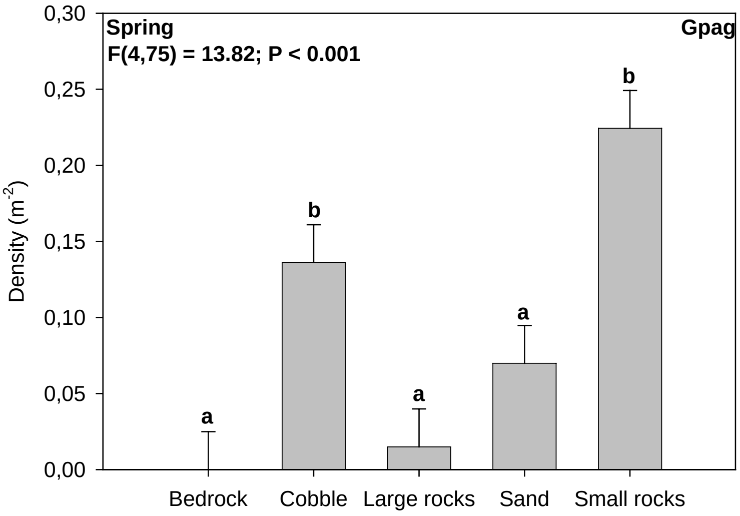

Autumn
F(4,75) = 32.59; P < 0.001
Gxan
Density (m-2)
0,8
0,6
0,4
0,2
0,0
a
a
a
b
b
Bedrock
Cobble
Large rocks
Sand
Small rocks

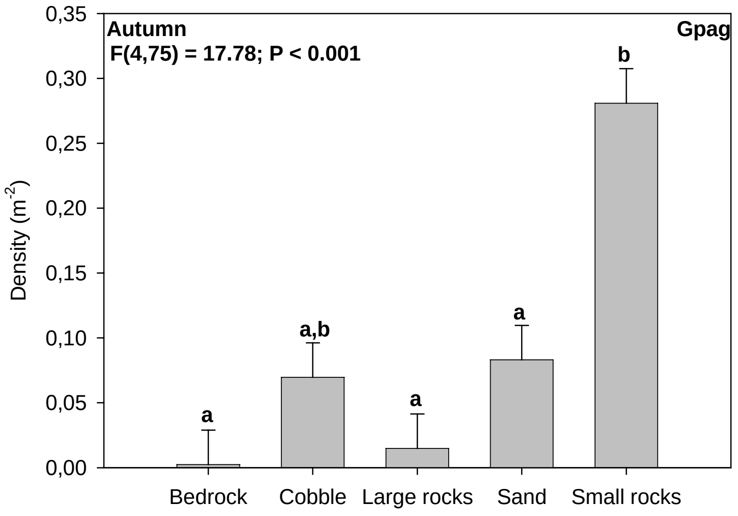

Winter
F(4,75) = 8.63; P < 0.001
Gcru
Density (m-2)
0,12
0,10
0,08
0,06
0,04
0,02
0,00
a
a
a
b
a
Bedrock
Cobble
Large rocks
Sand
Small rocks

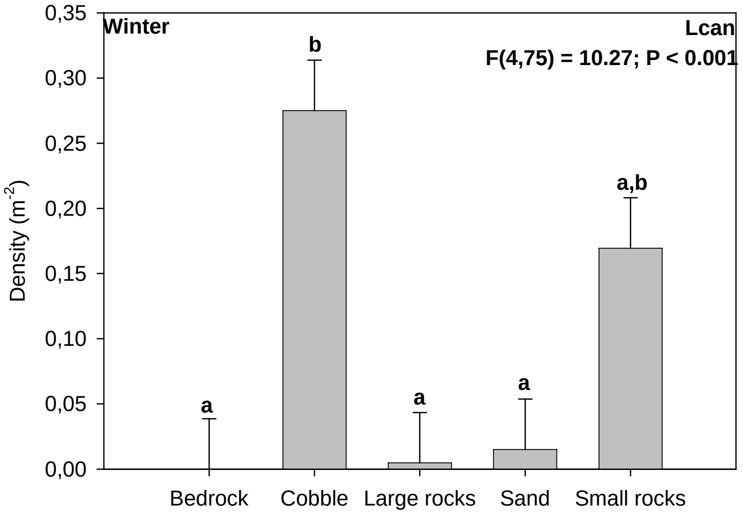

Spring
F(4,75) = 5.04; P < 0.01
Gcru
Density (m-2)
0,14
0,12
0,10
0,08
0,06
0,04
0,02
0,00
a
a,b
a
b
a
Bedrock
Cobble
Large rocks
Sand
Small rocks

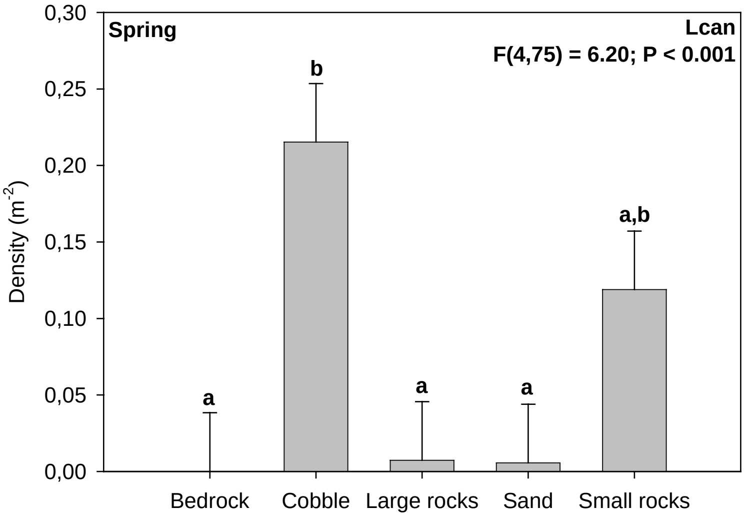

Autumn
F(4,75) = 20.71; P < 0.001
Gcru
Density (m-2)
0,18
0,16
0,14
0,12
0,10
0,08
0,06
0,04
0,02
0,00
a
a
a
b
a
Bedrock
Cobble
Large rocks
Sand
Small rocks

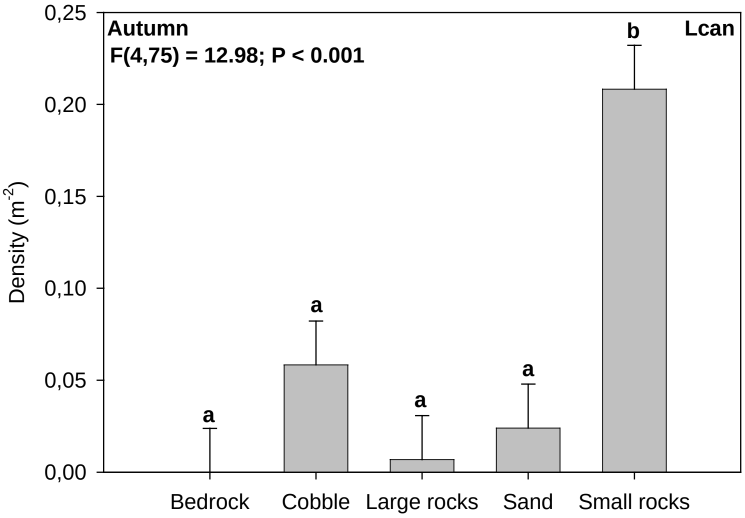

Winter
Gpag
F(4,75) = 8.78; P < 0.001
Density (m-2)
0,25
0,20
0,15
0,10
0,05
0,00
a
b
a
a
a,b
Bedrock
Cobble
Large rocks
Sand
Small rocks

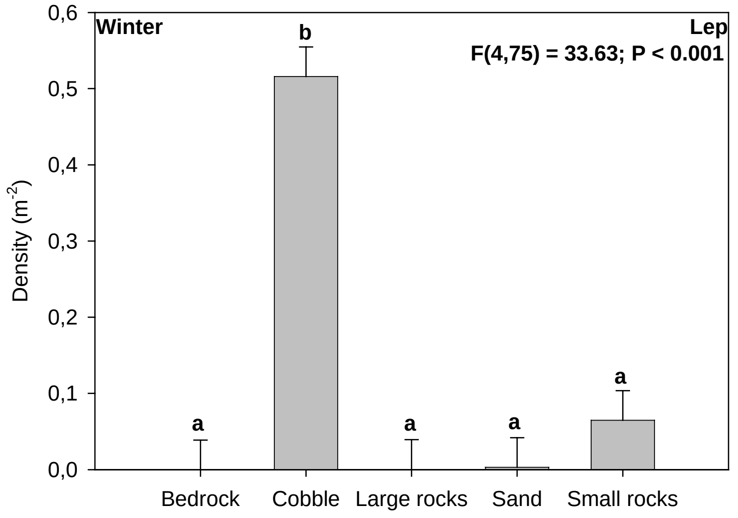

Spring
Gpag
F(4,75) = 13.82; P < 0.001
Density (m-2)
0,30
0,25
0,20
0,15
0,10
0,05
0,00
a
b
a
a
b
Bedrock
Cobble
Large rocks
Sand
Small rocks

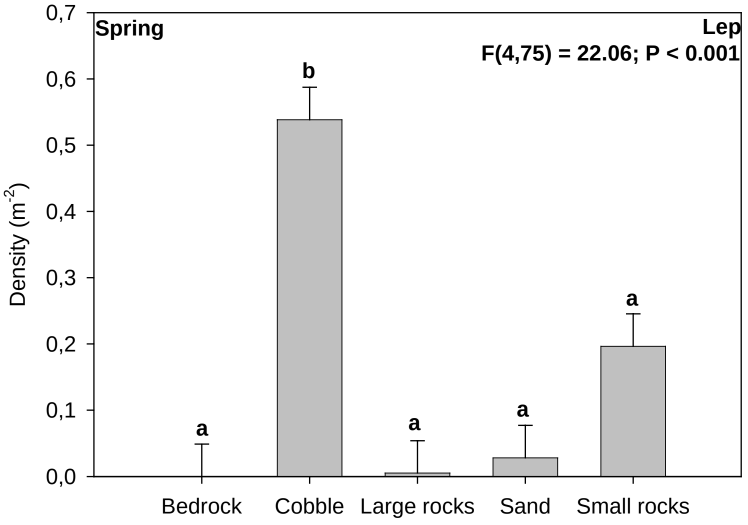

Autumn
Gpag
F(4,75) = 17.78; P < 0.001
Density (m-2)
0,35
0,30
0,25
0,20
0,15
0,10
0,05
0,00
a
a,b
a
a
b
Bedrock
Cobble
Large rocks
Sand
Small rocks

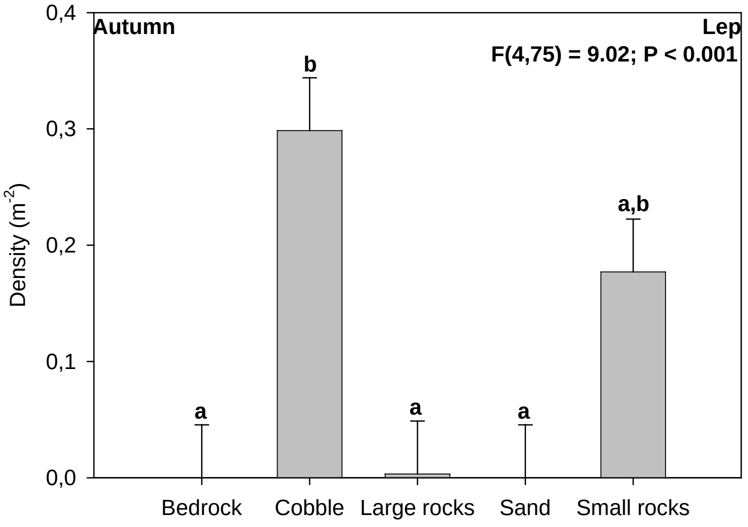

Winter
Lcan
F(4,75) = 10.27; P < 0.001
Density (m-2)
0,35
0,30
0,25
0,20
0,15
0,10
0,05
0,00
a
b
a
a
a,b
Bedrock
Cobble
Large rocks
Sand
Small rocks

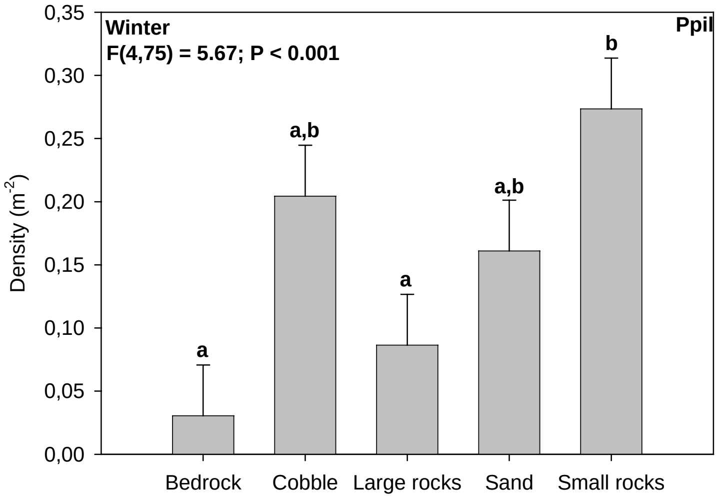

Spring
Lcan
F(4,75) = 6.20; P < 0.001
Density (m-2)
0,30
0,25
0,20
0,15
0,10
0,05
0,00
a
b
a
a
a,b
Bedrock
Cobble
Large rocks
Sand
Small rocks

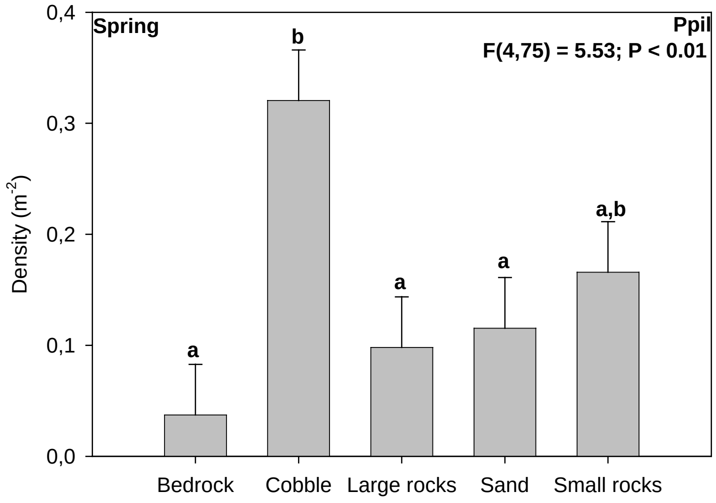

Autumn
F(4,75) = 12.98; P < 0.001
Lcan
Density (m-2)
0,25
0,20
0,15
0,10
0,05
0,00
a
a
a
a
b
Bedrock
Cobble
Large rocks
Sand
Small rocks

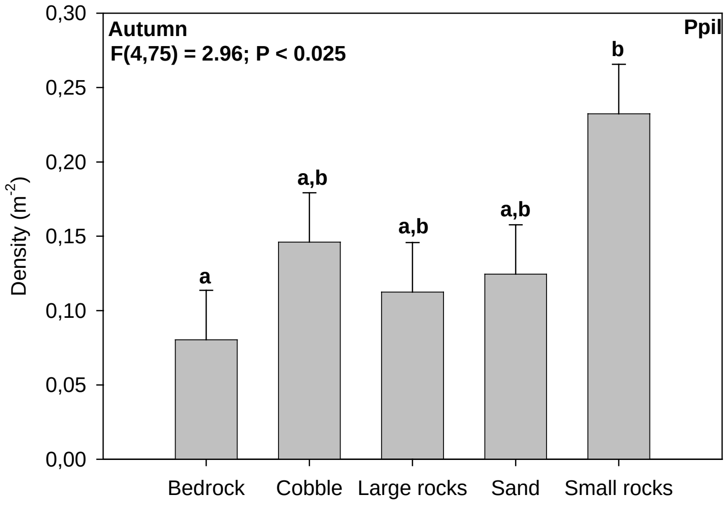

Winter
Lep
F(4,75) = 33.63; P < 0.001
Density (m-2)
0,0
0,1
0,2
0,3
0,4
0,5
0,6
a
b
a
a
a
Bedrock
Cobble
Large rocks
Sand
Small rocks

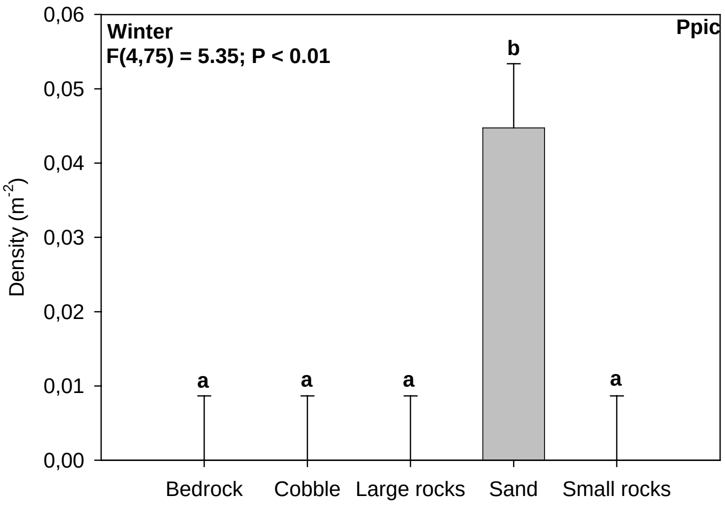

Spring
Lep
F(4,75) = 22.06; P < 0.001
Density (m-2)
0,0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
a
b
a
a
a
Bedrock
Cobble
Large rocks
Sand
Small rocks

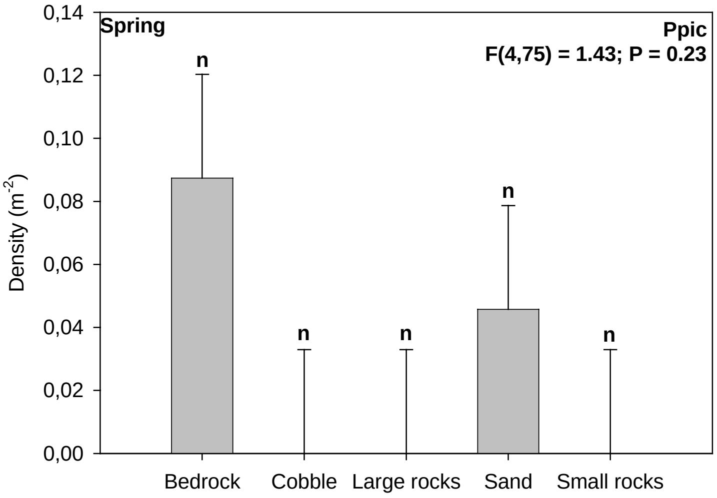

Autumn
Lep
F(4,75) = 9.02; P < 0.001
Density (m-2)
0,0
0,1
0,2
0,3
0,4
a
b
a
a
a,b
Bedrock
Cobble
Large rocks
Sand
Small rocks

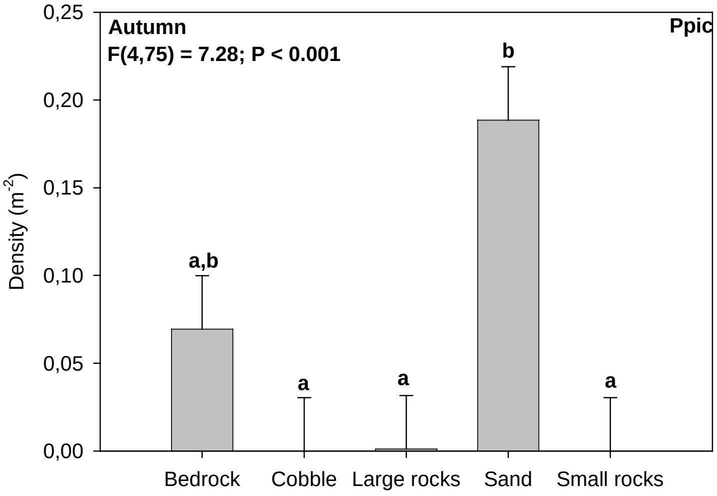

Winter
F(4,75) = 5.67; P < 0.001
Ppil
Density (m-2)
0,35
0,30
0,25
0,20
0,15
0,10
0,05
0,00
a
a,b
a
a,b
b
Bedrock
Cobble
Large rocks
Sand
Small rocks


Spring
Ppil
F(4,75) = 5.53; P < 0.01
Density (m-2)
0,4
0,3
0,2
0,1
0,0
a
b
a
a
a,b
Bedrock
Cobble
Large rocks
Sand
Small rocks


Autumn
F(4,75) = 2.96; P < 0.025
Ppil
Density (m-2)
0,30
0,25
0,20
0,15
0,10
0,05
0,00
a
a,b
a,b
a,b
b
Bedrock
Cobble
Large rocks
Sand
Small rocks


Winter
F(4,75) = 5.35; P < 0.01
Ppic
Density (m^{-2})
0,06
0,05
0,04
0,03
0,02
0,01
0,00
a
a
a
b
a
Bedrock
Cobble
Large rocks
Sand
Small rocks


Spring
Ppic
F(4,75) = 1.43; P = 0.23
Density (m^{-2})
0,14
0,12
0,10
0,08
0,06
0,04
0,02
0,00
n
n
n
n
n
Bedrock
Cobble
Large rocks
Sand
Small rocks


Autumn
F(4,75) = 7.28; P < 0.001
Ppic
Density (m^{-2})
0,25
0,20
0,15
0,10
0,05
0,00
a,b
a
a
b
a
Bedrock
Cobble
Large rocks
Sand
Small rocks

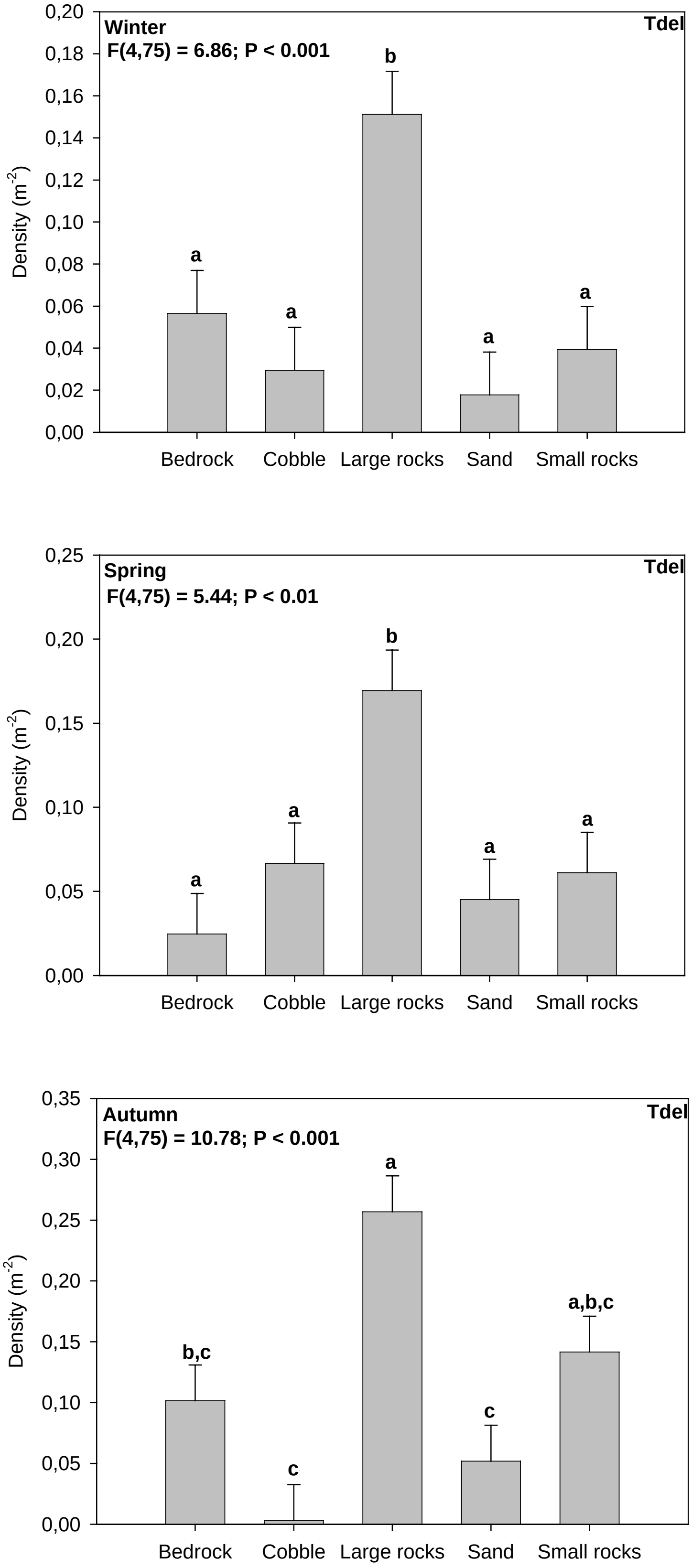


Fig. 4. Average density (column) and SE (whisker) for the nine most abundant species across habitats in the winter, spring and autumn. Species are in the top right hand corner

of each histogram (Gxan – *Gobius xanthocephalus*; Gcru – *Gobius cruentatus*; Gpag – *Gobius paganellus*; Lcan – *Lepadogaster candolii*; Llep – *Lepadogaster* spp.; Ppil – *Parablennius pilicornis*; Ppic – *Pomatoschistus pictus*; Tdel – *Tripterygion delaisi*). Results for comparison of densities across habitats are presented for each species at the top of each histogram and letters above the columns indicate post-hoc significant differences.

Discussion

The rocky subtidal in the Arrábida Marine Park is very diversified in comparison to other nearby locations. The overall diversity of cryptobenthic fishes (29 species in 11 families) is higher than what has been described for other nearby areas. In the Ciclopi Islands Marine Reserve, in Italy, La Mesa et al. (2004) detected 20 species in five families, whereas in north-eastern Spain Macpherson (1994) observed 17species in three families. The number of families found in each area is strikingly different. However, when comparing these results, one should take into account the definition of cryptobenthic fish followed and the method used. Even though Miller's definition of cryptobenthic fishes included solely species smaller than 10cm, most studies on temperate cryptobenthic species also include larger species such as the scorpaenidae (e.g. La Mesa et al. 2004). The method used in this study provided data for several species, which occur preferably or exclusively under cobble and small rocks (*Lepadogaster* spp., *Lepadogaster candolii*, *Gobius paganellus* and *Nerophis ophidion*), some of which were among the most abundant in this area.

The rate of common to rare species was similar to other studies in which a small group of numerical abundant species is common in these assemblages (e.g. Macpherson 1994, Willis 2001, Depczynski & Bellwood 2004, La Mesa et al. 2004). Total mean abundance (1.62 specimens/m^2) was higher than at other nearby locations: 0.87 specimensm^{-2} in the southern coast (La Mesa et al. 2004); 0.81 specimensm^{-2} (Macpherson 1994). This difference results, at least in part, from the method used in this study.

In this study we analysed the specific richness and density of a cryptobenthic fish assemblage across seasons and habitats. Across seasons no significant differences in richness or abundance were found. Overall density did not change seasonally even though there were some clear seasonal density changes for some of the species. This surprising lack of seasonal differences in total density is probably due to the fact that we were unable to sample during the summer when many local species recruit (Almada et al. 2000). In the strategy used, habitats that did not have any specimens were not used to calculate densities presented in table II. Therefore, the total density is lower than the sum of the densities for every species presented in that table.

Within each season, there were however significant differences in richness and abundance across habitats. These differences were however not consistent from season to season suggesting that there may be seasonal displacement of species across habitats. Furthermore, while one habitat may house a small number of individuals in one season it may gather the highest abundance in the other seasons, as was the case of the habitat cobble.

The distribution of the most abundant species by habitat was analysed to look at seasonal differences in habitat use. Among the nine most abundant species there were contrasting patterns in the utilization of habitat types. In fact, the analyses of the density variation by habitat, in each season, revealed the existence of two major categories of species. First, a specialist group used one or two of the available habitats, and can be further divided in sand dwelling gobies: *Gobius xanthocephalus*, *G. cruentatus*, *Pomatoschistus pictus*; associated with small rock and cobble there was a second group of species *G. paganellus*, *Lepadogaster* spp., *Lepadogaster candolii* and finally the large rock species *Tripterygion delaisi*. Secondly one generalist species, *Parablennius pilicornis*, used more than two habitats in every season. Similarly, in a coral reef cryptobenthic assemblage Depczynski & Bellwood (2004) found significant differences in abundance across more than two habitat types for a number of species, i.e. they identified a number of habitat specialists and generalists. Our results contrast to those of Greenfield and Johnson (1999) who found that tropical gobiidae had intermediate levels of habitat specificity while blenniidae (blenioidei) had higher levels. However, the majority of Blennidae studied by Macpherson (1994) used three habitat types while two gobies used fewer. Finally, Malavasi et al. (2005) found a group of gobies to be ubiquitous (the majority of species) across five habitat types. Nonetheless, these authors suggest a degree of spatial segregation within the goby assemblage. In the present study, some species were associated to different habitats from those described elsewhere. While our study shows that *G. cruentatus* is associated with sand, Wilkins & Myers (1992) described an association of the same species to boulders (rocks larger than 0.5m). At the Arrabida Marine Park, this species was found in the sand patches close to large rocks. The definition of each habitat type or the range of habitat types available at a given location may explain the differences between the two studies.

There seem to be several specialists and generalist species using the same habitat types which might suggest an overlap in habitat use and ecological niche. However, these species may be using particular niches separated in a smaller scale than the one used here. For example, our observations suggest that while *Gobius cruentatus* utilizes the sandy areas next to large rocks, *G. xanthocephalus* and *P. pictus* use the whole area of the sand patch (i.e. they also occur away from rocks). Similarly, while

Lepadogaster spp. dwell underneath cobbles, *Parablennius pilicornis* uses the space over the cobbles. Microhabitat types can play a crucial role in the abundance and distribution of cryptobenthic fishes (Syms 1995, Gonçalves et al. 1998, Willis & Andersen 2003, La Mesa et al. 2004). Spatial segregation at the microhabitat level need to be further investigated in order to have a more detailed idea about the fine-scale distribution of the cryptobenthic species. The spatial overlap for some of the species presented in this study may also be understood in light of diet, predation, temporal activity and interspecific competition (Costello 1992).

Even though we did not test specifically for seasonal changes in mean abundance across habitats, we observed clear seasonal variation in the abundance of some species. Similarly seasonal changes in density across habitats have been observed in other temperate gobies (Malavasi et al. 2005). Some more pronounced variations in density across habitats and seasons led us to compare the sizes (TL) of the specimens associated to different habitats. In particular, the recruits of those species could be responsible for the observed variation. However, we could not detect significant differences in size for two of the species that underwent higher abundance per habitat changes (*Gobius paganellus* and *Lepadogaster candolii*). There are several possible explanations for this result. On the one hand, both of these species also occur in the intertidal in the spawning season (Briggs 1990, Faria & Almada 1995) and may therefore have been underestimated in the subtidal. Other species may also occur in the intertidal (e.g. *G. paganellus;* Maze 2004). In fact they may be intertidal secondary residents, i.e. subtidal species that reside as juveniles in the intertidal for varying lengths of time and then as adults may enter the subtidal habitat for breeding or foraging (Horn & Martin 2006). On the other hand, we may have underestimated the number of juveniles in particular microhabitats. Specific habitat patches, such as gravel, need to be sampled using other techniques, such as anaesthetics, in order to find out whether these species are using them as recruits. Nonetheless, larger *G. xanthocephalus* specimens were found in the sand and smaller specimens were found in small rock and large rock habitats during the autumn season. However, we cannot exclude the possibility that this species recruits to the adult's habitats as well just by comparing the size across habitats. If this species uses the same habitat as the adults and if the recruits suffer higher mortality in that habitat, then the average size would not be significantly different from the other habitats. This has already been observed for other cryptobenthic species (Connell & Jones 1991). The presence of conspecific adults may play a significant part in the abundance of settlers (Macpherson & Zyka 1999). Further studies are needed to clearly identify which habitats recruits use.

In this study a modified visual sampling technique was applied to a cryptobenthic fish assemblage that returned higher overall abundance values than other studies in nearby geographic areas. No seasonal differences between species richness and abundance were detected across habitats at the assemblage level. Clear differences in the abundances and richness across habitats were detected within seasons. Among the most abundant species habitat utilization was variable but a large group of specialist species was identified. Seasonal abundance shifts in habitat utilization for a number of species are reported and ontogenetic habitat shifts associated to recruitment, for the most abundant species, is described. However, the possibility that species use other habitats as recruitment habitats cannot be ruled out.

Ackowledgements

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Composition and temporal dynamics of a temperate rocky cryptobenthic fish assemblage

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Abstract

Temporal variation in a temperate cryptobenthic fish assemblage at the Arrábida Marine Park (Portugal) was assessed by visual surveys during 2002 and 2003. A total of 9596 fishes from 11 families and 30 species were recorded. There were no changes in structure or density at the assemblage level between years, whereas diversity changed significantly due to a higher number of abundant species in the second year. A similar seasonal trend was found between years, with a significant overall density increase in autumn. This is partially explained by the arrival of new recruits of some of the most abundant species in the assemblage. Assemblage diversity and structure also changed across seasons. A group of species encompassing *Gobius xanthocephalus*, *Tripterygion delaisi*, *Parablennius pilicornis*, *Gobius paganellus*, *Lepadogaster candolii* and *Lepadogaster* spp. were analysed in detail. The temporal patterns of two of the most abundant species, *G. xanthocephalus* and *T. delaisi*, mimicked the overall temporal patterns of the assemblage. We suggest that the inter-annual stability in density of this subtidal fish assemblage may be similar to what has been reported for the intertidal and that strong post-settlement processes are probably shaping this assemblage.

Introduction

Fishes inhabiting the littoral rocky shores are not only valuable elements of coastal biodiversity, but they also exert an important ecological role in the functioning of littoral ecosystems (La Mesa et al. 2004). The ecological importance of cryptobenthic fishes, as energy mediators (Depczynski & Bellwood 2003), justifies an increased effort aimed at a deeper understanding of this overlooked component of the rocky coast fish assemblages.

In the North Atlantic and Mediterranean several studies have been done on rocky coastal fish assemblages (Jansson et al. 1985; Minchin 1987; Miniconi et al. 1990; Falcon et al. 1993; MacPherson 1994; Reñones et al. 1997; La Mesa & Vacchi 1999; Gonçalves et al. 2002; Magill & Sayer 2002; La Mesa et al. 2004; Ordines et al. 2005). Most of these studies used traditional visual census methods to assess the whole fish assemblage, including cryptobenthic fishes. These are "small bodied fishes (<10 cm) that exploit restricted habitats where food and shelter are obtained in, or in relation to, conditions of substrate complexity and/or restricted living space, with a physical barrier likely to be interposed between the small fish and sympatric predators" (cf. Miller 1979).

Limitations to visual sampling of cryptobenthic fishes have been recognised and tested by several authors (e.g. Harmelin-Vivien et al. 1985; Willis 2001). Many community studies have either excluded these species (Anderson & Millar 2004; García-Charton et al. 2004) or sampled them using traditional visual census techniques (e.g. Ilich & Kotrschall 1990; La Mesa & Vacchi 1999; La Mesa et al. 2004). Disregarding or miss-sampling the small specimens in a community has, however, the potential to change any theoretical conclusions based on the observed patterns

(Blackburn & Gaston 1996) due to the important role they can have on the overall community dynamics and functioning.

Studies on the temporal dynamics of coastal fish assemblages in temperate areas have shown strong seasonal and inter-annual variations in density for some species (Janson et al. 1985; Costello 1992; Wilkins & Myers 1992; Magill & Sayer 2002). Temporal dynamics of fish populations have broadly been related to the input of recruits (e.g. Doherty & Williams 1988; Cushing 1995) and to post-settlement mortality processes that may reshape initial settlement patterns (e.g. Forrester 1995; Macpherson & Zika 1999). Observational studies at the assemblage level can assess inter-specific variation and also provide new insights into the variables affecting the temporal dynamics of coastal assemblages.

The objectives of this study were to: 1. Describe the cryptobenthic fish assemblage occurring on the rocky shore of the Arrábida Marine Park; 2. Analyse the temporal diversity, density and structure variation of this assemblage.

Materials and Methods

Sampling location

This study was carried out in the Arrábida Marine Park (Portugal), from January 2002 to December 2003. The Arrábida Marine Park was created in 1998 but management and protection measures were only approved in 2005. Two sites, presently part of a full protection area, were sampled (Fig. 1) in the sector identified as having the highest biodiversity (Gonçalves et al. 2002). In this Marine Park, the underwater rocky habitats are highly heterogeneous and partially derive from the disintegration of the calcareous cliffs that border the coastline. The underwater rocky bottom extends offshore for several tens of metres and is composed of mixed patches of sand, gravel, cobbles, small rocks, large rocks and bedrock.

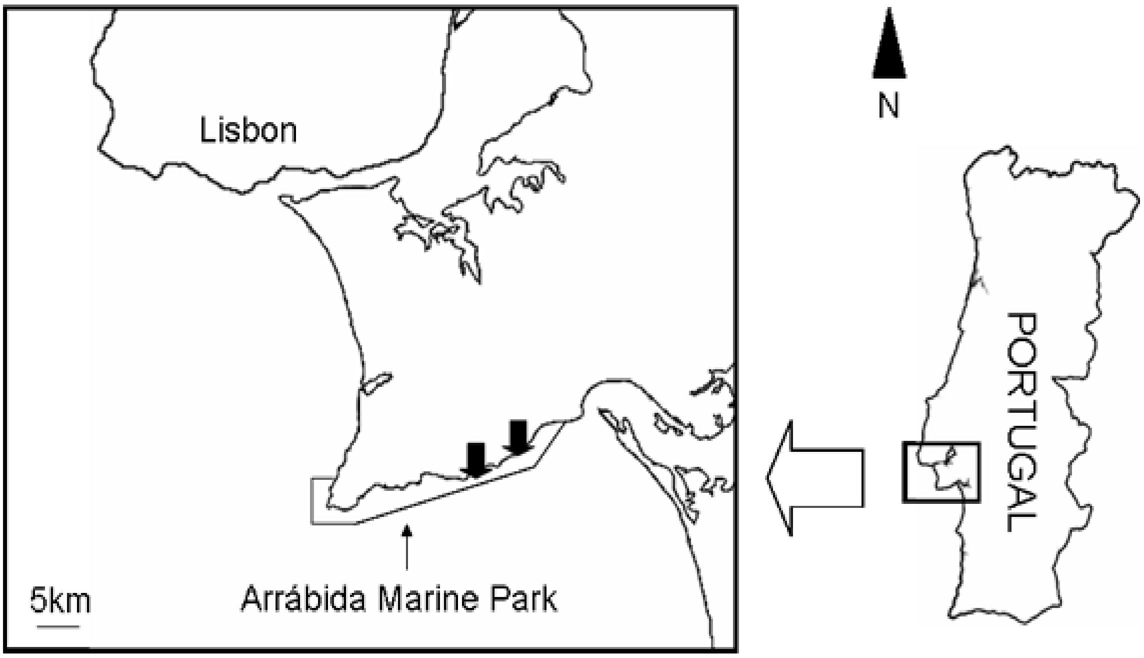


Fig. 1. Arrábida Marine Park location on the Portuguese coast and sampling sites (black arrows).

Data collection

To assess seasonal variation in diversity and abundance of the cryptobenthic fish assemblage, sampling activities were carried out in the winter (January-February), spring (April-May) and autumn (October-November). A total 91 h in 87 visual counts were performed (Table I). In the summer months, sampling was not possible due to the occurrence of fast growing macroalgae (*Cystoseira usneoides*, *Sacchorhiza polyshides* and *Laminaria digitata*). These algae cover the substrate in very high densities making any visually-based census method impracticable. Censuses were performed in the morning between 10-12:30h, except in one occasion when the census was performed at 17h. Strip transects were laid perpendicularly to the coastline, from the deeper rocky area (mean depth = 9.5 m; SD = 1.6) to the lower limit of the intertidal (mean depth = 2.5 m; SD = 0.8). Overall the average length of transects was 63.9m (SD = 9.8, range = 40 - 87.3 m) and width of 1m were surveyed. Transects were laid in the same areas in the different seasons to assure that the same proportions of habitat types were sampled in the different seasons. Water temperature, measured at the beginning of each census, was significantly higher in 2002 (Mean=16.42ºC, SD=1.48ºC) than in 2003 (Mean=15.08ºC, SD=0.24ºC) (One-way ANOVA: F=14.13, p<0.001).

Table I. Time and number of census performed in each sampling period. Census correspond to strip transects laid perpendicularly to the coastline (see Materials and Methods).

Year	**Season**	**Time (min)**	**Census (N)**
2002	Winter	940	12
	Spring	897	10
	Autumn	919	16
2003	Winter	913	17
	Spring	887	17
	Autumn	888	15

Each cryptobenthic fish inside the transects was recorded, and its total length (TL) visually estimated. We followed Miller's (1979) definition of cryptobenthic fish but included species larger than 10 cm. All fishes were easily identified according to distinct morphological and coloration characteristics except for *Lepadogaster lepadogaster* and *L. purpurea*, due to the difficulty in distinguishing them in the field (Henriques et al. 2002). These species were recorded as *Lepadogaster* spp.

Considering the importance of training in visual size estimation of small specimens (Edgar et al. 2004), we performed visual estimation tests prior to every sampling season. Correlations between estimated and real values were always higher than 0.90. The visual census technique used was an interference technique which involved disturbing certain microhabitats, like sand or gravel, and dismantling others, like small rocks or cobble, to look for fish in any accessible hideout that might be occupied (Beldade & Gonçalves in press). The visual counts performed are a modification of the timed counts used by Syms (1995). Each census was of variable duration given that length of transects and complexity of habitats was variable. Minimum sampling time per season was established on the basis of a cumulative number of species by time curve (with an asymptote at 90% of the number of species) in 2002. The time necessary to sample different microhabitat types varied, but density by time and by area were highly correlated in the 49 out of 87 censuses where both area and duration were recorded ($r = 0.934$, $p < 0.001$). All density values are expressed as number of fish.min^{-1}.

Data analysis

Assemblage composition and dynamics

Fish assemblage heterogeneity was specified by calculating species richness, diversity (Shannon-Wiener index) and evenness (Zar 1986). A two-way nested ANOVA was used to assess yearly and seasonal variations in the above mentioned assemblage parameters and in overall density (specimens.min^{-1}). Post-hoc tests were used to find out where seasonal differences lay. Density data were transformed following a $\log(x + 1)$ function to meet parametric assumptions (Zar 1986).

Assemblage structure dynamics

Multivariate analyses were used to assess yearly and seasonal differences in assemblage structure using the PRIMER software package (Clark & Warwick 2001). An overall matrix was built to assess yearly differences, and two other matrices, one for each year, were built to assess differences among seasons. The original density matrices of samples by species were transformed into a Bray-Curtis similarity matrix. Based on the relative abundance of each species, non-metric multidimensional scaling (MDS) diagrams were used to graphically display the inter-relationships among samples. In each plot, samples that are closer together are more similar to each other. Stress values smaller than 0.15 were considered a good portrayal of data (Clark 1993).

To test for differences between years and seasons, multivariate analysis of similarity (ANOSIM) was used to identify differences in assemblage groupings (Clark & Warwick 2001). Similarity percentages analysis (SIMPER) was used to identify the main taxa responsible for the yearly and seasonal groupings, assuming a cut-off at 90 %. Clarke & Warwick (2001) classified species for which the ratio similarity/SD is large as good discriminators or "typical" species, given that they consistently contribute to the similarity between groups. We use the term typical species to refer to cases where the similarity/SD value is higher than 1.86 (*sensu* Clarke & Warwick 2001).

Temporal variation in fish density

For each dominant species we used non-parametric statistical techniques to assess density differences between years (Mann-Whitney U Test) and seasons in each year (Kruskal-Wallis ANOVA) given that parametric assumptions could not be met. Dunn's post-hoc tests were used to find out were differences lay (Zar 1986) Density patterns for the different size classes were also analysed for each season in each year.

Results

Assemblage composition and diversity

A total of 9596 fishes from 11 families and 30 species were recorded (Table II). The assemblage was numerically dominated by seven species (*Gobius xanthocephalus*, *Gobius paganellus*, *Parablennius pilicornis*, *Tripterygion delaisi, Lepadogaster* spp. and *Lepadogaster candolii*) that comprised 90 % of counts, with 73 % of the species common to both years. Five species were only recorded in 2003 and one in 2002. An interesting result was the abundance rank change observed between years in *P. pilicornis*, which fell from the most abundant in 2002 to the third place in 2003. This change was accompanied by an increase in the number of other species such as *G. paganellus¸ Lepadogaster* spp. and *L. candolii*.

Species richness and density did not differ between years, whereas the diversity indexes were significantly higher in 2003, owing to changes in abundance of the main species (Tables II and III). In 2002, there were no differences in richness and Shannon index across seasons, but evenness was significantly higher in the autumn and density was significantly different across all seasons (Table IV; Fig. 2). In 2003, species richness did not change across seasons but significant differences separated the autumn from the other seasons for the Shannon index, evenness and density (Table IV and Fig. 2).

Table II. Number of specimens of the rocky cryptobenthic assemblage recorded for each species in each season (W winter, S spring, A autumn) in 2002 and 2003, at the Arrábida Marine Park.

Family	**Species**	**2002**			**2003**			
		W	**S**	**A**	**W**	**S**	**A**	**Total**
Blenniidae	*Parablennius pilicornis*	535	419	464	307	278	338	2341
	Parablennius gattorugine	18	16	66	28	29	27	184
	Lipophrys pholis					1		1
	Parablennius ruber						1	1
	Parablennius sanguinolentus					1		1
Callyonimidae	*Callionymus reticulatus*	1	1	24	5	3	6	40
Congridae	*Conger conger*				1	1		2
Gadidae	*Gaidropsarus mediterraneus*	1	4	9	2	7	1	24
	Gaidropsarus vulgaris				1	1	3	5
Gobiesocidae	*Lepadogaster* spp.	34	35	88	109	147	119	532
	Lepadogaster candolii	37	28	74	87	54	73	353
	Apletodon dentatus		3			4	4	11
	Diplecogaster bimaculata		3				2	5
Gobiidae	*Gobius xanthocephalus*	365	231	776	303	270	921	2866
	Gobius paganellus	41	32	136	103	99	113	524
	Gobius cruentatus	37	30	55	31	50	57	260
	Pomatoschistus pictus	16	5	68	15	32	89	225
	Thorogobius ephippiatus	2	1			2	4	9
	Chromogobius britoi						2	2
	Gobius cobitis		1					1
Muraenidae	*Muraena helena*	3	3	4	4	3	5	22
Scophthalmidae	*Phrynorhombus regius*	1		2	1	2		6
	Zeugopterus punctatus		1			1		2
Scorpaenidae	*Scorpaena notata*	21	3	10	3	4	3	44
	Scorpaena porcus	5	4	6	6	4	3	28
Syngnathidae	*Syngnathus acus*			3	10	3	8	24
	Nerophis lumbriciformis		1	1	9	7	4	22
	Entelurus aequoreus		1	4		1		6
Tripterygiidae	*Tripterygion delaisi*	264	209	395	280	310	597	2055

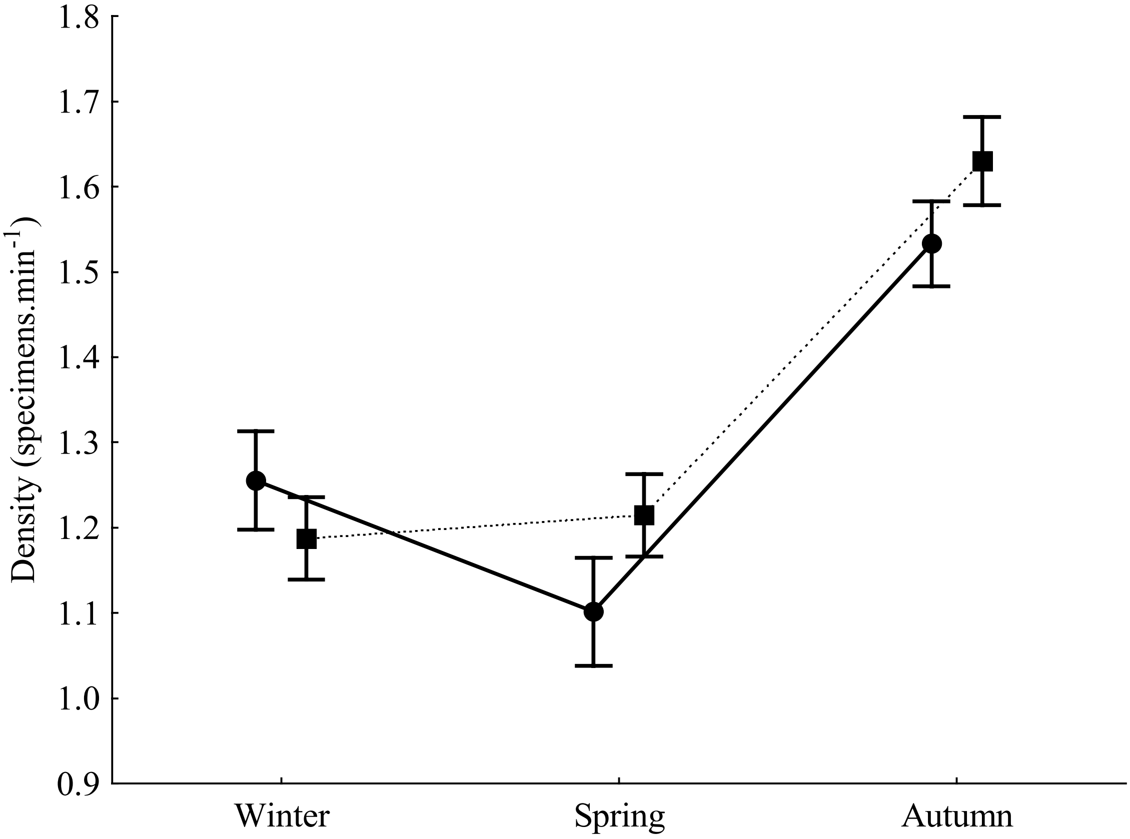


Fig. 2. Density (specimens.min^{-1}) values for the whole assemblage in the three sampling periods in 2002 (circles) and 2003 (squares). Vertical bars denote 0.95 confidence limits.

Table III. Two-way nested ANOVA results for species richness, Shannon diversity index, evenness index and density between years and across seasons within years.

Species Richness				
	df	MS	F	P
Year	1	0.199	0.093	0.093
Season(Year)	4	3.480	1.632	0.174
Error	81	2.132		

Table III. (continued) Two-way nested ANOVA results for species richness, Shannon diversity index, evenness index and density between years and across seasons within years.

Shannon index				
Year	1	0.071	21.08	< 0.001
Season(Year)	4	0.035	10.50	< 0.001
Error	81	0.003		
Eveness				
Year	1	0.483	26.00	< 0.001
Season(Year)	4	0.211	11.37	< 0.001
Error	81	0.018		
Density				
Year	1	0.008	46.24	0.055
Season(Year)	4	0.133	79.19	< 0.001
Error	81	0.002		

Table IV. Post-hoc comparisons for the Shannon diversity index, evenness index and density across seasons within each year.

Year	Season	Shannon	Evenness	Density
2002	winter-spring	n.s.	n.s.	P < 0.01
	autumn-winter	n.s.	P < 0.001	P < 0.001
	autumn-spring	n.s.	P < 0.05	P < 0.001
2003	winter-spring	n.s.	n.s.	n.s.
	autumn-winter	P < 0.001	P < 0.001	P < 0.001
	autumn-spring	P < 0.001	P < 0.001	P < 0.001

Assemblage structure dynamics

Assemblage structure changed both between years and across seasons. The non-metric MDS plot showed some degree of segregation among samples according to year (Fig. 3), which was confirmed by the ANOSIM results (global R value of 0.36 and $p < 0.001$). There was a clear separation of samples by season in each year mainly due to the autumn samples (Figs. 4A and B). Both in 2002 and 2003, significant differences among seasons in the assemblage composition were detected (2002: $R = 0.53$, $p < 0.001$; 2003: $R = 0.55$, $p < 0.001$). Pairwise comparisons showed that the autumn was significantly different from the other seasons in both years.

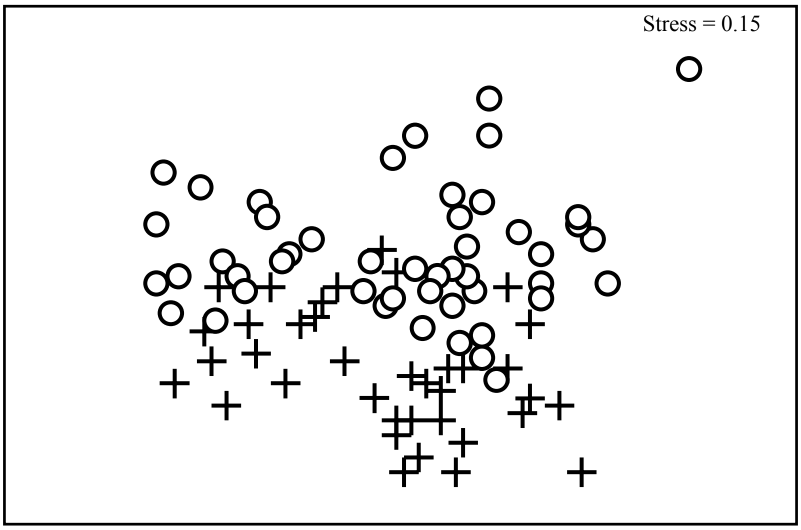


Fig. 3. MDS ordination showing differences between the cryptobenthic fish assemblage in each year. Each individual point represents a replicate sample (census). Circles: 2003; Crosses: 2002.

SIMPER results showed that in the yearly comparison, *Parablennius pilicornis*, *Gobius xanthocephalus*, *Tripterygion delaisi* and *Gobius paganellus* were considered as typical species (see Materials and Methods). In the seasonal analysis, typical species were: *P. pilicornis* and *G. xanthocephalus* in all seasons; *T. delaisi* in every season except in the spring 2002; *G. paganellus* in the winter 2002 and in all seasons in 2003; *Lepadogaster candolii* in the winter 2003; and *Lepadogaster* spp. in the spring 2003.

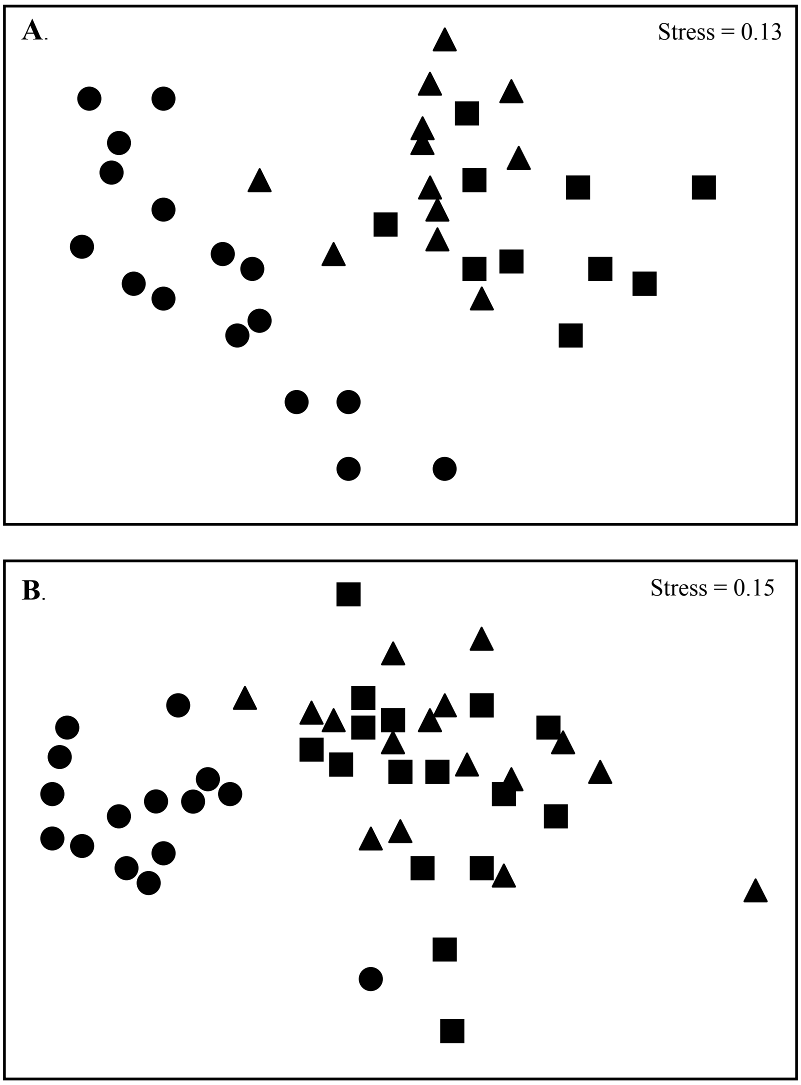


Fig. 4. MDS ordinations showing seasonal differences in 2002 (A) and 2003 (B). Each individual point represents a replicate sample (census). Triangles: winter; squares: spring; circles: autumn.

Temporal variation in fish density

Density variations of the typical species showed three different temporal patterns between years: 1) a significant density increase; 2) decrease; or 3) no significant density change. These species can additionally be grouped into three different categories of seasonal patterns: 1) a clear density increase in the autumn in both years; 2) a density increase in the autumn only in one year; or 3) other seasonal pattern.

Densities did not change significantly between years for *Gobius xanthocephalus* and *Tripterygion delaisi*, but there were significant seasonal changes for these species (Table V). In the autumn, significantly higher densities were recorded in both years (Table VI). The density of small individuals of *G. xanthocephalus* clearly increased in the autumn of the two years, whereas small *T. delaisi* only increased in the autumn of 2002 (Figs. 5A and B).

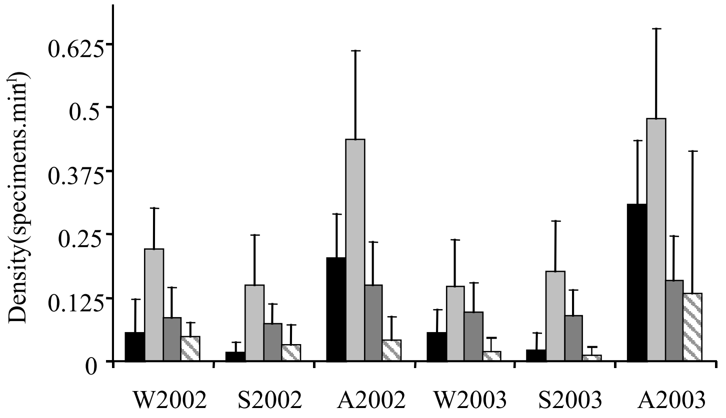
Table V. Density variations between years and across seasons within each year for the most abundant species. (Z) Mann-Whitney U test; (H) Kruskal-Wallis ANOVA.

	Yearly changes		Seasonal changes (2002)		Seasonal changes (2003)	
	Z	P	H	P	H	P
G. xanthocephalus	0.80	0.426	17.61	< 0.001	27.41	< 0.001
T. delaisi	1.75	0.08	18.01	< 0.001	24.05	< 0.001
P. pilicornis	6.11	<0.001	5.85	0.054	3.23	0.199
G. paganellus	2.98	<0.001	17.53	< 0.001	0.36	0.837
L. candolii	2.96	<0.001	5.45	0.066	8.67	<0.05
Lepadogaster spp.	3.51	<0.001				

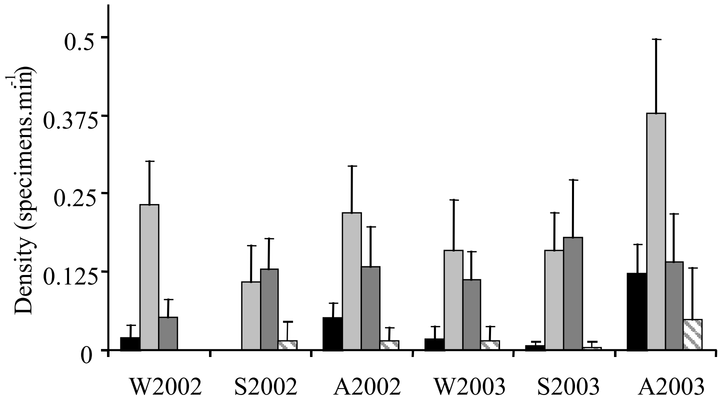
Table 6. Dunn's test results for the post-hoc comparisons of the seasonal density variation for the most abundant species. W winter; S spring, Aautumn.

Year	2002			2003		
Season comparison	W-S	A-W	A-S	W-S	A-W	A-S
G. xanthocephalus	n.s.	$P < 0.05$	$P < 0.001$	n.s.	$P < 0.001$	$P < 0.001$
T. delaisi	n.s.	$P < 0.05$	$P < 0.001$	n.s.	$P < 0.001$	$P < 0.001$
G. paganellus	n.s.	$P < 0.05$	$P < 0.001$	n.s.	n.s.	n.s.
L. candolii	n.s.	n.s.	n.s.	$P < 0.05$	n.s.	n.s.

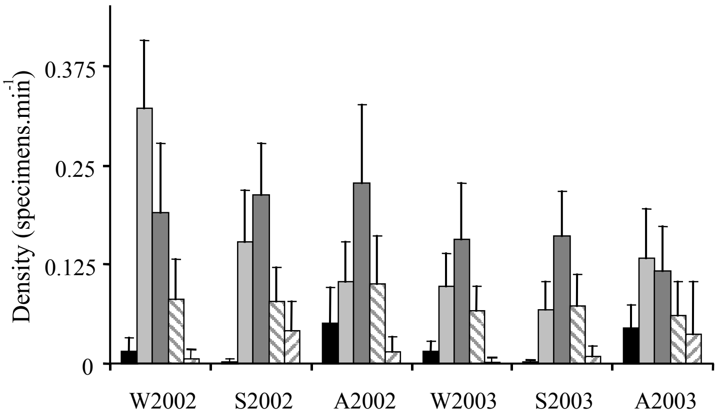
A. *Gobius xanthocephalus*



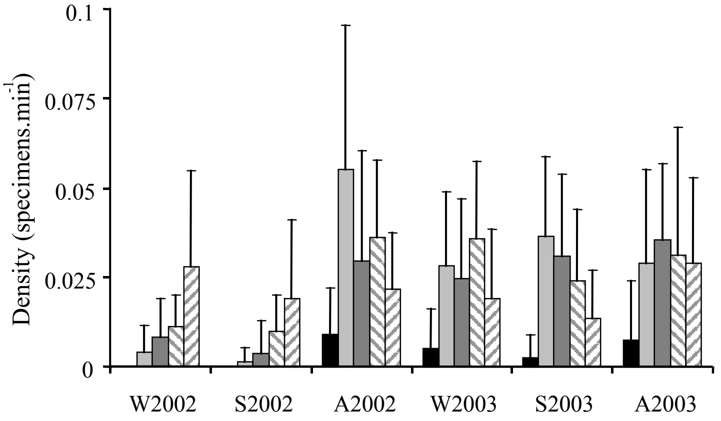
B. *Tripterygion delaisi*



C. *Parablennius pilicornis*



D. *Gobius paganellus*



E. *Lepadogaster candolii*

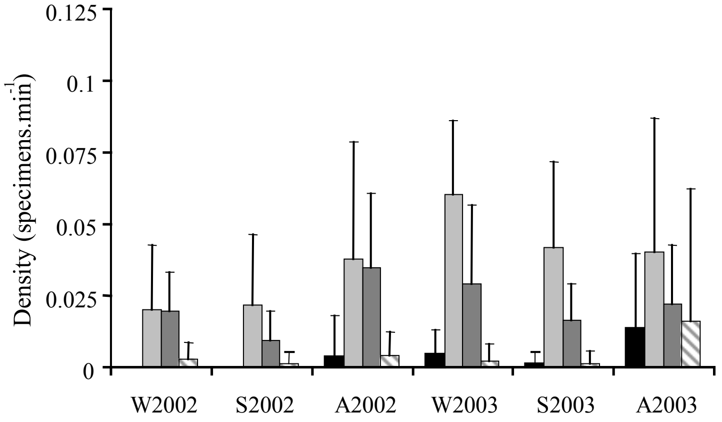


Fig 5. Two cm size class density variation (Mean ± SD) for A. *G. xanthocephalus*, B. *T. delaisi*, C. *P. pilicornis,* D. *G. paganellus* and E. *L. candolii* in each season for both sampled years. Only individuals larger than 2cm were included in the histogram. (2-3cm) black bars; (4-5cm) light grey bars; (6-7) dark grey and (8-9cm; 10-12cm) crossed bar. Years: 2002 and 2003; Seasons: W winter, S spring; A autumn

Parablennius pilicornis presented significant annual density variations, with lower values in 2003, but no seasonal variations (Table 5). Small specimens were more abundant in the winter in 2002 and in the autumn in 2003 (Fig. 5C).

Although there was a density increase in 2003 for *Gobius paganellus* and *Lepadogaster candolii* (Table 5), their seasonal patterns differed. There were significant seasonal changes in 2002 for *G. paganellus* but not for *L. candolii*. In 2003 this pattern reversed (Tables 5 and 6). The increase in density for *G. paganellus* in the autumn 2002 coincided with an increase in the number of small individuals (Figs. 5D and E). For *L. candolii*, there was a decrease in 2003 from the winter to the spring (Table 6).

Discussion

As in other studies on temperate cryptobenthic fishes (Willis 2001; La Mesa et al. 2004), relatively few taxa were numerically dominant in our assemblage. It was noteworthy the abundance of clingfishes (*Lepadogaster* spp. and *Lepadogaster candolii*) especially in comparison with data collected elsewhere (Falcón et al. 1993; Reñones et al. 1997; La Mesa & Vacchi 1999; Magill & Sayer 2002; La Mesa et al. 2004). This outcome was probably derived from the sampling technique used here. More reliable results on the abundance of clingfishes, which occur preferably under cobbles and rocks (Hofrichter & Patzner 2000; Henriques et al. 2002) and can be missed by traditional visual sampling techniques (Willis 2001), were likely ensured by habitat dismantling. Furthermore our sampling technique has a very transient impact on the environment and is therefore highly suited for temporal dynamics studies.

Species richness did not change significantly between years. However, significantly higher diversity indexes were recorded in the second year due to the higher number of individuals of abundant species, like *Lepadogaster* spp., *Lepadogaster candolii* and *Gobius paganellus*. Even though overall density was similar between years, there were significant increases (*G. paganellus*, *L. candolii* and *Lepadogaster* spp.) and decreases (*P. pilicornis*) among the most abundant species. Multivariate analyses did not showed strong differences between years and the same typical species (sensu Clark & Warwick 2001) were identified in each year (except for *G. paganellus*) in spite of the density variations between years.

The constant overall density values observed are in accordance with what has been described for the highly resilient and stable intertidal fish communities (Almada & Faria 2004). However, other studies on coastal subtidal fish assemblages (encompassing both pelagic and cryptobenthic species) have found strong seasonal density fluctuations (Magill & Sayer 2002). These density variations are probably more related to changes in pelagic species, which have strong inter-annual density fluctuations (Fogarty et al. 1991), than to variations in cryptobenthic fish assemblages. However, more studies are needed to determine the relative contribution of cryptobenthic species to the overall variation observed in fish coastal assemblages. Only two years of data have been analysed in the present work and results need therefore to be interpreted with caution.

Species richness did not change seasonally within each year. However in 2002, the higher number of abundant species in the autumn originated higher diversity indexes. These results contrast with the patterns described by La Mesa & Vacchi (1999) who found no seasonal effect on the same diversity indexes for a coastal fish community in Ustica Island Marine Reserve. In the present study, over two years, the density of the whole assemblage showed the same seasonal pattern in which density peaked in the autumn and was the lowest in the spring. Similar seasonal trends have been described for several other fish species in temperate regions (Wilkins & Myers 1992). Strong seasonal patterns are also commonly found in intertidal fishes (Almada & Faria 2004).

We could not sample the assemblage during the summer months. However, many of the cryptobenthic species spawn during the spring and summer and probably start to settle at the end of summer. Given that changes in density can be very sharp and concentrated in time, for example, Connell & Jones (1991) recorded a decrease of 60% within the first month of recruitment; fluctuations may be greater than the ones described here. Multivariate analyses clearly separated the autumn from the other seasons in each year. For *G. xanthocephalus*, *T. delaisi* and *G. paganellus*, the increases in density in the autumn coincided with an increase in the density of small individuals. Therefore, these fluctuations seem to be related with recruitment in these species. The smaller *G. paganellus* were absent from our counts, which could be explained by their known recruitment to the intertidal (Faria & Almada 1999).

The linkage between seasonal fluctuations in density and higher number of recruits, suggests that strong post-settlement processes are shaping the cryptobenthic assemblage. It is possible to speculate that population densities may be limited and regulated by post-settlement processes rather than by the input of settlers (Steele, 1997; Macphersen & Zika 1999). For instance, Depczynski & Bellwood (2005) have recently found high mortality rates for a cryptobenthic coral reef fish (the pygmy goby *Eviota sigillata*), a species with the shortest recorded lifespan among vertebrates. Besides the role as trophic links between lower and higher order predators that cryptobenthic fishes have, the high fluctuations in density across seasons observed for most species, implies a high-energy turnover that will certainly have a significant impact on the local coastal community (Depczynski & Bellwood 2003).

The implications of these results are relevant not only for understanding the dynamics of an often overlooked component of temperate reef fish populations, but also for biodiversity management of the Marine Park. Understanding the structure, composition and temporal variation of these assemblages and decoupling this natural variation from that derived from the protection measures applied, is a central aspect for management. This study preceded the approval of the Arrábida Marine Park legislation and may therefore be used in the future for comparative purposes.

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General discussion

Throughout their larval and benthic life-stages, cryptobenthic fishes display particular characteristics that influence the composition and diversity dynamics of these assemblages. Only through the study of the different life stages is possible to understand the population dynamics of these species. During the relatively short periods in the plankton as larvae, many cryptobenthic fishes are able to remain close to shore in coastal shallow subtidal areas. The newly settled juveniles may occur in particular low-abundance, high complexity habitats. As juveniles and adults, cryptobenthic fishes may be habitat specialists, using one or two habitat types, while others are generalists and use all types available. Furthermore, habitat use can change seasonally across habitat. These changes may be related either to migrations between habitats or by the arrival of young specimens from the plankton. Nonetheless, the temporal fluctuations in density revealed a constant trend for most of the cryptobenthic species.

Through sampling of all habitats during a two-year period a consistent seasonal trend in the density was observed. A significant density increase in the autumn, related to recruitment, was followed by a decrease in the following winter and spring. This pattern suggests that post-settlement processes shape this assemblage and that populations are not recruitment limited.

Each of the ontogenetic stages studied provided new information on the ecology of these fishes. Larvae of several cryptobenthic fishes like *Pomatoschistus pictus*, *Gobius xanthocephalus*, *Tripterygion delaisi*, as well as other coastal fish species were captured. Other studies that have sampled coastal waters have also found high abundances of coastal species in the nearshore (e.g. Brogan 1994; Sabatés et al. 2003; Vélez et al. 2005). Cryptobenthic species can be a major component of nearshore collections. Close to the substrate we found mostly late-stage larvae. This result generally agrees with previous observations of late-stage larvae close to the substrate (e.g. Breitburg et al. 1995). In fact it is not only the late larval stages that occur close to the bottom, but also the entire size range is found at shallow depths close to shore. Similar results were described for several fish species, called lagoon completers, in tropical lagoons (Leis 1991), and also for a few temperate species (Beyst et al. 1999). Marliave (1986) suggested that larvae of intertidal fishes might be able to reduce offshore dispersal by remaining close to high relief substrates, and avoiding areas with laminar currents. This may also be the case of some cryptobenthic species e.g. *P. pictus*, *G. xanthocephalus* (Beldade et al. submitted 1).

There are very significant implications for these results in the sense that they extend the findings of Swearer et al. (1999) and Jones et al. (1999), who estimated a relatively high percentage of larvae return to natal locations after spending some time in the plankton. At the Arrábida Marine Park, we found that a number of larvae of some cryptobenthic fishes may not be dispersing offshore at all. The implications of these findings to planning future conservation strategies are substantial. If some cryptobenthic species spend their larval phase close to shore, than conservation strategies need to be adjusted and should aim at specifically protecting these areas to insure that there is sufficient input to replenish local populations. Alternatively the conservation measures could protect certain areas at certain times to ensure sufficient recruitment. The use of new sampling techniques such as light traps or diver steered plankton tows will aid in nearshore sampling and may provide further insight into the spatial distribution of cryptobenthic fish larvae. The study of larval behaviors and the ontogeny of larval abilities may also provide essential information to understand the dispersal or lack there of in cryptobenthic fishes.

The dispersal period during which larvae are planktonic was assessed through the settlement marks on the otoliths of ten cryptobenthic fish species. The most common settlement mark detected was an abrupt single increment mark. Overall, the cryptobenthic fish's larval duration is not much different from that of epi- or nektobenthic coastal species (sensu Miller 1979) presented in Raventós & Macpherson (2001). However, the clingfishes (Gobiesocidae) have slightly shorter pelagic larval durations than the gobies and the blenny studied (Beldade et al. submitted 2). Even among the cryptobenthic species there is considerable variation in the average duration in the plankton. The assessment of the duration of life in the plankton by the identification of the settlement marks provides information about the dispersal potential of fishes. Although there are several examples that showed no correlation between the duration of life in the plankton to genetic structure (e.g. Victor & Wellington 2000), others have (e.g. Lester & Ruttenberg 2005).

The use of particular habitats by the initial benthic stages of cryptobenthic fishes is largely unknown. The clingfish *Lepadogaster lepadogaster* settled into gravel, one of the least abundant habitats in the study area. Adults were found in cobbles and there was practically no spatial overlap between the adults and the juveniles (Beldade & Gonçalves submitted 3). Several studies have shown that the presence of conspecifics may influence recruitment (Sweatman 1983; Webster 2004). In *L. lepadogaster* the absence of juveniles in the adult's habitats may result from inter-cohort competition or predation. The importance of these two factors needs to be assessed through experimental work. Habitat complexity can influence the survival of newly settled individuals (Connell & Jones 1991). In this study newly settled juveniles occurred in higher abundances in the habitat with higher number of items (high complexity) and lowest relief (low complexity). This result highlights the importance of using several measures of complexity to accurately assess habitat complexity. Only through an accurate evaluation of the spatial distribution of all ontogenetic stages will it be possible to develop a correct conservation plan. Managing a diverse assemblages and habitats is a demanding objective which depends on the scientist's ability to describe the species habitats associations across all development stages.

Many cryptobenthic species, such as clingfishes, use the underside of small movable items throughout their entire life cycle. Especially for this reason, traditional visual sampling techniques have been proven to return very biased results both in terms of composition and abundance (Kulbicki 1990; Edgar et al. 2004). Additionally, it is generally agreed that only through destructive sampling is it possible to adequately assess these fishes (Ackerman & Bellwood 2000; Willis 2001). We defined and tested a new non-destructive technique that involves habitat dismantling of small movable items. The densities estimated with this technique were similar to those resulting from anesthetic counts (Beldade & Gonçalves in press). The main differences were found for those species living under small movable items, such as clingfishes (Gobiesocidae). We believe that in the future this technique should be used preferentially in protocols that aim at sampling shallow bottom coastal fishes. In marine protected areas in particular, the study of fish assemblages should be based on non-destructive sampling methods, which do not alter the environmental integrity ensured by the protection regime (La Mesa & Vacchi 2005). The interference technique meets this requirement, since it allows diversity and abundance assessment without removal of the organisms. In particular, this technique provides data for those fishes that occur under movable items and is especially appropriate for temporal dynamics studies because of its' transient effects on the community.

The application of this sampling technique led to the discovery of yet another new record of cryptobenthic species in continental Europe, *Chromogobius britoi*. The observation of a new species during interference counts led to collections in order to identify this new species. The body proportions presented in Beldade et al. (2006) based on 27 specimens (25 from the Arrábida Marine Park and 2 from the Canaries) corrected some of the miscalculated values presented the first time the species was described in Van Tassell (2001) and encompasses new maximum and minimum values for the species. The application of adequate sampling techniques and the growing interest in the cryptobenthic fishes in coastal areas will increase the number of species found. The finding of more 'rare' cryptobenthic species in coastal areas (e.g. Ahnelt & Dorda 2004) will be an essential argument for marine conservation, given that one of the main factors that has driven the establishment of marine protected areas is biodiversity (Botsford et al. 2003; Roberts et al. 2003). Another interesting aspect is the ecological role of cryptobenthic species in coastal areas, for example as trophic links (Depczynski & Bellwood 2003). Only when the role of these species in coastal communities is fully understood will it be possible to adequately plan conservation measures.

We investigated the spatial variation of the cryptobenthic assemblage. Two categories of species arose from the number of habitats used: seven specialists and one generalist. It is curious to identify specialists at this scale because it is generally agreed that cryptobenthic species are usually associated to specific microhabitats, i.e. at a smaller scale (e.g. Patzner 1999) that, in turn, may or may not be related to larger spatial scales (Syms 1995). The specialist group encompassed four gobies, two clingfishes and one triplefin, and the generalist was a blenny. For three of the generalists there was a seasonal fluctuation in abundance between two habitats, cobble and small rocks. In the sampled sites cobble only occur in the shallower areas and *Gobius paganellus* is known to spawn in the intertidal (Faria & Almada 2001). So far it is not known if the same individuals use the subtidal and intertidal, but if the subtidal specimens spawn in the intertidal then the increase in the shallower habitats may be the result. Inversely, some temperate species in the intertidal show winter declines (e.g. Davis 2000) and it has been hypothesized that they may migrate to the subtidal (Horn & Martin 2006). Also, some temperate blennies are known to migrate to tide pools during high tide and back to the subtidal when tide is out (Stephens et al. 1970). *G. paganellus* recruits in the autumn and it was in that season that higher abundances were registered in the small rocks. Therefore small rocks may be one of the habitats for newly settled *G. paganellus*. *L. candolii* presented a very similar pattern to *G. paganellus* but the abundance fluctuation was more abrupt between the spring and the autumn. The recruitment season for *L. candolii* is also the autumn. This species abundance increased in small rocks and decreased in cobble, which may be due to the arrival of new recruits.

The diverse cryptobenthic fish assemblage in the no-take area of the Arrábida Marine Park underwent diversity and composition changes, at the assemblage level and of a few dominant species, measured across two temporal scales: years and seasons. At the assemblage level density remained constant from year to year as shown by assemblage parameters and multivariate results (Beldade et al. in press). This constancy resulted from the simultaneous decrease of the most abundant species and the increase in a number of other species. Considering that cryptobenthic fishes have very strong associations with the substrate we are led to believe that the overall constant density could be near the carrying capacity of the environment. In fact many of these species utilize similar habitats where a limited number of resources such as food or refuges may preclude a specific carrying capacity and thus an equilibrium density such as was observed here.

Over two years, local populations followed regular annual cycles of abundance that stemmed from seasonal recruitment in the autumn of both years. Multivariate analysis of the assemblage structure showed a strong seasonal variation. Recruitment occurred predominantly in the autumn for these species, but in the subsequent season there is a great decrease in density and especially in the number of small individuals. In this view, we are lead to believe that strong post-settlement events are reshaping the density fluctuations set by recruitment of these species. In temperate areas, the density fluctuations of small benthic associated species were proven to depend on post-settlement factors such as predation, competition and not only recruitment input (Forrester 1995; Steele 1997). Some of the species, for example *G. paganellus* and *L. candolii* showed density increases in the autumn of the first year and also relatively higher densities in the subsequent seasons of the second year and these increases were consistent with the increase in the number of small individuals. These results suggest that density may be set or at least be strongly dependent on the patterns set by recruitment events. However the simultaneous decrease in the abundance of *P. pilicornis* and the theoretical increase in the local carrying capacity, support the idea that post-settlement processes are mediating the density fluctuations of the different species. The observed patterns suggest that within the cryptobenthic guild there may be different population hypotheses explaining the observed patterns. Experimental studies based on the patterns described here will provide new insights into the factors affecting the density dynamics of these assemblages. However in diverse assemblages as the one studied here the number of possible specific interactions is great and the simplification of some sampling designs may not offer appropriate results to be applied in assemblage management. In other words, experimental studies of few ecological factors may not reflect the natural variations in composition and abundance observed at the assemblage level, especially in diverse assemblages.

Concluding remarks

The objectives proposed for this thesis were fully accomplished. The innovative sampling methods and techniques provided new insights into the composition, distribution and abundance of cryptobenthic fishes throughout their life-cycle. This is a particular fish assemblage that presents several ecological features that contribute to their high densities close to shore at all ontogenetic development stages. Results indicate that these fishes may be able to remain close to shore for short periods as larvae, select particular habitats in which to settle but also change habitats as adults. Some species use a small number of habitats whereas others use several. Finally, they seem to undergo consistent seasonal changes in habitat use that are related to recruitment. A two-year study on the population dynamics of the cryptobenthic assemblage at the no-take area in the Arrábida marine park revealed an overall constancy at the assemblage level despite the density variation observed for a group of abundant species. For most of these species, density variations were not consistent with the recruitment limitation hypothesis as the strong pulses in recruitment in the autumn, i.e. high number of settlers, were generally strongly modified in the subsequent seasons while there were no density differences between years. In this view the theoretical carrying capacity may have been reached and density dependent factors, such as refuge availability and predation will have had a determining part in setting the observed density levels. For *Parablennius pilicornis*, the most abundant species in the first year, a decreasing number of settlers and consequent lower adult density were observed. In this case the low input, i.e. number of settlers, may be the strongest factor in determining adult density. These results show that even within the cryptobenthic guild, contrasting population dynamics hypothesis may best explain the density variation of each given species.

In recent years the interest and number of publications on cryptobenthic subtidal fishes has increased. Despite growing awareness about the importance of these fishes in coastal ecosystems, the number of unanswered questions is still overwhelmingly high. Furthermore research in coastal areas, especially marine protected areas, will need to analyze whole fish communities to assess the actual local biodiversity, which will also fuel the study of cryptobenthic fishes.

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