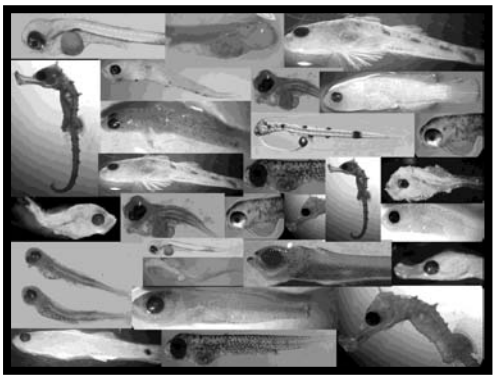
UNIVERSIDADE DO ALGARVE

FACULDADE DE CIÊNCIAS DO MAR E DO AMBIENTE

COMPOSITION, TEMPORAL AND SPATIAL PATTERNS OF VERY-NEARSHORE LARVAL FISH ASSEMBLAGES AT THE ARRÁBIDA MARINE PARK

(Tese para a obtenção do grau de doutor no ramo de Ciências do Mar, especialidade de Ecologia Marinha)



RITA ALEXANDRA DUARTE BORGES

Orientador: Doutor Emanuel João Flores Gonçalves (I.S.P.A.)
Co-orientadores: Doutora Maria Alexandra Anica Teodósio Chícharo (UAlg)
Doutor Pedro Miguel Alfaia Barcia Ré (F.C.U.L.)

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Doutor Karim Erzini
Doutor Luís Manuel Zambujal Chícharo
Doutora Maria Alexandra Anica Teodósio Chícharo
Doutor Henrique Manuel Roque Nogueira Cabral

FARO
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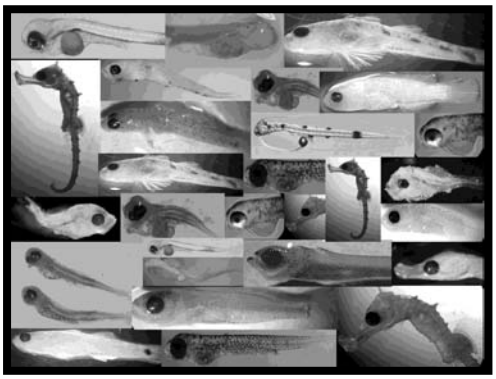

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NOME: RITA ALEXANDRA DUARTE BORGES
FACULDADE DE CIÊNCIAS DO MAR E DO AMBIENTE
ORIENTADOR: Prof. Doutor Emanuel João Flores Gonçalves
CO-ORIENTADORES: Prof. Doutora Maria Alexandra Chícharo;
Prof. Doutor Pedro Ré
DATA: 22 de Maio 2006

COMPOSIÇÃO E PADRÕES TEMPORAIS E ESPACIAIS DE VARIAÇÃO DAS ASSEMBLEIAS DE LARVAS DE PEIXES QUE OCORREM JUNTO À COSTA NO PARQUE MARINHO DA ARRÁBIDA

RESUMO

No presente trabalho pretendeu-se investigar alguns aspectos da ecologia e biologia da fase larvar dos peixes que vivem associados aos recifes rochosos no Parque Marinho da Arrábida. Com o objectivo geral de caracterização das assembleias de larvas e de determinação dos seus padrões de dispersão, investigou-se a composição das assembleias de larvas que ocorrem junto à costa, bem como os padrões temporais e espaciais (horizontal e vertical) de variação da sua estrutura, diversidade e densidade larvares. A diversidade foi máxima durante a época de reprodução da maioria das espécies e diminuiu, tal como a densidade larvar, com o afastamento de costa. Os padrões de dispersão foram variáveis de espécie para espécie, tendo a maioria das espécies ocorrido apenas junto à costa. Detectou-se um padrão vertical na estrutura das assembleias de larvas, tendo-se obtido junto ao fundo uma elevada densidade de um reduzido número de espécies. As larvas de algumas espécies parecem ficar retidas junto ao fundo durante toda a fase pelágica. Os resultados são discutidos face aos mecanismos que podem influenciar a dispersão ou retenção larvares no Parque Marinho da Arrábida. É ainda descrito o desenvolvimento embrionário e larvar para algumas espécies de recifes.

Palavras-chave: larvas de peixe, variação temporal, distribuição, retenção, desenvolvimento larvar, recifes temperados.

COMPOSITION, TEMPORAL AND SPATIAL PATTERNS OF VERY-NEARSHORE LARVAL FISH ASSEMBLAGES AT THE ARRÁBIDA MARINE PARK

ABSTRACT

In this thesis some aspects of the ecology and biology of reef fish larvae living at the Arrábida Marine Park were investigated. With the general goal of characterizing fish larval assemblages and their dispersal patterns, the composition, temporal and spatial (both horizontal and vertical) patterns of variation in larval diversity, abundance and in the structure of the assemblages was studied. Diversity was highest during the breeding season of most species. Both diversity and total abundance decreased with increasing distance from shore. The dispersal patterns obtained were species specific, with most species occurring exclusively in the very-nearshore. A vertical pattern of distribution could be found, with bottom assemblages being dominated by a small number of species, with very high larval densities. Larvae of some species seem to be retained nearshore during the whole pelagic phase by remaining at the bottom. Results are discussed considering possible mechanisms affecting larval dispersal or retention patterns, potentially acting at the Arrábida Marine Park. Embryonic and larval development of some reef fish species is also described.

Key-words: fish larvae, temporal variation, distribution, retention, larval development, temperate reefs.

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I. INTRODUCTION

The early life history stage of fishes extends from fertilization to the early part of the juvenile stage (Kendall, Jr. *et al*. 1984; Trippel and Chambers 1997; Fuiman and Higgs 1997). This period includes the embryonic and larval stage, during which the mortality is high and significant changes occur in their morphology (e.g. Moser 1981), behaviour and ecological aspects, including habitat use (Kendall, Jr. *et al*. 1984; Leis 1991a; Trippel and Chambers 1997).

These changes are particularly notorious in most reef associated fishes in which the pelagic larval phase alternates with a benthic life style when adults (Doherty and Williams 1988; Leis 1991a; Armsworth *et al*. 2001; James *et al*. 2002). The different environments in which these two stages live increase the number of factors that may limit population size (Irisson *et al*. 2004).

Fluctuations in fish populations have been considered, since the beginning of the 20th century, to depend on recruitment (for a detailed historical review see Sinclair 1997). The need to understand the processes underlying the fluctuations in fished populations led to the development of several hypotheses. Hjort (1914) proposed the "Critical Period Hypothesis" to explain inter-annual fluctuations in populations, relevant to fisheries (Sinclair 1997). Recruitment would depend on food availability for first feeding larvae during the critical period, after yolk absorption (Richards and Lindeman 1987; Sinclair 1997). He also proposed that larval survival would depend on advection or failure in the transport to nursery grounds at the end of the larval period (Doherty and Williams 1988; Sinclair 1997). His contribution was crucial as he was the first to emphasise the importance of the larval stage to fish population dynamics.

The Stock size can influence recruitment (Cushing 1973 *in* Richards and Lindeman 1987). It is assumed that the recruitment potential can be measured directly

by the spawning stock biomass (Trippel *et al*.1997). This has been extensively used in fisheries.

In 1975, Cushing proposed the "Match-Mismatch Hypothesis" which considers that spawning would be coincident with periods in which conditions would maximize larval survival (Cushing 1990). This theory also considered starvation and emphasised the importance of temporal and spatial overlapping between larval distribution and phytoplankton blooms. It also included larval stages other than only the newly hatched larvae (Cushing 1990).

The **"**Stable Ocean Hypothesis" (Lasker 1981), a particular case of the Hjort hypothesis, considered that successful larval survival would depend on aggregations of food items that usually occur in stable conditions; disturbance of this stability by oceanographic events (e.g. upwelling or storms) could lead to high larval mortality (Lasker 1981).

As an alternative to starvation hypothesis, predation has been proposed as the major cause of mortality controlling recruitment (Lasker 1981). Predation could act not just over the first larval stages, but during all development. In the case of reef fish larvae, recruitment would be also dependent on predation level over these late stages, in which larvae may explore the demersal habitats, being more exposed to predators (Richards and Lindeman 1987). However, most studies about the impact of predation have been conducted with clupeoid larvae and little is known about this factor in reef species (Richards and Lindeman 1987).

Models of recruitment have also integrated physical dispersal, considering that spawning and nursery grounds can be different**.** Parish *et al*. (1981) proposed the "Theory of the Larval Transport". This states that spawning locations in coastal zones

would occur in places that could minimize offshore displacement of eggs and larvae to areas with low food availability and that the recruitment level would thus depend on oceanographic factors. Parish *et al*. (1981) concluded that offshore transport of eggs and larvae of several coastal species could be important in the reproductive success of these species. In this case, larval mortality would depend both on predation and starvation.

The "Retention" or "Member/Vagrant Hypothesis" was proposed by Iles and Sinclair (1982). This considered that herring spawns in areas where water circulation generates retention of larvae near the spawning sites (Iles and Sinclair 1982; Sinclair 1988). Therefore, the recruitment would be dependent on the appropriate larval retention patterns near spawning grounds, where predation is low and food is available. This suggests that optimal spawning locations would be coincident with persistent and predictable hydrodynamic regimes, and considered the interaction between larval behaviour and oceanographic conditions.

Other models integrate growth (depending on food and temperature) and mortality considering that this is size selective (due to predation), with small individuals having higher probability of mortality than bigger ones (Anderson 1988 *in* Cowen and Sponaugle 1997). The "Bigger is Better Hypothesis" states that individuals that grow faster and get larger take advantages in avoiding predators and in getting food (Ware 1975; Shepherd and Cushing 1980, both *in* Cowen and Sponaugle 1997). The "Stage-Duration Hypothesis" defends that given that mortality is highest in the beginning of the pelagic period, the faster the development, the bigger will be the chance of surviving (Chambers and Legget 1987; Houde 1987, both *in* Cowen and Sponaugle 1997).

Recent models tend to incorporate several aspects of the interaction between larvae and the environment. Bakun (1996) has proposed the "Triad Hypothesis" which considers three major elements that combined provide favourable conditions for

reproduction: enrichment processes (e.g. upwelling), concentration processes (e.g. convergences and fronts) and drift to or retention within appropriate habitat. Where these three processes meet, locations are called "ocean triads" (Bakun 1996).

Small scale turbulence has also been proposed to affect the encounter rates between predators and preys, determining prey ingestion rates (Rothschild and Osborne 1988; MacKenzie and Miller 1994). However, more studies are needed to adequately evaluate the effect of turbulence on growth and mortality of larval fishes (Browman and Skiftesvik 1996). Trippel *et al*. (1997) proposed that future emphasis should be placed to include also aspects of the "parent-progeny" relationship on the stock production. These include the effects of adult age and body size and the duration and timing of spawning (Trippel *et al*. 1997). Recent models consider several other aspects like energetics (Winkle *et al*. 1997), offspring size, variable juvenile growth and age at maturity (Hutchings 1997). Recruitment transitions have been proposed to depend upon activating or inactivating processes of recruitment control, like "regime shifts" or species interactions as predation and competition (Duffy-Anderson *et al*. 2005).

1. Larval supply, recruitment and reef fish population dynamics

In contrast to the acceptance of variability in pelagic fish recruitment, coral reef fish assemblages have been considered as stable, without depending on recruitment (Doherty and Williams 1988).

Several models have been proposed to predict spatial patterns of recruitment and population dynamics in reef fish ecology at ecological or evolutionary time scales

(Doherty and Williams 1988). These can be grouped in equilibrium *vs* non-equilibrium models. Equilibrium models were traditionally accepted (reviews by Richards and Lindeman 1987, Doherty and Williams 1988, Doherty 1991, 2002). These models assume that there is a saturation of recruits in the environment and that post-settlement processes, which depend on resources at the benthic habitat, must act in limiting rates of settlement and post-settlement mortality (Doherty and Williams, 1988). In this case the structure of the assemblages is predictable. However, recent studies showed that reef fish recruitment can be highly variable in time and space (Sale 2004; reviews by Doherty and Williams 1988 and Caley *et al*. 1996).

Several studies have shown that larval supply can control adult populations (see next section; reviews by Doherty 1987; Richards and Lindeman 1987; Caley *et al*. 1996). These evidences led ecologists to start considering larval supply in attempts to understand reef fish recruitment variability (Hamer and Jenkins 1996). As larval supply depends on several factors, recruitment may not be enough to saturate benthic environments. The result is "non-equilibrial assemblages without predictable structure" (Sale 1980, *in* Doherty 1987). Non-equilibrium models state that resources are not the factors limiting the abundance of fishes but, instead, species are not saturated in their habitats due to fluctuations in recruitment.

The importance of the larval phase gained new force when reef fish ecologists recognized that these non-equilibrium models could explain the dynamics and structure of reef fish communities, depending on several biotic and abiotic factors affecting survival of the individuals during and after recruitment (see Caley *et al.* 1996).

Doherty (1981 *in* Caley *et al.* 1996) proposed the "recruitment-limitation" hypothesis, which considers that local reef populations are limited by the supply of recruits and that competition has little influence over population size. Later he

postulated that the limiting larval supply would maintain populations under their carrying capacity and that enhanced recruitment would promote higher abundance. Victor (1986b) defended that it is not clear whether it is the recruitment rate or the juvenile mortality that mostly affect the adult population. This author defended that population limitation may not be always explained by only one factor and proposed a model in which he distinguished primary recruitment from secondary recruitment limitations. Primary recruitment limitations occur when the input in larval supply is lower than the carrying capacity of the population. Secondary recruitment limitation act when the number of settling larvae is enough to reach the adult carrying capacity but juvenile mortality reduces these numbers below the carrying capacity (Victor 1986b).

Between the two extreme situations of non equilibrium and equilibrium models, a continuum may exist and the observations about the predictability of recruitment may vary according to the scale considered in sampling designs (Doherty 1987). Inconsistent variations in recruitment must indicate fluctuations in larval supply. On the other hand, when patterns are predictable they can reflect post-settlement mortality but also habitat selection by larvae (see Marliave 1977, 1986; Doherty, 1987; Breitburg 1991; Levin, 1991; Breitburg *et al.* 1995; Doherty *et al*. 1996; Risk 1997). To better understand temporal and spatial variation in recruitment, it is necessary to investigate variation in larval production and survival, patterns of larval transport and habitat selection (Caley *et al*. 1996).

The extent to which populations are more influenced by larval supply or by other factors occurring during or after the settlement process is controversial (Victor 1986b; Caley *et al*. 1996; Armsworth 2002). The difficulty in relating the supply of pre-settlement larvae with settlement regulation processes is in part due to the spatial and temporal scales analysed and to the sampling methods used (Steele *et al*. 2002). Most

recruitment studies have been done with organisms after settlement (e.g.: Victor 1986 b, Carr 1991; Levin 1991; Tolimieri *et al*. 1998; Petrik *et al*. 1999; Hsiao *et al*. 2003) and few studies have directly related larval supply with recruitment (Jenkins *et al*. 1998).

The recent development of new sampling methods (e.g. light traps or reef crest nets) allowed sampling directly over reefs and this has given new insights to understand small scale larval distribution and patterns of recruitment variability (e.g. Leis 1982; Leis 1986b; Leis and Goldman 1987; Smith *et al*. 1987; Robertson *et al*. 1988; Kobayashi 1989; Leis 1993; Leis 1994; Doherty and McIlwain 1996; Dufour *et al.* 1996, Sponaugle and Cowen 1996b; Leis *et al*. 1998; Tolimieri *et al*. 1998; Hendriks *et al*. 2001; Kingsford 2001; Wilson 2001;Valles *et al*. 2001; Sponaugle *et al*. 2003; Leis *et al*. 2003b; Wilson 2003).

1.1. Relationship between larval supply and settlement

Some studies have focused in the direct comparison between larval supply and settlement patterns.

Victor (1986b) found that the intensity of settlement of a coral reef fish was correlated to the diversity of larvae collected with night-lights in the night before. Although the intensity of settlement was variable, densities of recruits were consistent among reefs and well predicted with the exposure to the onshore current.

Robertson *et al*. (1988) studied spawning and settlement of a Caribbean reef fish. They found no correlation between these factors and proposed that variation in the planktonic phase could explain the high fluctuations in the settlement patterns found.

Milicich *et al*. (1992) caught late stage larvae at the Great Barrier Reef (GBR) with light traps and followed recruitment, collecting fish one week after settlement. They found that patterns of larval supply correlated well with recruitment levels observed in three coral reef species. For one of these species, Meekan *et al*. (1993) also found a good correlation between spawning patterns and temporal patterns of larval supply and recruitment in a damselfish; the magnitude of recruitment was related to factors affecting the larval stage.

Hamer and Jenkins (1996) found positive correlation between pre-settlement larval supply and post-settlement recruits of *Sillaginodes punctata*. They suggested that temporal variability in larval supply explained well the short-term variability in recruitment. However these authors pointed that at longer time scales post-settlement processes could also be acting.

Swearer *et al*. (1999) showed the existence of a high degree of self-recruitment in the leeward side of St Croix (where >70% of the annual recruitment of the island occurs) and suggested that a significant part of the local recruitment would result from the local retention of larvae.

Valles *et al*. (2001) investigated the relationship between the supply of 10 selected species larvae and their settlement patterns. The patterns obtained were species specific; for one of the species investigated there was a good correlation between larvae and settlers. They suggested that local variation of physical and biological factors could be influencing larval supply.

Breitburg (1991) and Breitburg *et al*. (1995) found that benthic schooling of larvae of the naked goby in an oyster temperate reef was related to distribution of fishes after settlement.

Although the above studies showed a good relationship between larval supply and settlement, others led to opposite results. Sponaugle and Cowen (1996 a) simultaneously sampled late stage larval supply and recruitment patterns of two Caribbean reef fishes. They found that for one of the species investigated, spatial and temporal patterns of recruitment were related to larval supply. For the other species, however, post-settlement processes seemed more important. Schmitt and Holbrook (1996) compared the patterns of larval supply and settlement of a damselfish settling into anemones that lacked residents (to test the effect of larval supply), with those where recruits accumulated (to test the effect of larval supply and interactions with residents). The obtained spatial patterns related to the larval supply were reduced after some time, possibly as a consequence of interactions between settlers and residents on the reef.

Levin (1996) investigated the relationship between late stage larval supply and settlement patterns of a temperate reef fish at a small spatial scale in the Gulf of Maine. He found the size of pre-settlement larvae to be correlated to the variability in the magnitude of settlement occurring in artificial substrata. Levin (1996) concluded that, at a scale of hundreds of meters to kilometres, processes occurring within the first hours of settlement, rather than larval supply, were influencing recruitment. However, at smaller spatial scales, the relationship between settlement and recruitment disappeared with time.

Jenkins *et al*. (1998) defended that in coral reefs, habitat selection would be of minor importance when compared to larval supply, but that in temperate zones recruitment would be related to the habitat complexity at small scales. They investigated the abundance of pre-settlement larvae and recruits in natural and artificial seagrass beds and concluded that settlement patterns were not related to larval supply but probably dependent on post-settlement processes at the seagrass beds. Steele *et al*.

(2002) used artificial substrate devices to investigate late stage larval supply and recruitment of a temperate reef fish, at 4 different sites. They found no influence of larval supply; instead, recruitment was correlated to 1-year old individuals. These authors proposed that post-settlement deterministic processes should regulate recruitment at large scales. They also found temporal consistency in larval supply at small spatial scales and suggested that deterministic mechanisms could regulate larval supply at small scales. However, these patterns were not consistent for every taxa.

McIlwan (2002) found a weak relationship between larval supply and spawning temporal patterns. He related this failure with the action of disturbing factors like cyclone activity.

Large pulses of recruitment may increase population sizes and the action of density-dependent regulating mechanisms (Caselle *et al*. 2003). If a population is limited by recruitment, higher recruitment will lead to increased population sizes and higher local production (Caselle *et al*. 2003). Caselle *et al*. (2003) studied the relationship between larval supply and retention, juvenile density and mortality of adult populations at three sites of St.Croix. The site with the highest larval retention and recruitment had the highest juvenile mortality and the site with the highest production had low retention and moderate recruitment rates. These authors proposed that sites with higher larval retention and recruitment may not be the most productive due to density-dependent processes.

From what was exposed, it can be concluded that the relative importance of larval supply to recruitment patterns can vary between species (Sponaugle and Cowen 1996b) and places. A possible combination of factors may be best suited to explain abundance patterns (Jones 1991, Shima 1999, 2001). Shima (2001) and Jones and

McCormick (2002) emphasized the need of quantifying the relative importance of each factor, considering multiscale studies.

2. Dispersal/ retention patterns as determinants of larval supply

Recruitment has five main components (according to Jenkins *et al*. 1998): 1) input of larvae to a given water body; 2) larval transport; 3) eggs and larval mortality; 4) settlement; 5) post-settlement growth and survival. The importance of larval transport to the dynamics of nearshore populations is the focus of the present study and will be emphasized.

The time spent in the plankton varies from no planktonic stage to many months (Victor 1991). The ability to disperse is higher in the planktonic stages when compared to the benthic adults living in reefs (Leis 2002). Therefore, larval dispersal has been considered to allow connectivity between populations of benthic marine organisms; recruitment to local populations would occur from the "pelagic larval pool" (Doherty 1991; Caley *et al.* 1996; Roberts 1997; Planes *et al*. 2000; Sponaugle *et al*. 2002; Sale 2004). The larval stages of reef fishes would have a higher influence in determining the geographical size of populations than adults (Leis 1991a; Shanks *et al*. 2003). This connectivity between populations through the passive dispersal of larval stages was generally assumed in the recruitment models and was called the "Open Population Paradigm". This was based on the following assumptions (Leis 2002): 1) reef-fish larvae are passive, having no control over their "trajectories"; 2) reef-fish populations are demographically open at large scales; 3) genetic panmixia gives evidence that reef

fish populations are open; 4) ”far-field” currents are appropriate for use in modelling dispersal.

Several hypotheses have been developed to explain possible ecological advantages of sending propagules away from reefs (Leis 1991a; Bonhomme and Planes 2000): larval dispersal has been proposed as a mechanism of avoiding the high predation pressure near reefs (Johannes 1978). In fact, several species spawn at night (Doherty and Williams 1988), which could be a mechanism of avoiding diurnal reef predators. However, data on predation rates near reefs *vs* offshore are inexistent (Leis 1991a). Bourret *et al*. (1979 *in* Bonhomme and Planes 2000) proposed that if larvae did not have to swim against currents near reefs, they would save energy since passive drifting expends little energy. Barlow (1981) has proposed that dispersal could avoid local extinction, since reefs are uncertain patchy environments. Dispersal has been also considered as a way of exploring resources in new environments where benthic adults, living in patchy food environments, could not reach (Doherty *et al.* 1985). But this would be unnecessary in unsaturated adult habitats, given the evidences of recruitment limitation (Leis, 1991a). Barlow also suggested that larvae from pelagic eggs would disperse more than larvae from demersal eggs, as demersal spawners cannot produce enough eggs for successful long-distance dispersal (Kobayashi 1989). Bakun (1986 *in* Bonhomme and Planes 2000) stated that larvae dispersing offshore could avoid the surf zone near reefs reducing loss through pulverisation. Dispersal has also been proposed to provide mobility among habitats (Bonhomme and Planes 2000; Strathmann *et al*. 2002). However, Strathmann *et al*. (2002) defended that to select new habitats larvae should be released as precompetent larvae and these larvae can only disperse for short distances.

These hypotheses have been criticized by lacking generality (Bonhomme and Planes 2000) and still remain to be tested (Leis 1991a).

Understanding the degree of dispersal of marine organisms and its effect in structuring marine populations is one of the central issues in marine ecology (Armsworth 2002; Largier 2003; Irisson *et al*. 2004). In fact, there is increasing evidence that the assumptions of the "open population paradigm" may not always meet and that local larval retention may be more prevalent than previously thought (e.g. Leis 1991a; Jones *et al*. 1999; Swearer *et al*. 1999; Cowen 2002; Leis 2002; Leis and McCormick 2002; Sponaugle *et al*. 2002, Swearer *et al*. 2002; Warner and Cowen 2002, Taylor and Hellberg 2003; Paris and Cowen 2004; Jones *et al*. 2005).

Larval retention near reefs could be adaptive for many coastal marine organisms with planktonic larvae given the more favourable environmental conditions for larval growth and survival and the higher probability of encountering suitable adult habitats to settle (Leis 1991a; Swearer *et al*. 1999). Advection from these habitats could lead to effective loss causing significant larval mortality (Kobayashi 1989). The evidences of larval retention near source populations have been reviewed by Cowen (2002), Leis and McCormick (2002), Swearer *et al*. (2002) and Sponaugle *et al*. (2002). These evidences were obtained from: 1) work on larval distribution in nearshore waters; 2) modelling larval dispersal; 3) tagging studies and 4) genetic evidence (reviewed by Planes 2002).

2.1. Distributional studies

Leis and Miller (1976) investigated the composition of larval assemblages sampled from very nearshore reefs to 12 Km offshore, in Hawaii. They found that larvae of several reef species were found offshore and that the most common patterns were for larvae from pelagic spawners to increase in density with distance from shore, and from demersal spawners to have the opposite pattern. Leis and Miller (1976) suggested that vertical migration of larvae from demersal eggs could contribute for this pattern of retention nearshore. A smaller spatial scale was investigated by Leis (1982), sampling at several distances from shore, from 0.2 to 3 Km. He found different patterns, depending on the species and on the ontogenetic stage considered. Reef larvae from pelagic eggs were not found near reefs. This author suggested that the currents regime could have influenced the observed patterns; however, not all distributions could be explained by passive drift. Leis (1982) suggested that inshore patterns of distribution should be maintained by larval behaviour. Latter, Kobayashi (1989) studied the distribution of larval fish around reefs at the opposite side of the same island, at a fine spatial scale. This author used vertical nets and found gobiid larvae to be significantly more abundant near reefs than offshore and related these patterns as a result of the interaction between vertical distribution and currents; other species were however present mainly offshore.

Around Lizard Island, Great Barrier Reef, Leis (1986b) obtained different spatial patterns of larval distribution, through vertical stratified horizontal tows and current measurements. The patterns found related well with local oceanographic conditions. Larval vertical patterns were also evident in locations where clear current regimes differed with depth and Leis suggested that differential vertical positioning by the larvae in face of the oceanographic conditions would result in retention near reefs at one

location, while favouring dispersal at the other. As most larvae avoided surface waters, he considered that this behaviour could contribute to retention. Later, Leis and Goldman (1987) studied the composition and temporal variation of the larval assemblages. They found that, although diverse, assemblages were dominated by larvae spawned from demersal eggs. However, larvae of some reef species were rare or absent from their catches. They found seasonal and distinct spatial patterns in assemblages and suggested that those patterns could reflect different retention patterns.

Smith *et al*. (1987) sampled very nearshore larvae in the Caribbean using lights and netting and obtained assemblages different from those previously collected offshore. Inshore collections had larvae within all developmental stages, indicating that those species could complete the entire planktonic cycle nearshore. These authors listed several larval specializations to planktonic life and suggested that the inshore larval assemblage was composed by unspecialized larvae that remained near the reefs.

Small scale patterns of vertical larval distribution were investigated at very nearshore waters, for the 50 most abundant species at Great Barrier Reef (Leis 1991b). Species specific patterns were found during the day which seemed to contribute to larval retention near reefs (Leis 1991 b). Leis (1993) summarized previous results for Indo-Pacific reef species, and included some new data. He analysed the patterns of horizontal and vertical distribution depending on the flexion stage of the larvae and on the taxa, with distance from reefs. Leis' general conclusions confirmed that vertically there was a strong structure during the day that was less clear at night. Along the horizontal axis, onshore-offshore patterns were more clear than alongshore ones. Leis (1993) related the observed patterns with several factors including currents, mode of spawning and spawning behaviour of adults, planktonic larval duration, habitat requirements and larval behaviour.

Cowen and Castro (1994) found very high nearshore densities of larval fishes at Barbados. They related the pattern found with physical features of the environment, given that onshore flow was stronger at depths where maximum larval densities were found. These authors suggested that larvae could be retained near reefs due to around island circulation patterns.

Hendriks *et al.* (2001) also found vertical patterns of distribution of larvae in nearshore waters, with older larvae occupying deeper strata; the authors emphasized that differences may exist in the response to light in species of different families. In the Florida Keys Sponaugle *et al*. (2003) also obtained near reef larval assemblages different from those from offshore collections. Although without vertical patterns of distribution, some taxa were found nearshore within several size classes. These authors proposed that currents could have influence over the temporal patterns observed and emphasized the need to more studies involving biophysical interactions.

Coral Sea atoll lagoons have also been investigated in relation to their larval composition. Leis (1994) found few oceanic taxa inside two atoll lagoons in the western Coral Sea, whereas several reef fish larvae dominated the catches. Leis identified 33 taxa with larvae of all size classes and developmental stages inside the lagoon, and concluded that these species were locally completing their pelagic phase. These taxa included Blennids, Tripterygids and Gobies among other reef fishes. Leis *et al*. (1998) also found six out of 18 taxa to be present within the full range of sizes (including both larvae from pelagic and benthic spawners) at Taiaro Atoll, in the French Polynesia. The other taxa, however, were only present when newly hatched, suggesting completion of their pelagic cycle off the lagoon. Similar results were obtained by Planes *et al*. (1998b) and by Leis *et al*. (2003b).

Taylor *et al*. (2004) identified larvae of several species of rock fishes through DNA analysis; they found that for some species, the southern California eddy could act as a retention mechanism that could maintain larvae near the adults' habitats; other species had more offshore distributions that could result from vertical distribution in the water column.

Although the above described studies are indicative of local retention, other studies have found evidence of dispersal. Planktonic larval duration (PLD) may vary from 7-10 days to several months (Victor 1991; Sale 2004). Species with long PLD often have specialized structures to live in the pelagic environment (Smith *et al*. 1987) and this has been related to the geographic range of species. It is frequently assumed that long PLD's are related to high connectivity between populations (e.g. Victor, 1987, Sale 2004).

Populations of species with longer PLD have also been found to be less genetically differentiated (review by Doherty and Williams 1988; Planes 2002). Therefore, the geographic range of reef species has been sometimes related to their dispersal ability, considering PLD (Mora *et al*., 2003). In a review of the studies that investigated PLD and geographic distribution in tropical reef fishes, Lester and Ruttenberg (2005) found that, although some times correlated with species range, PLD is not a universal predictor of range size (see e.g. Victor 1986, Thresher *et al.* 1989, Victor and Wellington 2000; Raventós and Macpherson 2001). Species with long PLD may also be retained nearshore (Leis *et al.* 2003b; Shanks *et al*. 2003).

At smaller relevant ecological scales, the probability of self-recruitment is higher for species with a short PLD (Raventós and Macpherson 2001; Sponaugle *et al*. 2002). Species developing faster have more chance of being locally retained by physical mechanisms, until the settlement stage.

2.2 Models of larval dispersal

The first models of larval dispersal considered larvae as passive particles. Williams *et al*. (1984 *in* Doherty and Williams 1988) showed that passive drifting organisms could be transported along several Kilometres in a few weeks (Doherty and Williams 1988). In the Caribbean, Roberts (1997) has modelled larval transport routes only based on current patterns and has proposed that the use of these patterns could help in the design of reserve networks in the area. Cowen *et al*. (2000) simulated passive larval dispersal potential around Barbados; their results indicated that larval exchange between closely located islands was not sufficient to sustain downstream populations over ecological time scales, even when all larvae produced left the source area. They suggested that mechanisms like vertical migration by larvae in stratified waters should exist to maximize retention near the source population. On the contrary, James *et al*. (2002) modelled the influence of regional physical oceanographic factors over the possible self-recruitment of reef fishes of the GBR. Their results predicted that most populations would depend mainly on external sources of larvae.

Hare *et al*. (2002) have incorporated a function of larval mortality in a model that considered the influence of physical factors on larval transport from southeast to the northeast US continental shelf; their results showed that modelled transport matched observed distributions.

While the first models of dispersal did not include behavioural information, sometimes overestimating larval exchange rates (Cowen *et al*. 2000), recent models started to incorporate the behavioural components and obtained more realistic results.

Werner *et al*. (1993 *in* Armsworth *et al*. 2001) and Armsworth (2000 *in* Armsworth *et al*. 2001) incorporated horizontal swimming into models; Wolansky *et al*. (1997) modelled larval dispersal including larval swimming with tidal, current and wind information. They considered three distinct behavioural patterns: passive larvae, and swimming larvae at two different speeds when approaching the reefs. Simulations that considered larval swimming fitted better the observed distributions of larvae collected in the field. The authors emphasized the need of better understand the sensorial and behavioural processes of larvae in order to improve more realistic results in models. Armsworth *et al.* (2001) developed two types of models of larval distribution focusing on larval behaviour of reef species: models that assumed larval return to the reefs and models that assumed dispersal; models outputs depended on the efficiency and sustainability of larval swimming.

Individual larval releases considering flow, PLD and settlement competency of larvae, were modelled by Siegel *et al*. (2003). Averaged patterns of dispersal varied from few to 100's of Km, and matched well with population genetic information.

Paris and Cowen (2004) collected *in situ* currents data and larvae of different ontogenetic stages, during the same period. They found that general patterns in larval transport determined by circulation were altered by the vertical distribution of larvae. Larvae actively responded to a vertically stratified flow with bigger larvae having distributions that minimized strong advective loss in the surface by 20% hence retaining c.a. 20-25% of the larvae in the study area after 15 days (Paris and Cowen 2004).

2.3. Otolith studies

Swearer *et al*. (1999) investigated trace elemental composition in larval otoliths of a Caribbean coral reef. This method can give information about larval dispersal, considering that concentrations between coastal and open oceans can result in differences in the growth rate and elemental composition between larvae developing in the two environments. Their findings indicated that recruitment was strongly influenced by "retention signatures".

At the GBR Jones *et al*. (1999) marked otoliths of embryos using tetracycline and recaptured juveniles; they showed that as many as 15-60% of juveniles could return to natal populations, showing a high degree of self-recruitment. More recently, Jones *et al*. (2005) marked all the larvae of one clownfish species produced in a population at Schumann Island, Papua New Guinea, and investigated parentage between adults and the new recruits. Microsatellite DNA analysis allowed them to measure dispersal distance and direction. Jones *et al*. (2005) found that most of the juveniles settled at a distance <100 m from their birth site. This was the smallest scale of dispersal studied, showing a significant degree of self-recruitment for the species considered.

Other studies have found no evidence of retention. For example, McIlwan (2002) estimated juveniles' birth date through otolith analysis; he found that most juveniles were spawned when local spawning activity was low, indicating that they were originated from external populations.

2.4. Genetic studies

Gene flow between populations can promote panmixis even with a small number of migrants. Nonetheless, genetic differences may reflect biological isolation in similar environments (Planes *et al*. 1998a,b).

Several studies indicated the existence of genetic homogeneity among populations while others showed genetic differentiation (see reviews by Planes *et al*. 1998a,b). Doherty *et al*. 1995 (*in* Planes *et al*. 1998a) genetically analysed seven species at the GBR; they found a good relationship between PLD and the genetic structure of the populations. On the contrary, Planes *et al*. (1998a), investigated allozyme variation in three reef species of New Caledonian lagoon, having different PLD, behaviour, size range and reproductive strategies. They showed evidence of limited genetic flow at a small scale, for two species. This genetic differentiation was not correlated to the PLD of the species considered. Planes *et al*. (1998a) explained the results considering the possible limited water flow between populations and climatic factors.

Taylor and Hellberg (2003) also found strong genetic differentiation between 17 populations of a Caribbean goby. They considered that although having an extended PLD of 21 days, the differences obtained could reflect "absent or restricted" gene flow among populations.

2.5. Retention in temperate nearshore environments

In temperate waters most work on ichthyoplankton composition and distribution has been performed in oceanic or shelf waters (Australia: Gray 1993, 1998; Smith 1999; Gray and Miskiewicz 2000; Neira and Sporcic 2002; Canada: Suthers and Frank 1991;

North Sea/ Irish Sea/Galway Bay: Russell 1973; Fives and O'Brien 1976; Southward and Barret 1983; Riley *et al*. 1986; Tully and O'Céidigh 1989; Conway *et al*. 1997; Grioche *et al*. 1999; Coombs *et al*. 2001; Lee *et al*. 2005; Celtic Sea: Southward and Bary 1980; Horstman and Fives 1994; Acevedo *et al*. 2002; Baltic: Parmanne and Lindström 2003; Black Sea: Satilmis *et al*. 2003; Gordina *et al*. 2005; N Spain: Suau and Vives 1979; Dicenta 1984; NW Mediterranean: Sabatés 1990; Palomera 1991, 1992; Sabatés and Masó 1992; Sabatés and Olivar 1996; Olivar and Sabatés 1997; Olivar *et al*. 1998, 2001; Sabatés 2004; Greece: Somarakis *et al*. 2002; Koutrakis *et al*. 2004; Peru: Vélez *et al*. 2005; Canaria: Rodríguez *et al*. 2001; Chile: Hernandéz-Miranda *et al*. 2003; Arabian Sea: Röpke 1993; NE Pacific: Boehlert *et al*. 1985; Brewer and Kleppel 1986; Doyle *et al*. 2002; Eastern coast USA: Grothues and Cowen 1999; Reiss and McConaugha 1999; Powell *et al*. 2000; Hare *et al*. 2001; Grothues *et al*. 2002; SE Atlantic/South Africa: Olivar 1990; Olivar and Fortuño 1991; Harris *et al*. 1999; Mid-Atlantic Bight: Kendall and Naplin 1981. Along the Portuguese coast, the ichthyoplankton studies include those of Ré 1984, 1986; Afonso 1989, 1995; Ré *et al*. 1990; Andres *et al*. 1992; John and Ré 1993; Afonso and Lopes 1994; Lopes and Afonso 1995).

The above studies have been conducted over large temporal and spatial scales. Fewer ichthyoplankton studies exist in nearshore waters due to the difficulty in sampling close to shore and over heterogeneous substratum (Smith *et al*. 1987; Kingsford and Choat 1989).

In temperate systems, nearshore studies of composition and distribution of larval assemblages included sampling over soft bottoms (e.g. Brewer and Kleppel 1986, Walker Jr. *et al*. 1987; Grioche *et al*. 1999), near estuaries (e.g. Olney and Boehlert 1988; Harris *et al*. 1999, at the surf zone (e.g. Whitfield 1989; Watt-Pringle and

Strydom 2003; Strydom and d'Hotman 2005), in coastal inlets (e.g. Drake and Arias 1991) or in coastal lagoons (e.g. Pérez-Ruzafa *et al*. 2004).

There are fewer studies near rocky reefs, which include those of Walker Jr *et al*. (1987), Marliave (1986), Kingsford and Choat (1989), Suthers and Frank (1991), Brogan (1994), Palomera and Olivar (1996), Yoklavich *et al*. (1996), Tilney *et al*. (1996), Hernández *et al*. (2003), Hickford and Schiel (2003) and Sabatés *et al*. (2003). Some of these studies also obtained larval distributional patterns that can be indicative of retention.

Marliave (1986) sampled at a fine scale at extreme nearshore waters of a rocky shore in the British Columbia. Using plankton tows and diver observations, he concluded that larvae of some families as the Gobiesocidae were predominant in inshore waters, and were never found in the more offshore samples. Other families had predominantly offshore distributions. Marliave related the inshore distribution of larvae with schooling behaviour and proposed that larvae could actively respond to "visual landmarks" and velocity gradients, promoting their retention nearshore.

In New Zealand, Kingsford and Choat (1989) sampled at several distances from reefs, using oblique towing. Most larvae caught were from reef fishes. Tripterygiids of different sizes were captured at all distances and with high densities in bays and near reefs. Other families including Gobiesocidae, Labridae and Gobiidae, although less abundant, were also present in bays and near reefs. They also used visual censing of late stage larvae by SCUBA diving near the bottom. Large numbers of tripterygiids accumulated in small gutters along the reef close to the surface, whereas gobiesocids were mainly at very shallow waters (1-2m), at mid-depths or near the bottom. Gobiesocidae larvae were only found nearshore, indicating retention near reefs.

Suthers and Frank (1991) found a more uniform distribution of larvae from pelagic eggs, than of larvae hatching from benthic eggs which were predominantly distributed in inshore waters near spawning sites, at South-western Nova Scotia. They proposed that the larger larvae from benthic eggs could have vertical migration behaviours which could influence their retention.

In the Gulf of California, Brogan (1994) sampled along transects perpendicular to the shoreline, over depths that ranged from 1 to 30 m; light traps and net tows during daytime were used over reefs and over sand in the more offshore station. Brogan obtained different patterns of horizontal distribution, depending on the species. The most represented larvae near reefs were from reef fishes with demersal spawning, including larvae of the families Blenniidae, Gobiesocidae, Gobiidae and Tripterygiidae. From these, the author concluded that Gobiesocidae, Tripterygiidae and possibly some Gobiidae larvae were retained near reefs.

At the Tsitsikamma National Park, South Africa, Tilney *et al*. (1996) compared larval abundance over reefs and sand patches at very-nearshore waters, and over different water depths (between 20 and 80 m, located respectively 0,35 to 3,83 Km offshore). Gobiesocidae, Blenniidae, Engraulidae and Sparidae were the most abundant families, mostly with larvae in the preflexion stage. While Gobiesocidae larvae were most abundant in deeper waters near reefs, Engraulidae and Sparidae showed a more homogeneous distribution. Gobiesocids and blenniids were more abundant inshore. From these, the former were significantly more abundant over reefs than over sand; the fact that these larvae were present near reefs in different size classes, seemed to indicate active larval retention nearshore. Tilney *et al*. (1996) also concluded that blennies, although distributed at surface waters, also showed retention patterns.

Yoklavich *et al*. (1996) investigated abundance and distribution patterns of rockfish larvae off California; they related larval retention nearshore with onshore flow during one El-Niño event; in the subsequent year, density of larvae nearshore decreased due to upwelling. Miller and Shanks (2004) have analysed the geochemichal signatures in rockfish larvae; their results indicated a reduced flow of larvae between locations separated by hundreds of Kilometres.

In the NW Mediterranean, larval fish assemblages at very shallow waters (7-9 m depth) off the Costa Brava were composed mainly by larvae of demersal spawners (Palomera and Olivar 1996). Later, Sabatés *et al*. (2003) compared the composition and abundance of larval assemblages in this area with larvae from nearshore waters at the Medes islands Marine Reserve and from shelf waters. For some reef fish species with larvae hatching from demersal eggs as blenniids and gobies, they found patterns of larval dispersal offshore. Others, as were tripterygiids and gobiesocids, seemed to be retained near reefs.

At more exposed nearshore waters of New Zealand, Hickford and Schiel (2003) analysed larval distribution, with perpendicular and parallel to shore sampling. No pattern of retention/ dispersal related to the pelagic vs. demersal mode of spawning of the most abundant species could be found. Even larvae usually retained as tripterygiids were well dispersed in the nearshore-offshore axis. Hickford and Schiel (2003) proposed that in coasts more exposed to oceanographic conditions, retention of larvae from demersal eggs near reefs may not be the pattern.

3. Factors affecting larval retention

Pineda (2000) proposed that the settlement processes of marine organisms are hierarchic. Determinants of larval supply, settlement and population abundance would be different levels in this hierarchy. The factors influencing larval supply would include the interaction between the larval pool and physical and larval transport processes. The first would be influenced by large scale oceanographic factors, while smaller scale processes would act during the transport. Variation in large-scale processes could produce large fluctuations in larval supply (Pineda 2000). More close to settlement, other factors would act at a microscale.

Although some studies found no correlation between the structure of larval assemblages of reef fishes and physical factors (see Leis 1993, Wilson 2003, Sampey, 2004), there is evidence of the influence of these factors over larval transport. Several physical mechanisms can influence transport from and towards the shore, retention patterns and larval growth and survival nearshore (reviewed by Norcross and Shaw 1984; Leis 1991a, Pineda 2000; Cowen 2002 and Sponaugle *et al.* 2002).

3.1. Physical factors

Oceanographic factors can influence the distribution of larvae, their retention/ transport, and their interaction with prey or with predators (Kingsford and Cowen 1996). Among the physical factors that may influence larval transport from and towards the shore are the intensity and direction of currents (e.g. Hare and Cowen 1991; Cowen and Castro 1994; Roberts 1997; Hare *et al*. 2002). These can promote long distance transport of larvae. Interaction between currents and wind may also be important. For

example, upwelling can influence offshore or shoreward transport of planktonic organisms (Olivar 1990; Yoklavich *et al*. 1996; Pitts 1999); eddies can retain larvae (Nishimoto and Washburn 2002; Taylor *et al.* 2004). Mesoscale circulation patterns interacting with bathymetry and fronts (Sabatés and Olivar 1996) can create retention or transport mechanisms of planktonic organisms.

Although large-scale oceanographic processes may influence larval dispersal, nearshore environments offer distinct characteristics due to the shallow depths and shoreline proximity (Pineda 2000; Cowen 2002; Sponaugle *et al.* 2002; Largier 2003). Hydrodynamic patterns associated with shallow depths can "restrict" the transport mechanisms and create unique patterns: interaction with shallow bottoms originates currents alongshore that are more energetic that across shore currents (Pineda 2000, Largier 2003). Tides are known to have a crucial influence in larval transport and retention in estuaries (see Norcross and Shaw 1984; Boehlert and Mundy 1988). Tidal fluxes can have a strong influence at nearshore shallow waters and the interaction between tides and bottom topography may create small-scale patterns of circulation (Pineda 2000; Cowen 2002; Largier 2003). Fronts may be created, between less productive offshore waters and more productive nearshore (e.g. Munk *et al*. 1995) or estuarine environments. Tidal and saline fronts often create convergence zones where larvae can be retained (Thorrold and McKinnon 1995; Grimes and Kingsford, 1996; Reiss and McConaugha 1999). Thermal or saline stratification, can promote different flux conditions.

Site location is also important. More isolated populations are more likely to be maintained by self-recruitment, considering at the extreme the case of endemic populations (Sponaugle *et al*. 2002).

Wind speed and direction can create surface currents that transport organisms (Nakata *et al.* 2000; Wilson and Meekan 2001; Voss and Hinrichsen 2003). In shallow environments wind currents and other flow speeds may be slowed through bottom friction; the "coastal boundary layer" may retain larvae produced nearshore for some time, near the natal environments (Largier 2003).

The coastline orientation can have influence in retention patterns. Upwelling shadows along a coast may form in the lee of headlands that may retain organisms (Graham *et al*. 1992 *in* Cowen, 2002; Marín *et al*. 2003; Largier 2003; Roughan *et al.* 2005a,b).

Internal tidal bores can also be important in the shoreward transport of planktonic organisms (Pineda 1991, 1994, 1999, 2000; Lamb 1997; Leichter *et al*. 1998; Lennert-Cody and Franks 1999).

3.2. Biological factors

The physical factors may promote passive transport or retention of larvae. Several biological factors can interact with those factors at different scales, influencing dispersal patterns (reviews by Cowen and Sponaugle 1997; Sponaugle and Cowen, 1997; Cowen 2002; Leis and McCormick 2002; Sponaugle *et al.* 2002).

Several features of the adults may indirectly influence larval transport dynamics. These include adult fecundity, mobility and spawning patterns (e.g. when adults select spawning sites that enhance retention/ dispersal). Several coral reef species have spawning patterns synchronized with tidal cycle or with the moon phase (e.g. Robertson *et al*. 1988). Temporal patterns of spawning (e.g. seasonal cycles) and their relation to larval feeding conditions and physical environment can affect larval survival.

The spawning mode of fishes can also have a strong influence over dispersal patterns: reef fishes that spawn demersal eggs have usually parental care that leads to larger and more developed larvae at hatching, when compared to larvae hatching from pelagic eggs. These more developed larvae may be able of swimming early in development (Thresher 1984; Leis 1993; Sponaugle *et al*. 2002; Hickford and Schiel 2003). Size at hatching and growth during the planktonic phase can also influence the success of recruitment onto reefs (Bergenius *et al*. 2002; Vigliogla and Meekan 2002; Meekan *et al*. 2003; Raventós and Macpherson 2005). Larval growth rates can, in turn, be variable depending on the environmental conditions (Victor 1986 a; Houde and Zastrow 1993; Arvedlund *et al*. 2000; Searcy and Sponaugle 2000; Meekan *et al*. 2003; Sponaugle and Pinkard 2004; Bergenius *et al.* 2005).

The extent of the planktonic larval duration, as already focused, is another factor that can give different dispersal potential for different species.

Considering larvae as passive organisms, different patterns of larval positioning in the water column can occur. These can be explained for instance by differential buoyancy patterns that can vary from species to species and that can change with development (e.g. Adlansvik *et al*. 2001). Furthermore, environmental conditions like different salinities or feeding can have impact over buoyancy responses (Miller 1988).

3.3. Larval behaviour and environmental cues

Larval fishes are seldom passive particles; their ability to interact with the environment can influence their position in the water column and alter significantly larval dispersal.

The ability of larvae to vertically migrate in the pelagic environment has been well documented (see review by Neilson and Perry 1990). Vertical patterns of distribution have been related to water thermal stratification, often associated with thermocline positioning (Southward and Barry 1980; John and Ré 1993; Flores-Coto *et al*. 2001) but also in mixed waters (Sabatés 2004), being influenced by the light regime (Blaxter 1973; Trotter *et al*. 2003; Sabatés 2004) or food availability (Fortier and Harris 1989; Clay *et al*. 2004; Sabatés 2004).

The interaction between vertical positioning behaviour and physical factors may influence horizontal displacement of larvae affecting their shoreward transport (Hill, 1995; Stobutzki 2001; Paris and Cowen 2004). Vertical migration behaviour has been related to the ability of larvae to be retained within estuaries (Laprise and Dodson 1989; Dauvin and Dodson 1990; Ré 1990; Forward Jr *et al*. 1996a,b; reviews by Norcross and Shaw 1984 and Boehlert and Mundy 1988).

As described above, several studies have detected vertical patterns of larval distribution at a small spatial scale in reef environments, (see reviews by Leis 1991a; Cowen 2002; Leis and McCormick 2002; Sponaugle *et al.* 2002; **IV**). Remaining near the bottom, where usually currents are slower, may also allow larvae to stay locally retained (e.g. Leis 1986a, 1991a,b; Steffe 1990; Breitburg, 1991; Breitburg *et al.* 1995).

Other examples of larval active behaviour are the settlement patterns observed at night (Kingsford 2001; Wilson 2001, 2003), often in relation to tidal or moon phase condition (e.g. Robertson *et al*. 1988; Sponaugle and Cowen 1996 a, b; Lozano and Zapata 2003; McIlwain 2003). Settling larvae may also be able of responding to habitat specific cues, both in tropical reefs and in temperate reefs (Marliave 1977). Habitat

selection during the fish larval phase was already referred to occur frequently in reef environments. Other evidences of larval behavioural capabilities include the ability of larvae to detect predators by chemical and visual cues (e.g. Lehtiniemi 2005).

The control of the larval position, both vertical and horizontal, depends on the larval swimming abilities. The swimming abilities of reef fishes have been subject of recent research. Swimming speeds, direction and sustained swimming have been investigated, mainly in coral reef species, both under controlled conditions (Leis and Stobutzki 1999; Stobutzki 1998; Fisher *et al*. 2000; Fisher and Bellwood 2002, 2003; Fisher and Wilson 2004; Fisher 2005) and with *in situ* observations (Leis *et al*. 1996; Leis and Carson-Ewart 1997, 2000a; Leis and Stobutzki 1999). The results have shown that reef fish larvae can be good swimmers and that swimming behaviour could strongly affect dispersal patterns. Fisher (2005) showed that reef fish larvae of several families were able to swim faster than currents around reefs reinforcing the potential for self-recruitment. Similar results were found for 95% of 89 species, from the Caribbean and from the GBR, investigated by Fisher *et al.* (2005). These authors also found intra and inter-specific variation in swimming performance between locations.

The swimming abilities of temperate species in nearshore environments are less understood. Leis and McCormick (2002) compared coral reef fish larvae with temperate fish larvae; from the existent literature, coral reef larvae are better swimmers than temperate larvae. However, most of the work in temperate environments has been directed to larvae hatching from pelagic eggs (e.g. Blaxter 1986). Some studies have investigated swimming abilities of temperate larvae exploring nearshore environments for species recruiting to seagrass (Hindell *et al*. 2001) and for species spawned at the coast and settling in estuaries (Trnski 2002). Dudley *et al*. (2000) investigated the swimming abilities of seven reef and two non-reef species. Five of these species were

strong swimmers; the other two reef species swam less, but were better swimmers when compared to the non-reef species (Dudley *et al*. 2000). Leis *et al.* (2006) reared three species of temperate reef fish in laboratory. Larvae with different ontogenetic stages of development were released and followed in the natural environment. In all stages larvae showed directional swimming, and the swimming speeds increased linearly with development. Sparidae larvae swum towards the shore until about the settlement stage; with this size, larvae changed direction and swum parallel to shore.

These results indicate that temperate reef fish larvae also have the potential to influence their dispersal patterns.

Reef fish larvae also seem to be able of orientation behaviours in the pelagic environment, at least prior to settlement (Stobutzki and Bellwood 1998; Leis and Carson-Ewart 2003). It is important to understand which sensory capabilities and environmental cues allow larvae to present these behaviours; settlement patterns depend on these capabilities as it occurs only after the development of the main sensory systems (Myrberg and Fuiman 2002).

Larval fishes are able of responding to different environmental cues (reviews by Montgomery *et al.* 2001, Kingsford *et al.* 2002 and Myrberg and Fuiman 2002). Given that at least late stage larvae can hear and smell reef cues (e.g. Myrberg and Fuiman 2002; Wright *et al.* 2005), odours and sounds may play an important role for some species to detect reefs, since they are perceived at longer spatial scales than, for instance, visual cues. Vision can be important at smaller spatial scales, near the reefs (Montgomery *et al.* 2001; Kingsford *et al*. 2002; Myrberg and Fuiman 2002).

The first evidence of nocturnal orientation to reefs was given by Stobutzki and Bellwood (1998). They used behavioural cages where larvae could swim towards or

against reef direction; they deployed the cages at night at the GBR. The number of larvae entering cages towards the reef was always higher than in those away from the reef, both in Pomacentridae and Apogonidae larvae. Stobutzki and Bellwood (1998) proposed that the sound rather than olfaction was the main used cue. Later, Atema *et al*. (2002) collected late stage larvae from nature; fish were allowed to swim in a choice-flume with two distinct waters flowing: lagoon water x oceanic water. They also analysed the olfactory system of larvae. Pomacentrids had no odour choice, but apogonids moved to the lagoon water. These larvae, that showed well developed olfactive organs, could use turbidity plumes that extend from the lagoon for several Km as a cue to find reefs, including the possible recognition of reef particular odours. This is indicative that chemical cues can facilitate retention or navigation towards reefs.

Larval orientation behaviour of seven reef fish species was investigated by Leis and Carson-Ewart (2003). Larvae were individually followed after release and swimming speeds were calculated; all the species showed good orientation behaviour and were good swimmers. While some larvae seemed to use a sun compass, others seemed to use "reef based cues".

Light traps have been coupled with underwater loudspeakers emitting previously recorded New Zealand temperate reef sounds (Tolimieri *et al*. 2000) Among the two most abundant taxa, Tripterygiidae larvae were primarily caught in sound traps, indicating that sound must be important as a navigation cue used by these reef fish larvae. Leis *et al*. (2002) released damselfish larvae at several distances from a boat with a speaker simulating reef sounds and artificial sounds; divers followed the larvae, recording swimming direction. Their results indicated that larvae were able to distinguish among natural and artificial sounds. The same sounds were coupled with light traps by Leis *et al.* (2003a), to compare catches between noisy and silent traps.

From the five most abundant families, Blenniids were the only larvae without reaction to the sound. Tolimieri *et al.* (2004) proved that Pomacentridae larvae are able not just of responding to reef sounds (using nocturnal chorus, a portion of reef sound produced by fishes, Leis and Lockett 2005), but also of locating the sound sources. They used behavioural cages at the field to test if larvae directed swimming towards a sound source or to the opposite side. During the night, larvae moved preferably towards reef sounds and during the day they showed no preference in swimming direction. Leis and Lockett (2005) also used five species of coral reef fishes in field test choice experiments. They found differences among species, even within the same family. Temporal variations in behaviour were also proposed to explain some patterns observed.

The ability of reacting to sound seems to be widespread among coral reef species. In a total of 20 families caught, Simpson *et al.* (2004) found that larvae of the 10 most abundant reacted to the sound. These also included Pomacentridae and Blenniidae. Simpson *et al.* (2005) coupled speakers with patch reefs built from dead coral. Larvae settled mainly on noisy patch reefs rather than on silent reefs. This was true for Apogonidae and Pomacentridae larvae but also for other less common fishes.

At least settlement stage larvae probably can use multiple sensorial systems simultaneously (Kingsford *et al*. 2002; Myrberg and Fuiman 2002). For example, Pomacentridae larvae can use solar cues during day (Leis and Carson Ewart 2003; Leis *et al*. 2003a) and reef sounds at night (Stobutzki and Bellwood 1998; Simpson *et al*. 2005).

All the above described evidences of larval retention, the awareness of the bio-physical mechanisms which can act to influence larval retention at small scales, and in

particular the role of larval behaviour, motivated Leis (2002) to propose a "New Emerging View", in opposition to the traditional "Open Population Paradigm". This new perspective considers that at ecological scales, populations can be more closed than previously assumed; at evolutionary scales, they should be considered open.

4. Ecological consequences of larval retention

Adults of many reef species are often associated with particular patchy reefs distributed in metapopulations. These can be seen as a system of discrete local populations, with a local dynamics to a large extent, but having a degree of demographic influence from other populations that is achieved by dispersal of organisms (Kritzer and Sale 2004). As it was already referred, connectivity between populations may be achieved by dispersing larvae (James *et al*. 2002; Irisson *et al*. 2004). This concept started to be recently applied in marine ecology and resource management (review by Kritzer and Sale 2004). For instance, given the potential for dispersal, interest has grown on the use of marine reserves as tools in fisheries management (Stobutzki 2001) with the source protected populations replenishing depleted populations due to fisheries.

Knowing the extent to which reef fish larvae suffer dispersal or the extent of self-recruitment and its temporal and spatial scales, is crucial to understand those patterns of connectivity among populations (Kritzer and Sale 2004). This must be taken in account when deciding the size of Marine Protected Areas (MPA´s), which must consider aspects of the population's structure and the extent of dispersal. If dispersal is

high, connectivity between protected (source) populations and sink populations can be high. Protective measures acting on the source population, will not just improve local biodiversity, but also the flux of larvae towards exploited populations enhancing their replenishment (Planes *et al*. 2000, Stobutzki 2001, Leis 2003, Shanks *et al.* 2003). In more closed populations with higher degrees of self-recruitment, the export of larvae to external fished populations is lower and the MPA will protect local populations but their will not be an increased recruitment in the fished populations (Roberts 1997; Stobutzki 2001; Leis 2002; Kritzer and Sale 2004). In this situation, the recruitment to exploited populations could be much lower than currently assumed (Swearer *et al*. 1999). On the other hand, MPA could provide a significant increase in recruitment of those self-seeding populations (Planes *et al.* 2000; Jones *et al*. 2005). It is necessary to understand differences between populations and to investigate the extent to which particular populations within a metapopulation are more closed or open (James *et al.* 2002), in order to understand patterns of connectivity and larval fluxes. Knowing these patterns, the size, positioning and number of MPA's can be decided (Cowen *et al*. 2000; Planes *et al.* 2000; Stobutzki 2001; Mora and Sale 2002; Shanks *et al*. 2003; Miller and Shanks 2004). If in theory all the populations were totally self-recruited, than the metapopulation model could not be applied in the management of MPA's.

5. Importance of larval descriptions

Given the recognized difficulty in identifying fish larval stages (Aboussouan 1989; Leis 2000), errors in identifications can lead to misinterpretations of ecological processes (Powles and Markle 1984). In particular, dispersal patterns are species specific with related species having different behavioural patterns that may affect their dispersal (Leis 1991a; Leis and McCormick 2002). However, there is a lack of good larval descriptions (Leis 2000) that should precede the construction of identification keys (Balon 1984) and could help clarifying these questions.

Ahlstrom and Moser (1981) and Moser *et al.* (1984) reviewed early work on larval development and systematics. There are good recent identification guides with descriptions for Indic and Pacific species (Leis and Carson-Ewart 2000b; Neira *et al*. 1998; Moser *et al*.1984, 1996), Western Atlantic (Fahay 1983; Richards 2005), Mid-Atlantic Bight (Several authors,1978); Southeast Atlantic (Olivar and Fortuño 1991). Except for the recent book of Munk and Nielsen (2005), in which descriptions are made for several North Sea fishes, for the Northeast Atlantic and Mediterranean, there were good descriptions in the first half of the 20th century; most of this work was grouped in Fauna Flora Golfo Napoli (LoBianco 1956) and has been reviewed by Russell (1976), and by Ré (1999) for the Portuguese species. Since then, information is very scattered and descriptions available covered mainly commercial species. Aboussouan (1989) referred, for the Mediterranean, the lack of descriptions for 209 of the 569 existent species. This lack of descriptions is particular true for nearshore reef species. For example, from the existent 1169 Blennioidei species 177 have known larvae and from the 2220 Gobioidei species 26 were described by 1985 (review by Richards 1985).

The only way of solving this problem is through complete descriptions of larval development for the different species. These descriptions may be made from larval rearing under controlled conditions (Ahlstrom and Moser 1981; Leis and McCormick 2002, **V-A, B, C**). For reef species spawning benthic eggs it is relatively easy to identify the males guarding the eggs. Batches are easily transported to the laboratory, and subsequent larval rearing is a good way to obtain complete descriptions. Analysing series from plankton collections using the series method (Neira *et al*., 1998) and the dynamic approach method to describe ontogenetic events (Ahlstrom and Ball 1954; **V-D**) is another way of obtaining detailed descriptions. This technique combined with genetic molecular tools to assign certainty in the species identification (by comparing genetic information of the larvae to reference data of known adults) is a promising tool for future research (**V-D**, Taylor *et al.* 2004).

6. Study Area

The Arrábida Marine Park is located in the Portuguese high energetic western coast, but presents calm conditions all year, making it a suitable study area of nearshore communities. There is a highly diverse fish community: 106 of the known 230 species usually occurring in shallow waters could be found at the Arrábida Marine Park (Henriques *et al*. 1999). The sheltered location, the high diversity of rocky habitats alternating with sand areas and the proximity to the Sado estuary have been proposed to explain the high diversity found (Henriques *et al*. 1999). Another possible explanation could be the geographic location of this area: it is the southern limit of distribution for

some cold water species and the northern limit for others, making the region a "transition zone" (Henriques *et al*. 1999).

These reef fish populations are separated from other rocky areas by sandy bottom areas extending north and south by several Kilometres. It can thus be considered to have a certain degree of isolation (*sensu* Sponaugle *et al*. 2002). There are several species reproducing in the spring/summer period, when upwelling occurs and fertilizes waters (Fiúza 1984). Most of these species spawn demersal eggs having male parental care. Large schools of larvae of these species have been observed by divers very close to the adults' habitats. These observations motivated the investigation of the larval dispersal patterns, considering the scale of our study area as the range of the local populations of the rocky reef fishes.

7. Objectives

With the general goal of studying composition of the assemblages and investigating possible retention patterns for some reef species, the specific objectives developed in this study were to:

1- investigate composition of larval assemblages and larval abundance occurring at the extreme nearshore, near the reefs (Chapters **II, III, IV**);

2- understand if the temporal patterns of larval assemblages reflect the breeding seasons of nearshore reef associated species (Chapters **II**, **III**);

3- study the horizontal distribution of reef fish larvae with an increased distance from shore (Chapter **III**);

4- search for specific and ontogenetic larval vertical patterns of distribution near the reefs that could potentially help reducing offshore dispersal (Chapter **IV**);

5- investigate if the pelagic cycle of some reef species possibly retained nearshore can be completed near reefs (Chapter **II, III** and **IV**);

6- describe larval stages of reef fish species present at the Arrábida Marine Park (**V**).

Larval composition and temporal patterns of larval assemblages were investigated for the first time at the very nearshore environment of Arrábida Marine Park. Sampling was performed during 29 months. This allowed the identification of clear seasonal patterns in the assemblage composition, abundance and diversity patterns. Results are discussed in the light of other nearshore studies and with the species composition and reproductive patterns of the adults (Chapter **II**).

Horizontal patterns of larval dispersal were studied (Chapter **III**) focusing the spring and summer period. Larval assemblages were investigated in relation to their composition, abundance and diversity in samples taken along the shore line, at two distances from shore: a) the very nearshore and b) at two miles from shore. Their temporal structure was also studied, from May to October. Variation of larval assemblages along transepts perpendicular to the shore was also investigated in July. The results are discussed in relation to possible larval retention nearshore.

In chapter **IV** specific and ontogenetic vertical patterns of reef fish larvae were studied. This was possible comparing, at a very small spatial scale, the structure of surface and bottom larval assemblages, near the reefs where the adults live. The results are discussed in relation to possible larval retention near reefs.

Chapter **V** gives a small contribute to the best understanding of the pelagic phase of reef fishes. Embryonic and larval development for some reef species is described in detail. This is the first step to solve the problem of larval identification, which is crucial to best understand dispersal patterns at the species level. These kinds of studies may also be considered as a baseline for future research on the ontogenetic development of sensory structures and behaviour that may influence dispersal patterns of these larvae.

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II. TEMPORAL DYNAMICS OF VERY-NEARSHORE ICHTHYOPLANKTON ASSEMBLAGES AT THE ARRÁBIDA MARINE PARK

Rita Borges, Radhouan Ben-Hamadou, M. Alexandra Chícharo, Pedro Ré & Emanuel J. Gonçalves (*to be submitted*)

ABSTRACT

In order to investigate composition, spatial and temporal dynamics of very-nearshore fish larval assemblages at the Arrábida Marine Park, monthly sampling was performed at depths shallower than 13 m over two sampling periods (May 1999-October 2000 and December 2002- November 2003). Spatial homogeneity between stations existed, although alongshore currents prevail at the Arrábida Marine Park. The highly diverse larval assemblage was composed mainly by larvae of nearshore and coastal species. Inter-annual differences were detected, with lower values of larval abundance and diversity in the year 2000. Higher values of diversity were found in the spring and summer period. Total larval abundance was also high during this period and reached the maximum during the sardine peak in autumn months. Temporal succession of larval assemblages showed a clear seasonal pattern. Most larvae were in the pre-flexion stage, indicating that the Arrábida Marine Park is a spawning location for several species. Results are discussed in relation to possible factors explaining inter-annual differences and species breeding seasons.

INTRODUCTION

In temperate waters most work on fish larvae has been centred on pelagic or soft bottom associated species, rather than rocky reef species (Leis and McCormick 2002). In the North-Eastern Atlantic and Western Mediterranean most studies dealt mainly with commercial pelagic species, in particular clupeoids (e.g. Palomera 1991, 1992; Coombs *et al*. 2001; Olivar *et al*. 2001). Additionally, larval composition and temporal fluctuations of offshore assemblages have also been studied by several authors (Russell 1973; Fives and O'Brien 1976; Suau and Vives 1979; Dicenta 1984; Tully and O'Céidigh 1989; Sabatés 1990; Horstman and Fives 1994; Olivar and Sabatés 1997; Acevedo *et al*. 2002; Lee *et al*. 2005). On coral reefs, there are a few studies on temporal fluctuations of larval abundance which show that those fluctuations are highly correlated with the patterns of reproduction of species in the assemblages, although the magnitude of this influence is less predictable (Cowen 2002). Clear seasonal or monthly patterns of variation in species composition have thus been described for coastal systems, but most studies have largely ignored the very-nearshore component of the larval assemblages.

Nearshore waters are many times difficult to sample due to shallow depths, complex bottom topography and wave action, which challenge the use of traditional sampling methods (Smith *et al.* 1987). In temperate systems, recent work focusing on nearshore assemblages have shown that assemblage composition may be very different from nearby offshore waters and that both spatial and temporal patterns of variation are operating at different scales (Palomera and Olivar 1996; Tilney *et al*. 1996; Harris *et al*. 1999; Hernández-Miranda *et al*. 2003; Sabatés *et al*. 2003; Velez *et al*. 2005). In some

cases, nearshore assemblages contained species that were never found in adjacent offshore waters.

Small scale studies have been identified as more appropriate to sample all species and developmental stages in these nearshore assemblages (Kobayashi 1989). Small scale studies are also crucial to better understand the patterns of dispersal and temporal variation. In particular, results obtained by large-scale studies may be confounded by a number of factors operating at a smaller scale in these larval assemblages (Gray 1996). Further research is however needed to evaluate the importance of the temporal dynamics in assemblage composition on the spatial distributional patterns of species at ecologically relevant scales (Gray 1996; Lee *et al*. 2005).

A central factor influencing the temporal and spatial patterns of variation in larval assemblages is the duration of the spawning season for each species. It is expected that species with broader spawning seasons will present higher recruitment variability, a factor which can be influenced by a large number of physical processes occurring at small to medium spatial scales (Cowen 2002). There is therefore a need to increase our knowledge on the scales of temporal and spatial variation of the very-nearshore fish larval assemblages which will help to understand the mechanisms operating on these systems.

In Portuguese waters several ichthyoplankton studies have been conducted in estuarine environments (Ramos *et al*. 2006; older work reviewed by Ré 1999) or

offshore waters (Ré 1984, Afonso 1989, 1995; John and Ré 1993; Lopes and Afonso 1995). Nearshore larval assemblages have not been studied. The rocky temperate shore of the Arrábida Marine Park presents excellent conditions to undertake this type of studies. In this paper we aim at: i) describe the composition, abundance and diversity of the very-nearshore fish larval assemblage; ii) study the spatial and temporal patterns of variation in the assemblages; iii) investigate the occurrence and distribution of larval developmental patterns for the most abundant species.

MATERIALS AND METHODS

Study site

Located on the Portuguese west coast (30 Km south of Lisbon), the Arrábida coastline faces south, being protected from the prevailing north and northwest winds and waves (Figure 1). Samples were collected at a total of 17 stations in the Arrábida Marine Park, between Sesimbra and Portinho da Arrábida (8º58'40" to 9º04'20" W and 38º26' to 38º27' N). Relatively calm conditions exist throughout the year, allowing sampling very close to the shoreline since wave action is negligible. Tidal currents parallel to shore prevail in this area. The adjacent mountain chain of Arrábida is characterized by high vertical calcareous cliffs which promote the occurrence of highly heterogeneous rocky subtidal habitats composed of boulders of many different sizes, resulting from the disintegration of these cliffs. Coastline orientation, habitat diversity and biogeographic position of this part of the Portuguese coast (an important biogeographic transition zone between warm and cold temperate fish faunas) sustain high levels of biodiversity for rocky shore fish assemblages (Henriques *et al*. 1999; Gonçalves *et al*. 2003).

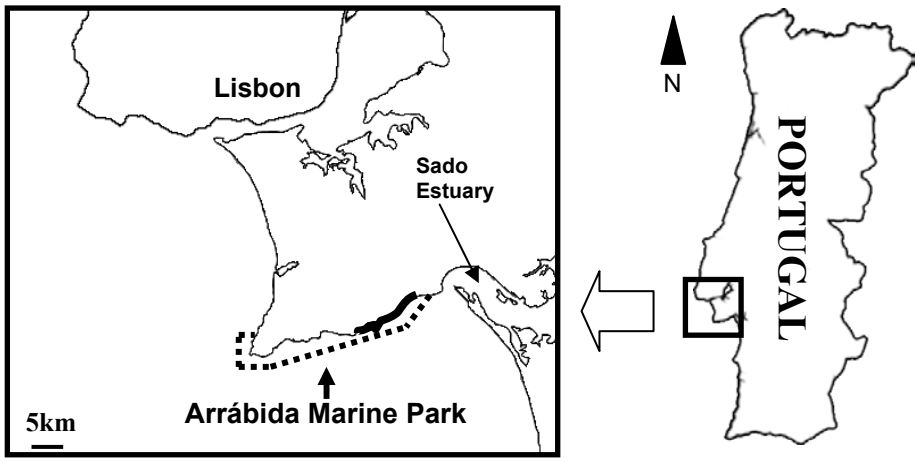

Figure 1 - Study site location. Dashed line indicates the limit of the Marine Park. Solid line signals the study location

Sampling Procedures

Monthly sampling was performed in the very-nearshore (<50m from shore) over rocky reefs at depths shallower than 13m, over two sampling periods: from May 1999 to October 2000 (with the exception of October 1999 which was not sampled due to logistical constraints) and from December 2002 to November 2003 (Table 1). In 1999 and 2000, 15 to 31 samples were taken each month. In the 2002/2003 period, sampling was reduced to 9 samples per month (sampling was only done in the central part of the study area). All samples were taken during the day and consisted of five minute sub-superficial (1 m depth) trawls using a standard plankton net with a 350µm mesh size, 0.30 m mouth diameter and a 1:5 mouth diameter to net length ratio. A small 4.6 m semi-rigid inflatable boat towed the net at a distance of 20 m and at a speed of approximately 1.5 knots. One Hydrobios flowmeter was attached to the net. Filtered volumes, and numbers of larvae caught are shown in Table 1.

Laboratory Procedures

Plankton was preserved in 4% saline formalin buffered with sodium borate for at least one month before larvae were sorted and identified under a stereomicroscope to the lowest taxonomic level possible (species level when achievable). We identified 96.5% of the larvae to family level, 77.7% to genus level and 61.2% to species level.

Table 1- Sampling dates, volume filtered and mean number of larvae for the three years studied

			Volume				N larvae			
Month	**Sampling Days**	**N**	**Mean**	**SD**	**Range**		**Mean**	**SD**	**Range**	**Total**
May 99	26 May, 2 Jun	16	**31.31**	6.54	18.34 - 42.58		**39.13**	35.25	12 - 164	626
Jun 99	22 Jun	15	**22.83**	6.04	12.87 - 34.99		**14.80**	6.12	3 - 24	222
Jul 99	27-28 Jul	20	**22.60**	5.22	14.65 - 32.30		**19.65**	9.75	5 - 37	393
Aug 99	30 Aug	16	**22.41**	5.91	14.12 - 34.90		**9.25**	6.21	0 - 20	148
Sep 99	29 Sep, 6 Oct	31	**23.60**	6.10	13.23 - 36.07		**6.81**	5.78	0 - 26	211
Nov 99	13 Nov	15	**18.26**	7.92	8.12 - 32.15		**27.00**	21.07	6 - 91	405
Dec 99	7-8 Dec	16	**21.53**	6.54	11.56 - 35.14		**7.56**	7.30	0 - 22	121
Jan 00	05 Jan	15	**27.33**	4.04	20.82 - 33.82		**9.33**	9.83	1 - 28	140
Feb 00	04 Feb	15	**29.05**	5.17	24.24 - 41.16		**42.93**	45.52	8 - 170	644
Mar 00	01 Mar	15	**21.44**	3.54	15.23 - 28.01		**5.93**	4.04	1 - 14	89
Apr 00	29 Mar	15	**33.27**	3.42	29.45 - 43.39		**6.47**	5.94	1 - 22	97
May 00	03 May	15	**28.96**	2.39	25.34 - 33.87		**16.80**	7.66	6 - 31	252
Jun 00	31 May, 08 Jun	15	**29.87**	7.71	18.41 - 49.43		**9.93**	5.04	5 - 23	149
Jul 00	11 Jul	15	**26.87**	5.43	15.12 - 36.75		**21.33**	10.24	6 - 41	320
Aug 00	01 Aug	15	**28.73**	3.52	23.45 - 36.56		**5.13**	3.44	1 - 15	77
Sep 00	4, 6 Sep	29	**28.90**	3.20	20.95 - 33.89		**0.34**	0.72	0 - 3	10
Oct 00	10, 18 Oct	15	**29.66**	5.04	19.40 - 37.15		**2.33**	2.06	0 - 7	35
Dec 02	04 Dec	9	**32.50**	1.61	29.84 - 35.27		**6.78**	7.19	1 - 24	61
Jan 03	14 Jan	9	**29.63**	3.11	24.68 - 34.06		**3.00**	2.12	0 - 7	27
Feb 03	12 Feb	9	**28.70**	1.96	24.90 - 31.45		**5.56**	2.13	3 - 10	50
Mar 03	12 Mar	9	**24.34**	2.33	21.12 - 27.14		**4.78**	4.89	0 - 15	43
Apr 03	20 Apr	9	**27.04**	1.73	25.11 - 29.90		**29.78**	19.94	5 - 60	268
May 03	13 May	9	**17.54**	5.49	11.94 - 30.41		**9.78**	5.78	3 - 18	88
Jun 03	11 Jun	9	**33.04**	1.18	30.92 - 34.61		**21.44**	11.78	11 - 46	193
Jul 03	09 Jul	9	**22.08**	3.86	16.31 - 28.31		**30.89**	17.00	9 - 56	278
Aug 03	21 Aug	9	**31.66**	4.91	25.96 - 42.75		**9.78**	8.79	1 - 24	88
Sep 03	24 Sep	9	**18.69**	11.07	11.26 - 47.65		**9.67**	7.73	4 - 27	87
Oct 03	21 Oct	9	**21.14**	5.22	15.78 - 29.79		**43.22**	20.76	20 - 83	389
Nov 03	12 Nov	9	**27.47**	4.81	19.98 - 34.69		**85.11**	53.08	27 - 171	766
	Total	401	**26.17**	6.62	8.12 - 49.43		**15.65**	22.33	0 - 171	6277

To help in the identifications, photographs were made using a digital camera attached to a stereomicroscope. Body length (BL), corresponding to notochord length in pre-flexion larvae or to standard length in post-flexion larvae, was measured to the nearest 0.01 mm using a stereomicroscope and a micrometer scale. All larval stages from hatching, including yolk-sac larvae, were considered. Only larvae of *Sardina pilchardus* from October and November 2003 were not measured. This was due to the great abundance of these larvae in those months, given time constraints and the fact that 99.6% of the larvae were in pre-flexion stage. All sardine larvae smaller than 2.75 mm BL were considered as free embryos since these larvae are known to hatch with 3.0-4.0 mm (Ré 1999) and several individuals still had the empty egg capsule attached. For the other species this distinction was not possible so every individual caught was considered as a larva. Excluding *S. pilchardus*, a total of 10.1% of larvae were damaged and were not measured. A developmental stage was ascribed to each larva depending on the notochord flexion stage in: pre-flexion, incomplete flexion and complete flexion, according to Leis and Carson-Ewart (2000).

Data analysis

The steps for data analysis are resumed in Figure 2 and are described in more detail in the following sections. Q- and R-mode clustering, their respective analysis of variance and the Indicator Values Index (Indval) calculation were performed using the MatLab statistical software. The PRIMER 5 programme was used for the calculation of diversity indices, ANOSIM and SIMPER analysis. STATISTICA 7 (StatSoft, Inc. 2004) was used for all other statistics.

Spatial homogeneity

In order to investigate spatial homogeneity of stations, each sample was assigned to one of the 17 alongshore stations (A to R) and a matrix with stations x months was created expressing mean larval density at each station for each month. For this purpose, only data from 1999 and 2000 (17 months) were used, since a broader spectrum of stations was sampled in this period. There were some missing data in the matrix since in each month not every station was sampled. Therefore, five stations located at the extremes of the sampling area were eliminated since they had a high number of missing values. The other missing data were interpolated after removing extreme values using the Kriging method (Legendre & Legendre 1998). This method allows estimation of missing data using the values of neighbouring stations as well as the values of neighbouring months. The spatial inter-relationship between these variables was measured by a cross-variogram. Q-mode clustering (Legendre & Legendre 1998) with contiguity constraint for each month was calculated based on the Bray-Curtis similarity index. Taking into account that the minimum grouping similarity among sites was 0.14 %, prior to multivariate analysis, transformations were applied and, to assess the multinormality of the transformed data, the Mahalanobis generalized distance D^2 was computed. A normal probability plot was used to test the good-fitting of the Mahalanobis distances to normality (Dagnelie, 1975).

Seasonal patterns

After determining that spatial homogeneity between stations existed, means were calculated between stations to obtain a 2-D matrix with species x months (Figure 2), in order to investigate temporal patterns of variation. A Q- mode clustering

(Legendre & Legendre 1998) was used to group months in seasons, based on the Bray-Curtis similarity index.

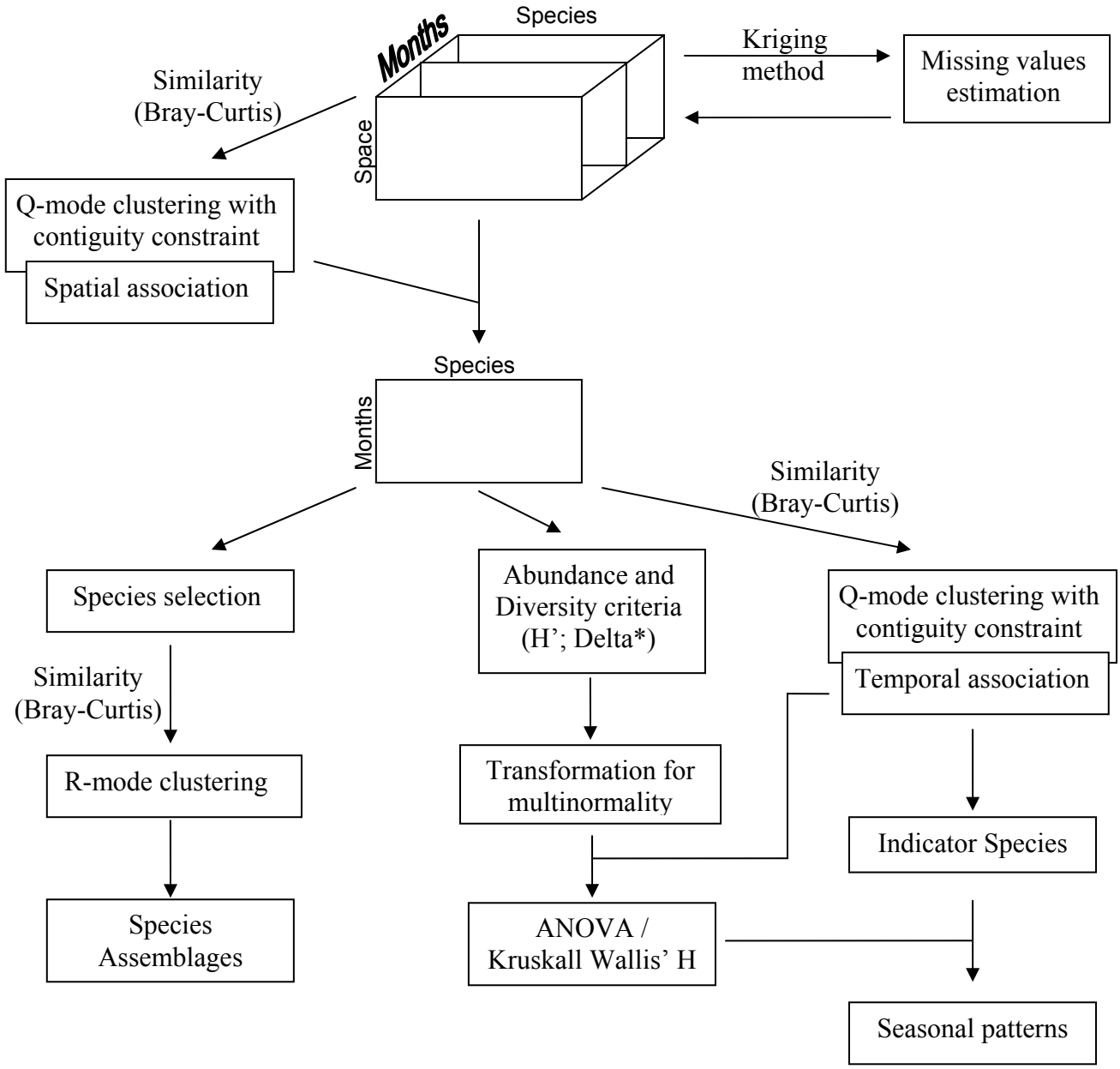

Figure 2 - Resume of the overall analysis used in this study.

To test the significance of the clustering cut-off, the between to within ratio of variances was calculated. The pooled variance-covariance matrix was used to compute overall variances of the multivariate data. The Pillai-Bartlett trace criterion was applied, because of its robustness versus the Wilk's lambda test (Olson 1976). Groups of species were associated to each season using the Indicator Values Index (IndVal). This index is computed using a measure of specificity and a measure of fidelity of each species in each cluster of months (Legendre & Legendre 1998).

Abundance and Diversity

Larval abundance was calculated for every taxa identified in each sample and is expressed as the number of larvae per 1000 m^3. Two biodiversity indices were calculated for each sample: the Shannon Diversity Index (H') using the natural logarithm in its formulation and the Average Taxonomic Distinctness Index (Delta*) which reflects the taxonomic spread of species among samples (Clarke and Warwick 2001). Delta* is based not just on the species abundances but also in the taxonomic distances between every pair of individuals. When compared to other biodiversity indices based on species richness, it presents advantages since it does not dependent on sample size. Since this index reflects phylogenetic diversity in samples, it can be used for instance in comparisons between different habitats (Clarke and Warwick 2001). High Delta* values (max=100) are associated with high taxonomic diversity in the assemblage (Clarke and Warwick 2001). Equal step-lengths were assumed between each taxonomic level, with four levels used (from species to order).

Months were grouped in seasons according to the seasonal patterns obtained in the temporal association. Seasonal differences in total larval abundances and diversity *indices* were tested using One-way ANOVA or Kruskall-Wallis ANOVA when heteroscedascity assumptions were not met even after log (x+1) transformation. Post-hoc comparisons were performed with the Newman Keuls or Dunn's test, respectively. Data from all years were pooled together in these comparisons since the same months were not always sampled in each year, which could confound a between-year comparison. Inter-annual differences in total larval abundance and diversity *indices* were calculated for each season or individual months (when the same month was not sampled in all years) using the same criteria. For each season or month, inter-annual differences in assemblage composition were tested using ANOSIM, based on a triangular matrix of Bray-Curtis similarities between samples. When differences between groups were detected, the SIMPER procedure was used to find which species better explained the observed differences (Clarke and Warwick 2001).

Assemblage structure

A matrix with the mean density for each species at each of the 29 sampled months was created to analyse assemblage structure. Only the most frequent and abundant species were used, selected following the criteria of Souissi *et al*. (2001): i) species present in less than 5% of the samples were eliminated; ii) from the remaining species, the sum of the total abundance for each species was computed; iii) after this, species were ranked following their contributions to the global sum of the data and species contributing for less than 0.5 % of the total abundance were eliminated. Species R-mode clustering based on the Bray-Curtis similarity index was applied in order to

identify groups of species. A MANOVA of the pooled variance-covariance matrix was used to compute overall variances and the Pillai-Bartlett trace criterion was applied.

RESULTS

Spatial and temporal association

Homogeneity between stations was tested with a Q-mode cluster (Figure 3). No multivariate outliers were detected from the normal probability plot. These results indicate that there was high degree of homogeneity between stations, which validates their use as replicates.

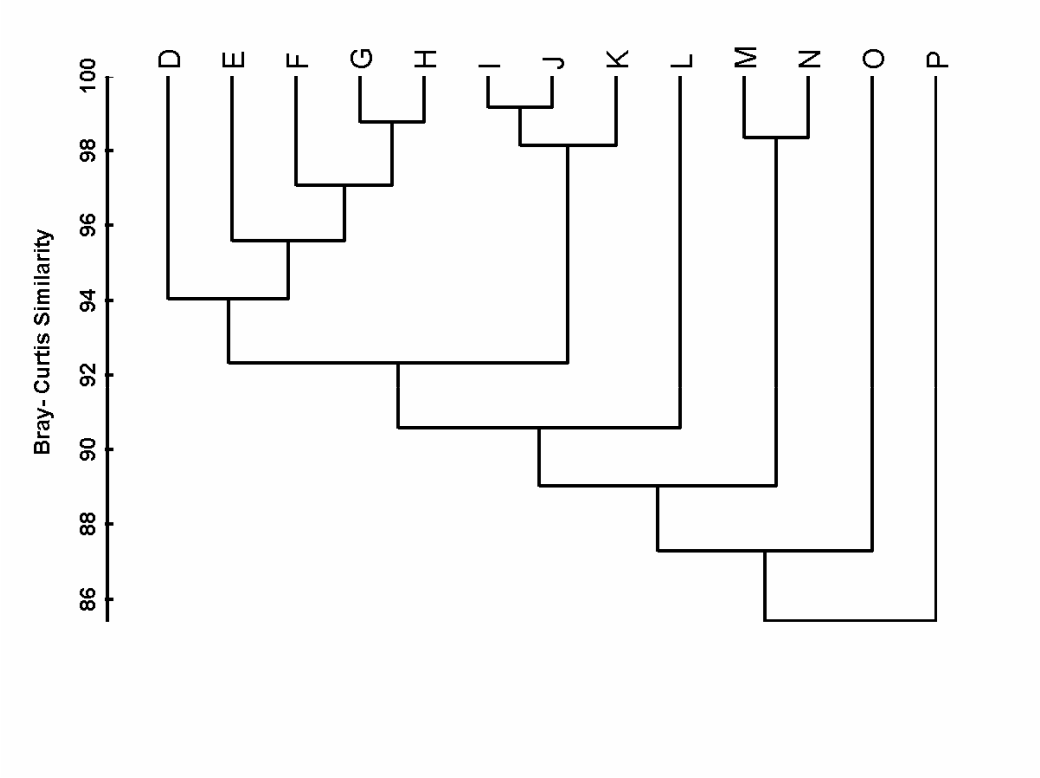

Figure 3 - Spatial aggregation of sampling stations as defined by the Q- mode clustering.

A cluster analysis of the temporal data clearly separated 5 groups of months (Figure 4). Season 1 isolated March from the other months. Season 2, contained the

spring and early summer months (April-July). Late summer months (August-September) were aggregated in Season 3. Season 4 contained the autumn months (October-November), and Season 5 included the winter months (December- February).

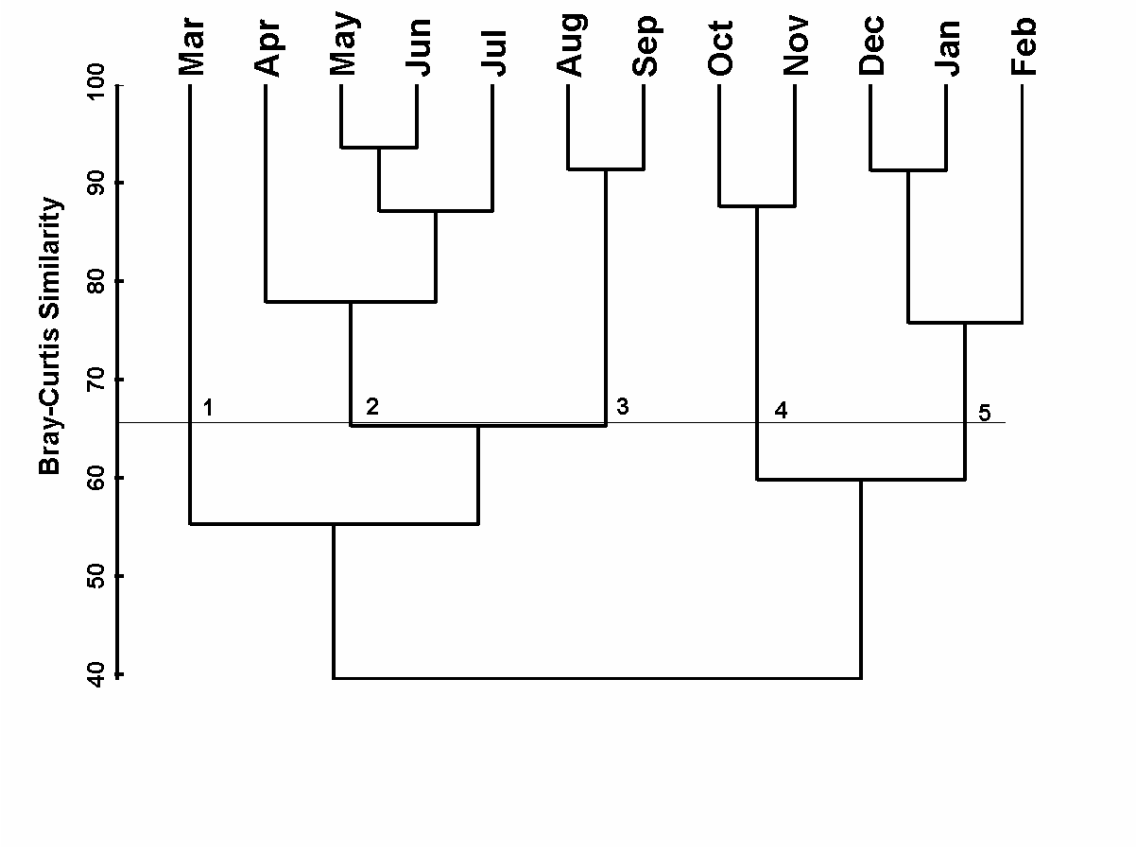

Figure 4 - Seasonal grouping of months as defined by the Q-mode clustering (Pillai's trace = 0.665 ($P< 0.01$)

Assemblage Composition

A total of 6277 larvae were identified, belonging to 85 taxa from 29 families (Table 2). The assemblage was composed mostly by coastal species. Larvae from species living in the pelagic environment over shelf waters (e.g. *S. pilchardus* and carangids) and in estuaries (*E.encrasicolus*) were present, but most larvae belonged to species whose adults live in nearshore rocky bottoms (blennies, gobies, labrids, among

Table 2- Species composition and abundance (expressed as number of larvae 1000^{-3}) of larval assemblages in each season as defined by the Q-mode clustering.

	Season	Apr-Jul 99 (N=51)		Aug-Sep 99 (N=47)		Oct-Nov 99 (N=15)		Dec 99- Feb 00 (N=46)		Mar 00 (N=15)		Apr-Jul 00 (N=60)		Aug-Sep 00 (N=44)		Oct-Nov 00 (N=15)		Dec 02- Feb 03 (N=27)		Mar 03 (N=9)		Apr-Jul 03 (N=36)		Aug-Sep 03 (N=18)		Oct-Nov 03 (N=18)		
		Mean	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	Relative Abund
Ammodytidae	*Ammodytes tobianus*			**0.59**	4.04	**6.99**	27.06	**376.26**	795.93	**2.49**	9.66							**29.27**	52.66	**19.41**	33.12							0.0492
	Ammodytidae n.i.			**0.89**	6.13			**2.67**	14.23	**3.56**	13.79																	0.00080
	Gymnammodytes semisquamatus									**2.38**	9.22	**1.68**	7.38															0.00046
Atherinidae	*Atherina presbyter*	**4.76**	14.03							**3.77**	14.58	**4.15**	13.04									**12.22**	21.09					0.00280
Belonidae	*Belone* spp.	**1.02**	7.26	**0.90**	6.14			**0.99**	6.71			**3.56**	14.15									**0.86**	5.18					0.00082
Blenniidae	*Coryphoblennius galerita*	**6.42**	19.14									**16.21**	33.58	**6.26**	14.84	**1.96**	7.60					**9.45**	28.71	**25.52**	65.94			0.00740
	Lipophrys pholis									**6.22**	16.44							**1.28**	6.66			**0.86**	5.18					0.00094
	Lipophrys spp.																	**1.25**	6.49									0.00014
	Lipophrys trigloides									**3.77**	14.58																	0.00042
	Parablennius gattorugine	**4.46**	16.39							**3.56**	13.79	**5.66**	20.43									**10.31**	34.23					0.00270
	Parablennius pilicornis	**41.08**	76.76	**11.14**	27.25	**2.07**	8.03	**0.66**	4.47			**104.99**	159.67	**5.29**	13.81			**3.72**	10.85	**73.65**	107.04	**176.80**	378.82	**33.33**	81.96			0.05092
Bothidae	*Arnoglossus* spp.	**19.78**	33.31					**4.76**	14.86			**9.30**	23.98	**0.84**	5.58							**4.70**	16.07	**3.58**	15.21	**2.02**	8.58	0.00506
Callionymidae	*Callionymus* spp.	**32.09**	93.30					**6.77**	17.02	**10.74**	22.99	**4.98**	14.79	**1.73**	8.02							**8.50**	23.08					0.00729
Caproidae	*Capros aper*	**9.11**	25.29	**2.80**	13.46							**1.44**	8.00											**3.72**	15.77			0.00192
Carangidae	Carangidae sp1							**2.34**	11.23			**0.71**	5.50													**32.56**	62.52	0.00400
	Carangidae sp2																	**1.05**	5.46									0.00012
	Trachurus mediterraneus													**0.88**	5.84													0.00010
	Trachurus spp.	**4.22**	13.09					**8.36**	20.70	**2.38**	9.22	**0.59**	4.55													**4.05**	17.17	0.00220
	Trachurus trachurus	**9.70**	25.12									**0.58**	4.51	**3.32**	10.69							**5.28**	13.87	**9.40**	20.67			0.00318
Clupeidae	Clupeidae n.i.			**0.83**	5.66																							0.00009
	Sardina pilchardus	**108.55**	156.14	**121.71**	160.07	**1555.05**	1514.36	**168.89**	236.40	**129.84**	139.61	**53.65**	86.94	**10.24**	29.05	**27.49**	44.00	**59.33**	136.39	**58.93**	85.75	**91.85**	184.45	**122.99**	223.98	**2242.52**	1413.02	0.53430
Engraulidae	*Engraulis encrasicolus*	**49.17**	79.44	**90.30**	132.77			**7.09**	27.36			**0.58**	4.47	**0.75**	4.98	**2.64**	10.22					**31.92**	72.05	**9.61**	19.56			0.02160
Gadidae	Gadidae sp1									**3.14**	12.18																	0.00035
	Gadidae sp2							**0.67**	4.54									**2.71**	9.80									0.00038
	Pollachius pollachius									**4.76**	18.43							**1.14**	5.94									0.00066
Gobiesocidae	*Lepadogaster lepadogaster*	**4.74**	15.51									**0.58**	4.53							**9.04**	18.01	**4.89**	15.25					0.00217
	Lepadogaster purpurea																	**6.43**	19.65							**1.86**	7.87	0.00093
Gobiidae	Gobiidae n.i.	**4.70**	14.98	**2.29**	11.06	**3.17**	12.27			**3.26**	12.63	**2.15**	8.24	**0.79**	5.27							**3.73**	16.10	**5.64**	13.00			0.00289
	Gobiidae type1	**6.92**	27.49	**4.28**	14.83			**1.68**	7.96			**1.53**	6.75							**15.78**	47.35	**6.33**	20.29	**6.54**	23.05			0.00484
	Gobius cruentatus type			**0.87**	5.93	**3.42**	13.23	**3.42**	14.99			**1.58**	6.97															0.00104
	Gobius niger type	**38.39**	60.38	**2.22**	8.70			**0.80**	5.44	**6.07**	16.02	**16.50**	53.25	**1.84**	8.65			**9.89**	18.28	**5.26**	15.78	**41.22**	149.10	**5.51**	17.10	**14.14**	21.82	0.01595
	Gobius spp.											**0.50**	3.88	**0.67**	4.48							**2.42**	14.54					0.00040
	Gobius xanthocephalus																					**1.69**	7.09					0.00019
	Gobiusculus flavescens											**1.12**	6.16															0.00013
	Pomatoschistus pictus	**11.58**	26.96	**26.32**	80.29	**5.45**	21.10	**0.80**	5.44			**6.82**	14.03	**0.71**	4.72							**21.90**	38.09	**3.61**	10.50	**5.87**	17.75	0.00934
	Pomatoschistus spp.			**0.76**	5.24			**1.21**	8.18					**0.87**	5.78	**3.44**	13.31											0.00071

Table 2 (cont).

	Season	Apr-Jul 99 (N=51)	Aug-Sep 99 (N=47)	Oct-Nov 99 (N=15)	Dec 99-Feb 00 (N=46)	Mar 00 (N=15)	Apr-Jul 00 (N=60)	Aug-Sep 00 (N=44)	Oct-Nov 00 (N=15)	Dec 02- Feb 03 (N=27)	Mar 03 (N=9)	Apr-Jul 03 (N=36)	Aug-Sep 03 (N=18)	Oct-Nov 03 (N=18)	
		Mean SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	Relative Abund
Labridae	*Coris julis*	**52.49** 78.45	**4.26** 17.60				**13.19** 30.40	**1.59** 7.39				**16.82** 37.52	**11.12** 22.45		0.01119
	Ctenolabrus rupestris	**0.46** 3.29						**2.45** 9.18							0.00033
	Symphodus bailloni	**0.67** 4.81													0.00008
Labridae	*Symphodus melops* type	**11.93** 33.97	**2.44** 11.80				**5.56** 15.04			**2.51** 9.05		**100.74** 133.36	**3.79** 16.09	**6.07** 25.75	0.01496
	Symphodus roissali	**6.36** 24.38					**9.78** 23.56					**5.95** 17.96			0.00248
	Symphodus spp.						**1.70** 9.73								0.00019
Lotidae	*Gaidropsarus mediterraneus*						**0.61** 4.75								0.00007
Macrorhamphosidae	*Macrorhamphosus scolopax*				**94.13** 153.74	**3.11** 12.04			**1.96** 7.60	**34.23** 72.03					0.01501
Mugilidae	*Liza ramada*			**4.90** 18.97	**2.86** 14.24				**8.53** 17.90				**4.85** 16.13	**53.14** 74.69	0.00835
	Mugilidae n.i.	**6.48** 27.85	**16.84** 28.71	**2.31** 8.94			**2.29** 10.21		**8.38** 24.23				**19.23** 38.28		0.00624
Mullidae	*Mullus surmuletus*	**2.90** 10.51					**6.64** 21.45	**0.67** 4.48				**0.86** 5.16			0.00125
Myctophidae	Myctophidae n.i.								**2.00** 7.75	**9.05** 22.55	**9.50** 28.50				0.00231
	Myctophum punctatum				**0.66** 4.47										0.00007
n.i.	Gadoide n.i.											**1.21** 7.27			0.00014
	no id. Sp 2	**0.83** 5.92													0.00009
	no id. Sp1												**4.27** 18.13	**3.71** 15.74	0.00090
Phycidae	Phycidae n.i.				**1.18** 5.63										0.00013
Scombridae	*Scomber japonicus*							**0.67** 4.48							0.00008
	Scomber spp.	**1.88** 13.44													0.00021
Scorpaenidae	*Scorpaena porcus*						**0.64** 4.93						**1.30** 5.51		0.00022
Serranidae	*Serranus atricauda*						**0.56** 4.30								0.00006
	Serranus cabrilla	**3.17** 22.64					**0.52** 4.00					**1.40** 8.42	**1.82** 7.71		0.00078
	Serranus hepatus	**1.71** 12.18	**0.74** 5.11									**0.98** 5.87			0.00039
	Serranus spp.	**97.14** 100.70	**9.75** 27.34			**2.49** 9.66	**36.63** 82.43	**7.41** 19.38			**4.87** 14.60	**16.65** 37.95	**23.14** 48.19		0.02227
Soleidae	*Microchirus variegatus*	**0.76** 5.46													0.00009
	Solea lascaris	**1.56** 7.89					**0.62** 4.80					**1.40** 8.42			0.00040
	Solea senegalensis	**9.09** 35.01	**1.91** 9.17	**5.03** 13.33		**5.45** 14.45						**2.44** 10.35			0.00269
	Solea spp.	**1.07** 7.63					**0.64** 4.93								0.00019
	Solea vulgaris								**2.00** 7.75					**14.28** 29.11	0.00183
	Soleidae n.i.	**9.06** 27.80	**1.66** 8.04	**4.15** 16.06			**1.27** 6.92					**2.40** 10.20			0.00208
Sparidae	*Boops boops*						**4.06** 23.32	**0.81** 5.38				**9.14** 37.40			0.00158
	Diplodus spp.	**18.78** 34.94	**1.71** 8.19				**14.99** 31.37		**3.44** 13.31	**1.20** 6.25		**20.80** 58.57	**40.88** 86.94	**1.86** 7.91	0.01166
	Pagellus bogaraveo				**1.88** 12.76									**1.86** 7.91	0.00042
	Pagellus sp1	**3.66** 15.75	**4.62** 17.23				**6.46** 15.93	**0.78** 5.19	**10.07** 19.02				**22.58** 45.30	**31.49** 53.52	0.00896
	Sparidae n.i.	**30.64** 51.36	**6.26** 18.54	**11.26** 30.70	**1.87** 9.71	**9.02** 18.71	**10.32** 20.45	**0.75** 4.98				**4.06** 11.73	**8.88** 20.92	**23.14** 47.83	0.01194
	Sparidae sp1	**108.31** 129.56	**6.64** 17.67		**2.41** 16.36	**20.95** 33.52	**52.61** 59.35	**2.97** 9.55				**138.65** 164.70	**14.65** 43.13	**22.99** 46.27	0.04163
	Sparidae sp2	**26.21** 41.85	**4.02** 13.35	**19.69** 37.44		**10.07** 26.61	**11.85** 29.65					**14.21** 35.09	**21.87** 49.17	**12.03** 21.15	0.01349
	Sparidae sp3		**0.89** 6.13											**21.88** 60.67	0.00256
	Sparus aurata				**0.67** 4.54										0.00008
	Spondyliosoma cantharus						**0.61** 4.69								0.00007

Table 2 (cont).

	Season	Apr-Jul 99 (N=51)	Aug-Sep 99 (N=47)	Oct-Nov 99 (N=15)	Dec 99- Feb 00 (N=46)	Mar 00 (N=15)	Apr-Jul 00 (N=60)	Aug-Sep 00 (N=44)	Oct-Nov 00 (N=15)	Dec 02- Feb 03 (N=27)	Mar 03 (N=9)	Apr-Jul 03 (N=36)	Aug-Sep 03 (N=18)	Oct-Nov 03 (N=18)	
		Mean SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	**Mean** SD	Relative Abund
Syngnathidae	*Entelurus aequoreus*							**0.81** 5.38							0.00009
	Hippocampus hippocampus				**1.88** 12.76								**1.72** 7.28		0.00040
	Hippocampus ramulosus		**1.07** 7.34		**1.34** 9.10		**0.65** 5.04	**0.90** 5.94					**4.93** 20.93	**2.78** 11.80	0.00131
	Syngnathus typhle		**0.59** 4.04												0.00007
Trachinidae	*Echiichthys vipera*	**0.92** 6.58					**0.66** 5.09					**0.90** 5.39			0.00028
	Trachinus draco	**1.05** 7.51	**3.16** 12.22				**1.38** 7.48	**3.90** 16.66				**1.21** 7.27	**19.37** 40.05		0.00338
Triglidae	Triglidae n.i.					**3.14** 12.18	**1.52** 8.41					**2.58** 11.42			0.00082
	Trigloporus lastoviza				**1.34** 9.10									**2.02** 8.58	0.00038
Tripterygiidae	*Tripterygion delaisi*	**63.02** 164.00					**14.88** 35.14				**8.19** 24.56	**148.19** 232.98	**3.02** 8.87		0.02669
n.i.	n i.	**139.97** 107.72	**20.37** 33.16		**4.88** 14.43	**25.72** 31.77	**49.19** 65.10	**10.19** 24.10	**9.29** 15.98	**7.02** 13.40	**4.09** 12.28	**50.02** 83.98	**37.35** 32.34	**23.67** 34.84	0.04293
Grand Total		**956.81** 629.19	**352.82** 305.18	**1623.49** 1535.14	**702.48** 845.69	**265.90** 157.81	**488.69** 365.63	**68.11** 100.44	**81.19** 66.16	**170.07** 149.78	**208.73** 230.74	**975.57** 752.89	**474.22** 416.26	**2523.95** 1441.46	

others). From the species frequently found offshore and in deeper water (John and Ré 1993) only the sporadic presence of Myctophidae larvae was detected.

In Table 3, a comparison of the number of taxa in each taxonomic level present in this work with other studies performed on the North-Eastern Atlantic temperate regions or in very-nearshore waters is presented. There is a higher number of common families between our data and the nearshore studies performed in the Northwest Mediterranean. The great variability in the detail to which each study goes in terms of species identification, renders however any comparison of species numbers meaningless at this stage. This is an important shortcoming since better knowledge of the nearshore larval assemblages and their biogeographic variations can only be achieved with comparisons at the species level.

Table 3 – Comparison between taxa occurring at the Arrábida Marine Park (present study) and published information for Portugal and other temperate regions. First ( ) = number of species in common with the present study; second ( ) = number of species hatching from demersal eggs in common with the present study. The last seven studies investigated nearshore assemblages of rocky reefs.

NºFamilies	Nº Genera	Nº Species	Location	Season	Reference
38 (17)	54 (15)	59 (13)(0)	Aegean sea	Jun	Somarakis *et al.*, 2002
30 (18)	64 (21)	76 (18)(8)	Galway Bay, Ireland	All year	Tully and OCéidigh, 1989
30 (13)	59 (8)	73 (6)(0)	Celtic sea	Apr-Jun	Horstman and Fives, 1994
29 (18)	58 (20)	62 (15)(3)	England Coast	All year	Riley *et al.*, 1986
28 (18)	45 (15)	45 (12)(3)	Plymouth	All year	Russell, 1973
23 (13)	47 (10)	47(7)(2)	Celtic Sea	Mar-Jun	Acevedo *et al.*, 2002
22 (17)	38 (19)	45 (17)(3)	Vasque coast	All year	Dicenta, 1984
22 (16)	55 (21)	67 (16)(8)	Galway Bay, Ireland	Without winter	Fives and O'Brien , 1976
22 (14)	39 (16)	44(11)(0)	NW Mediterranean	Without winter	Sabatés, 1990
21(12)	46 (10)	53(8)(4)	Irish sea	Mar-Jul	Lee *et al.*, 2005
21 (19)	22(14)	23 (13)(0)	NW Mediterranean	May -Jun	Olivar and Sabates, 1997
19 (16)	27 (16)	27(7)(0)	Cantabric	All year	Suau and Vives, 1979
≥26 (≥7)				Summer and autumn	Afonso and Lopes, 1994
14 (11)				Autumn	Lopes and Afonso, 1995
22(21)	32 (21)	23(12)(3)		March-Nov	Afonso, 1995
29	43	49-52			**Present study**
28 (20)	31 (12)	36 (10)(0)	NW Mediterranean	All year	**Sabatés *et al.*, 2003 (only nearshore)**
28 (20)	29 (13)	29 (10)(0)	NW Mediterranean	All year	**Palomera and Olivar, 1996**
≥16≥ (8)			Gulf California	Jun-Aug	Brogan, 1994 (only near reefs)
16 (10)	18 (1)	15	New Zealand	Dec-Jan	Kingsford and Choat, 1989
32(≥8)	>17 (≥1)	≥18	New Zealand	October-May	Hickford and Schiel, 2003 (only alongshore)
14 (6)	≥23	≥23	Vancouver	Spring	Marliave, 1986

Larval Abundance

Overall mean larval abundance was higher in 2003 than in the other years. Abundance values were lower in 2000, particularly in seasons 2 and 3 (Table 2; Figure 5). Inter-annual differences in total larval abundance were significant for all seasons or months, except March (Table 4). A comparison between the five groups of seasons obtained in the cluster analysis (Figure 4) showed a significant variation in the temporal patterns of larval abundance (Table 5). Post-hoc comparisons revealed that larval density between every two consecutive seasons was significantly different, except between the winter and March.

Table 4- Inter-annual comparison of total abundance (expressed as number of larvae 1000 m-3) and diversity (Shannon H' and Delta*) for each month/season. H= value of Kruskal-Wallis ANOVA (Dunn post-hoc test); F= value of One Way ANOVA (Newman-Keuls post-hoc test); t = value of t-test for independent samples; Z = value of Mann-Whitney U test; *ns* not significant, * P< 0.05, ** P<0.01,*** P<0.001

		1999			2000			2003			Statistics		Post-hoc
		Mean	SD	N	Mean	SD	N	Mean	SD	N			
Abundance	March				**265.90**	157.81	15	**208.73**	230.74	9	t=0.72	*ns*	
	April				**198.59**	183.15	15	**1129.77**	788.14	9	t= -4.49	***	
	May-Jul	**956.81**	629.19	51	**585.39**	361.00	45	**924.17**	748.97	27	H =12.55	**	1999>2000 **
	Aug-Sep	**352.82**	305.18	47	**68.11**	100.44	44	**474.22**	416.25	18	H =44.62	***	1999>2000***; 2000<2003***
	Oct				**81.19**	66.16	15	**1995.67**	693.26	9	Z=4.02	***	
	Nov	**1623.49**	1535.14	15				**3052.23**	1818.45	9	t=2.06	*	
	Dec-Feb				**702.48**	845.69	46	**170.07**	149.78	27	t=3.19	**	
H'	March				**0.83**	0.60	15	**0.61**	0.56	8	t=0.84	*ns*	
	April				**0.91**	0.55	15	**1.55**	0.25	9	Z= -3.13	***	
	May-Jul	**1.79**	0.43	51	**1.54**	0.39	45	**1.44**	0.50	27	F =7.19	***	1999>2000 *; 1999>2003***
	Aug-Sep	**0.98**	0.47	45	**0.78**	0.62	22	**1.36**	0.41	17	H =10.07	***	1999<2003*; 2000<2003***
	Oct				**0.52**	0.54	11	**0.56**	0.30	9	Z=0.57	*ns*	
	Nov	**0.21**	0.25	15				**0.39**	0.17	9	t=-1.94	*ns*	
	Dec-Feb				**0.67**	0.46	45	**0.55**	0.43	25	t=1.10	*ns*	
Delta*	March				**75.30**	39.60	15	**59.58**	49.60	8	t=0.83	*ns*	
	April				**69.02**	37.57	15	**89.58**	2.95	9	Z= -0.66	*ns*	
	May-Jul	**83.85**	13.44	51	**79.92**	6.70	45	**77.80**	16.50	27	F =2.46	*ns*	
	Aug-Sep	**79.44**	30.32	45	**54.90**	39.24	22	**83.57**	10.09	17	H =14.16	***	1999>2000 ***
	Oct				**52.12**	50.40	11	**98.17**	1.80	9	Z=-0.87	*ns*	
	Nov	**59.81**	50.55	15				**99.4**	0.75	9	Z=-0.8	*ns*	
	Dec-Feb				**82.19**	35.82	45	**69.00**	44.46	25	Z=0.58	*ns*	

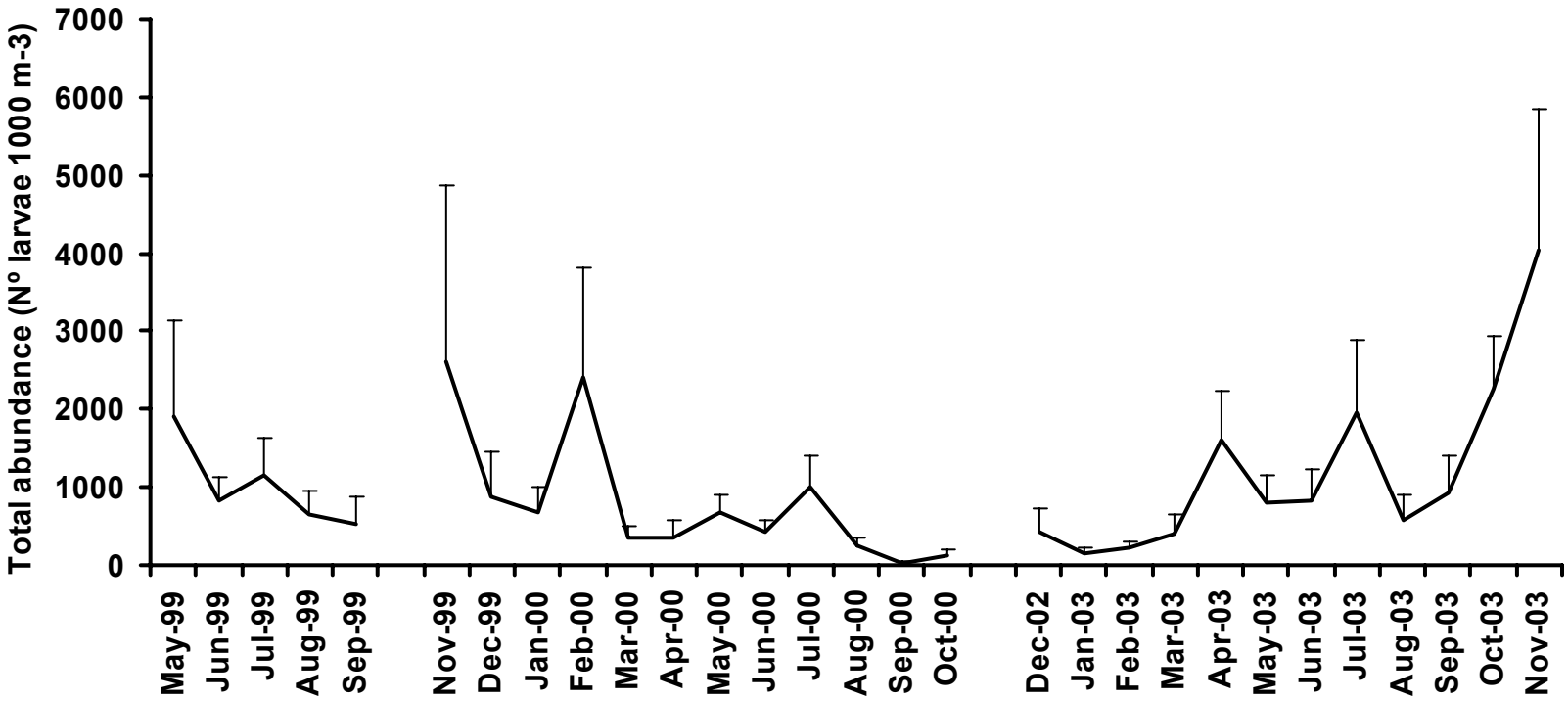

Figure 5 - Monthly variation of mean total larval abundance (expressed as number of larvae $1000m^{-3}$) for the three years studied (error bars represent standard deviation).

The temporal patterns of variation for the most representative species shown in Table 2 revealed that *Sardina pilchardus* was the most abundant species with a clear peak in the autumn. *Ammodytes tobianus* was particularly abundant in the winter months. In the spring-summer period (season 2), *Parablennius pilicornis* and *Tripterygion delaisi*, both hatching from demersal eggs, were among the most abundant species. Although 2000 was the year with the lowest overall larval abundance, some species only occurred during this year, like *L. trigloides* and *G. flavescens*, while others were more abundant, like *S. roissali*, *L. pholis*, *M. surmulletus* and *M. scolopax*. The peak in larval abundance in February 2000 corresponded to *A. tobianus*, which did not occur in 2003. Other species like *C. julis*, *C. aper*, *Callionymus* spp. and *E. encrasicolus* were more abundant in 1999.

Diversity Indices

The Shannon Diversity Index (H') revealed significant interannual differences in the spring/summer months (April-July) with 1999 higher than 2000 and 2003, and in the late summer (August-September) with 2003 higher than 1999 and 2000 (Table 4). Despite these interannual differences, the seasonal pattern of variation of H' was clear and followed similar trends between years (Tables 4 and 5). Post-hoc comparisons revealed significant differences among all seasons with the exception of the winter (season 5) which did not differ from any other seasons. There was a significant increase from March to the spring-summer months, where maximum values occurred. H' decreased to the late summer and autumn where the lowest values were observed.

Table 5- Abundance (expressed as number of larvae 1000-3 and Diversity at each season. H= value of Kruskal-Wallis ANOVA (Dunn post-hoc test); F= value of One Way ANOVA (Newman-Keuls post-hoc test) * P< 0.05, ** P<0.01,*** P<0.001.

Season	Abundance			H'			Delta*		
	Mean	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**
1	**244.46**	185.68	24	**0.76**	0.59	23	**69.83**	42.89	23
2	**770.34**	616.94	147	**1.55**	0.50	147	**80.37**	16.84	147
3	**257.94**	312.02	109	**1.01**	0.54	84	**73.85**	32.06	84
4	**1479.19**	1582.17	48	**0.40**	0.36	44	**73.83**	43.29	44
5	**505.56**	722.55	73	**0.63**	0.45	70	**77.48**	39.32	70
Statistics	H (4, N=401)= 96.94 ***			F (4, N=368)= 71.76 ***			H (4, N=368)= 47.88 ***		
Post-hoc	1<2***, 2>3 ***, 3<4*** 4>5***,1<4***,2>5***			1<2***, 1<3*, 1>4 *** 2>3***, 2>4***, 2>5*** 3>4***,3>5***,4<5*			2>4 ***, 2>5***, 3<4**, 3<5 ***		

The Average Taxonomic Distinctness Index (Delta*) varied somewhat differently. The only significant interannual difference occurred in the late summer (August-September), with a decrease from 1999 to 2000 (Table 4). Although not so evident, there was also variation across seasons. The spring/summer and late summer months differed significantly from the autumn and winter (Table 5).

Comparing the two biodiversity indices it can be seen that although H' in the autumn was about half the value found in the late summer months, mean Delta* values were very similar, although significantly different. This means that the few species which occurred in the autumn were not taxonomically related. On the other hand, spring/summer months presented the highest species diversity and taxonomic richness.

Assemblage Structure

Interannual variation in the assemblage structure was only detected in October (between 2000 and 2003) and November (between 1999 and 2003) (Table 6). SIMPER results showed that *S. pilchardus* and *Liza ramada* explained respectively 84.3% and 5.6% of the dissimilarities between 1999 and 2003 samples (average dissimilarity = 42.9), given their higher abundance in 2003 (see Table 2).

Table 6- Summary of the one-way analysis of similarity (ANOSIM) with pair-wise comparisons of larval assemblages between years for each month/season. 999 permutations were used for each test. The value of R and its significance are shown. Numbers in bold represent R> 0.5. *** P<0.001, ns not significant.

Season	Years	R	Signif.
March	2000-2003	0.33	***
April	2000-2003	0.14	*ns*
May-Jul	1999-2000	0.19	***
	1999-2003	0.30	***
	2000-2003	0.17	***
Aug-Sep	1999-2000	0.47	***
	1999-2003	0.38	***
	2000-2003	0.02	*ns*
Oct	2000-2003	**0.53**	***
Nov	1999-2003	**0.58**	***
Dec-Feb	2000-2003	0.28	***

When considering the most frequent species following the criteria described in the methods section, 24 species were considered the most abundant. A cluster analysis grouped these species in four distinct groups (Figure 6). Group I was composed of nine species that, individually, present abundances between 0.3 and 1.3 % of the total. Group II contained less abundant species. Group III was mainly composed by the Spring-Summer spawners with higher values of abundance (presenting abundances between 1 and 5% of the total). Two sub-groups within Group III could be identified: one with species having a clear peak of larval abundance in July (*P. pilicornis*, Sparidae sp1, *T. delaisi*, *C. julis* and *Serranus* spp.), and another with a broader temporal spectrum of

occurrence in the samples that peaked in May-June (e.g. *S. melops* type, *G. niger* type and *E. encrasicolus*). The fourth group was composed by autumn (*S. pilchardus*) and winter (*M. scolopax* and *A. tobianus*) spawners. The temporal variation for a few species of each group is shown in Figure 7.

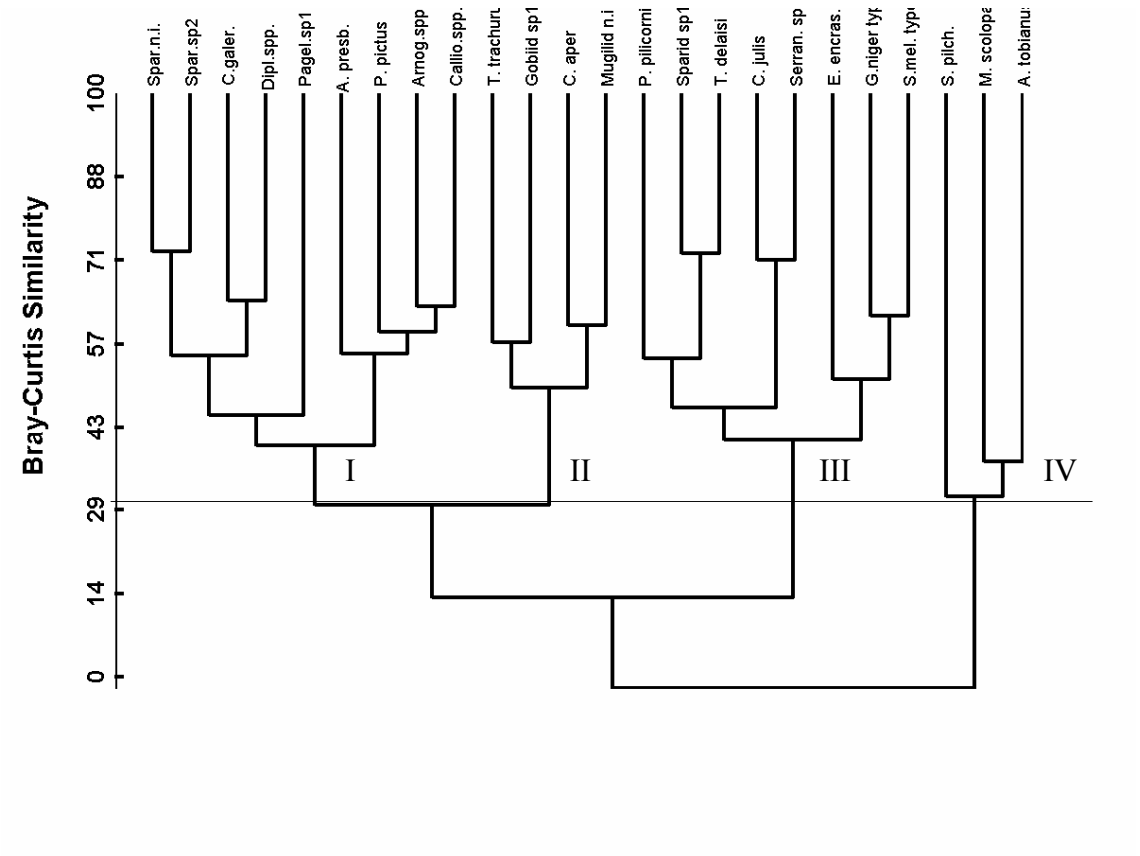

Figure 6 - Species assemblages considering the most representative species, as defined by the R-mode clustering (Pillai's trace = 0.23 ($P<0.05$).

The groupings of months obtained (Figure 4) generally agreed with the temporal occurrence of the most representative species composing the assemblages. This can be seen in Figure 8 where the association between species and seasons based on the Indicator Values Index is shown. *S. pilchardus* was clearly associated with the autumn, together with other rare species like *L. ramada* and *S. vulgaris*.

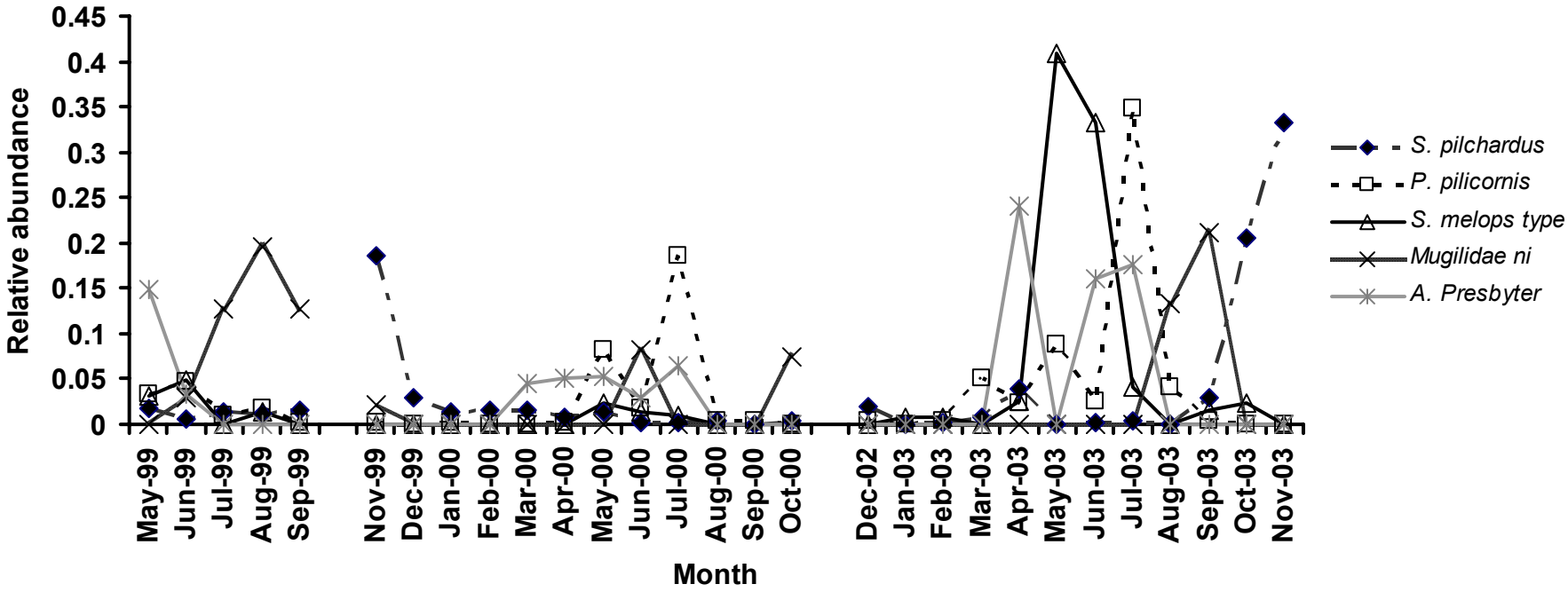

Figure 7- Monthly variation of larval abundance for selected species, from the different species assemblages.

In the winter months *A. tobianus* appeared, together with a few other species. Although March was a month with low density and diversity, it also had some indicator species, two of which are blennies from the same genus (*Lipophrys*). Associated with the spring and early summer months were species from groups I and III (Figure 6).

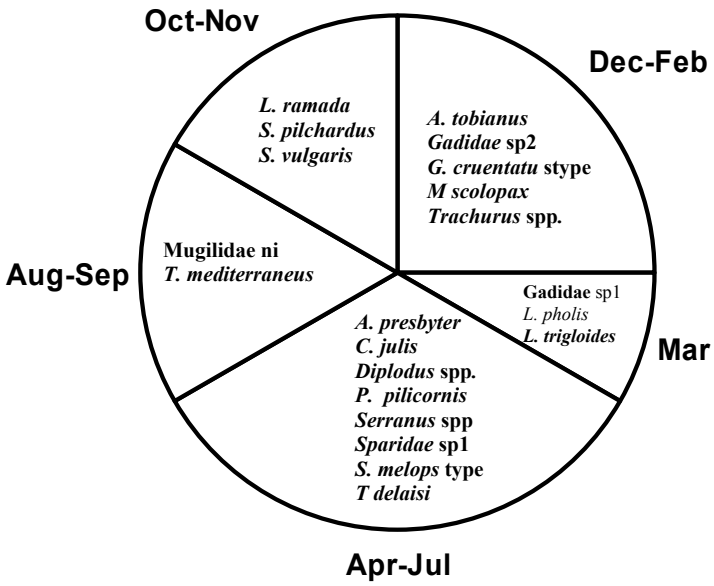

Figure 8 - Species association with seasons, according to the Indval results.

Developmental stage

Size of larvae for species with a total occurrence of more than 20 individuals is shown in Figure 9a. For most species, mean larval size (BL) was smaller than 4.0 mm BL, and the majority of larvae were in the pre-flexion stage (Figure 9b). Only a few species (e.g. *Atherina presbyter*, *Parablennius gattorugine*, *Tripterygion delaisi*, *Gobius niger* type and *Pomastoschistus pictus*) had flexion or post-flexion stage larvae.

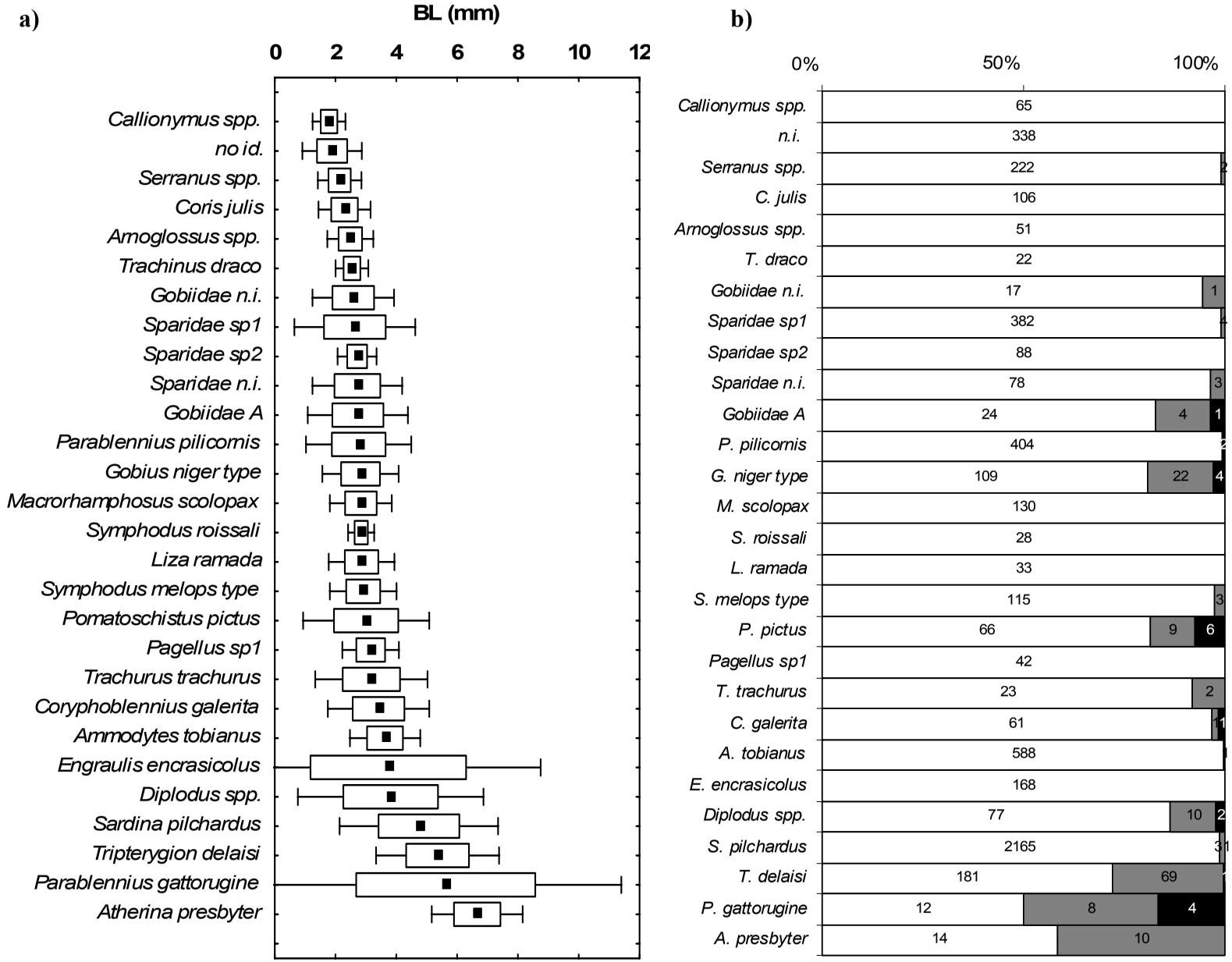

Figure 9- Developmental stage for larvae of species with 20 or more individuals. a) Mean Body Length (in mm). Central square = mean; large rectangle= mean ± S.D.; whiskers = Mean ± 1.96 S.D. ; b) Flexion stage for the larvae of the same species. White = number of pre-flexion stage larvae; grey = number of flexion stage larvae; black = number of post-flexion larvae..

DISCUSSION

In nearshore environments alongshore dispersal of planktonic organisms can occur due to advection promoted by prevailing alongshore currents near-surface (Largier 2003). At the Arrábida Marine Park alongshore tidal currents prevail (E.J. Gonçalves, personal observations). This could lead to small scale differences in larval abundance from the spawning sites to other areas. However, the results of the spatial aggregation obtained in the present study showed that alongshore surface distribution of larval abundance was homogeneous. This probably reflects the fact that all the samples were taken over the rocky bottom habitats, at a small spatial scale.

Temporal association isolated 5 groups of months, corresponding approximately to seasons; March was the only isolated month. This probably reflected the period of transition between the end of the breeding season of winter spawners and the beginning of the reproduction of Spring-Summer spawners.

Composition of the assemblages revealed a high number of taxa. The number of families was similar to the results obtained by some studies in the North Sea in more offshore waters, and higher than in other nearshore studies over rocky reefs from New Zealand (Kingsford and Choat 1989; Hickford and Schiel 2003) or Canada (Marliave 1986). This difference could be due to the fact that those studies only investigated the spring/summer period. Although some species can be only found in other seasons, it is during this period that a higher number of species is found. In our study only two families were not represented in the spring-summer period, namely Ammodytidae and

Macrorhamphosidae. This seems to indicate that there are differences in the number of families that are possibly explained by the different geographic location of these studies.

When comparing our results to those from offshore studies in the North Sea, the number of common families was lower than the number of common families between the Arrábida Marine Park and the NW Mediterranean. In fact Sabatés *et al.* (2003) and Palomera and Olivar (1996) obtained similar number of families when compared to our results, at nearshore rocky environments in the NW Mediterranean. However, a higher number of genera and species could be identified at the Arrábida Marine Park. These differences could be explained by the difficulty in identifying larvae to the species level. Palomera and Olivar (1996) and Sabatés *et al*. (2003) identified 10 species that we also caught, but also *Arnoglossus* spp, *Callionymus* spp, *Lipophrys* spp, and Blenniidae, Gobiesocidae, Gobiidae, Labridae, Mugilidae, Sparidae and Tripterygiidae larvae that were not identified to the genus or species level. These families have several species living in nearshore environments and identification to the species level would probably have increased the number of taxa in common with those of the Arrábida Marine Park.

Another possible explanation to the higher number of species found at the Arrábida Marine Park could be related to the geographic range of the species. The the northern limit of several species living in warm waters (Henriques *et al*. 1999), and the southern limit of several cold water species. This is why the adult community is so diversified (Henriques *et al*. 1999; Gonçalves *et al*. 2003). Despite these differences, the composition of larval assemblages was similar in these similar environments, with a high number of taxa common to both locations, and must reflect the typical composition of nearshore rocky reef assemblages at the latitudes considered.

Several of the species occurring at the Arrábida Marine Park, were also found in Portuguese estuaries: Ramos *et al*. 2006 found **17** species in common with our study in the Lima estuary and **22** of the Arrábida Marine Park species could also been found in other Portuguese estuaries (see review by Ré 1999). From the species frequently found offshore (John and Ré 1993) only the sporadic presence of Myctophidae larvae and *C. aper* was found. There were larvae from species that also live along the continental shelf (e.g. sardine, carangids, mugilids) and estuarine spawning species were also found (e.g. *E. encrasicolus*). The majority of the larvae occurring at the Arrábida Marine Park were, however, of nearshore living species whose adults have been classified as common or very common in the study area, living associated with the rocky bottom habitats (Henriques *et al*. 1999).

Larvae from species whose adults are considered rare at the Arrábida Marine park, could also be found in our samples (e.g. *M. scolopax*, *S. senegalensis*, or *S. hepatus*). It was the case of Syngnathidae larvae of the species *H. ramulosus*, *H. Hippocampus* and *E. aequoreus*. Henriques *et al*. (1999) registered the presence of *S. typhle* after the first record, more than a hundred years before. The presence of larvae of these species indicates that, although rare, they keep spawning at the Arrábida Marine Park.

Considering mean total abundance, values found were high, when compared to mean values obtained by Sabatés *et al*. 2003 for the NW Mediterranean (these authors found the highest winter peak in January to be less than 2000 larvae $1000m^{-3}$ versus more than 4000 larvae 1000 m^{-3} in our study, in November) and the spring peak occurred in June with about 1000 larvae $1000m^{-3}$ (vs 2000 in July 2003, in the present study). We must consider, however, that those results included not just larvae collected

nearshore but also from more offshore waters, which probably reduced the mean abundance values.

Inter-annual differences were found in total abundance, with the lowest values occurring in 2000; the overall seasonal pattern was generally similar between years: Abundance increased from March to April-July with a higher peak in July; in August-September there was always a decrease in larval abundance; In October-November the highest values of abundance were found.

Like total abundance, diversity was also lower in 2000 when compared to the other years, although taxonomic diversity did not change (except a significant decrease in August- September 2000). One possible explanation for the differences found between years could be the inter-annual fluctuation of the Atlantic North Oscillation (NAO). In fact, the year 2000 had particular high winter NAO Index (NAOI) values (2.80 against 1.70 in 1999 and 0.20 in 2003). This means that 1999 and in particular 2000 were years of strong winds, which caused strong winter upwelling (Ribeiro *et al*. 2005; Santos *et al*. 2005), while 2003 was a "normal" NAOI year. Low values of NAOI are associated with increased temperatures and decreased wind frequency and intensity; this in turn stabilizes water masses promoting a phytoplankton and zooplankton increase. NAO is known to affect temperature, wind or tidal flow that could have a more direct effect over the larval or adult populations, affecting their reproductive cycle. Inter-annual variation of the adult assemblage has also been related to the winter NAOI (M. Henriques *et al.* unpublished data). The differences found in NAO index for the years considered could be a possible explanation to the lower abundance in 2000 and higher abundance in 2003 for several species.

Maximum values of diversity occurred in April-July, in every year investigated. The highest number of taxa in the Spring-Summer has also been found in several studies (e.g. Russell. 1973; Suau and Vives 1979; Dicenta 1984; Tully and O'Ceidig 1989; Palomera and Olivar 1996; Sabatés *et al*. 2003). Ré (1984) and Afonso (1995) also obtained maximum diversity values in May and June 1981 along the Portuguese coast. These results also agree with the spawning seasons for most of the coastal species reproducing at the Arrábida marine Park (Henriques *et al*. 1999; Gonçalves et *al*. 2003)

The structure of the October-November assemblages changed inter-annually. This was mainly explained by the higher abundance of *S. pilchardus* larvae found in 2003. The lower sardine abundances of 1999 and 2000 could be related with the high value of NAO Indices in those years. Off the Portuguese coast, Borges *et al*. 2003 found the sardine recruitment to be strongly dependant of NAOI index, with a decrease in recruitment occurring during high NAO years. The strong winter upwelling event of 2000 did not affect, however, increased sardine recruitment off the northern Portuguese shore due to a less saline riverine plume (Ribeiro *et al*. 2005).

Despite the differences in abundance, the fact that the structure of the spring-summer period assemblages did not differ among years, can reflect a lower dependency of these species in relation to oceanographic factors that can vary temporally (as the NAOI). In species having more restricted spawning seasons, larvae are less exposed to those factors. This can reduce the variability in recruitment patterns (Cowen 2002).

Five distinct assemblages could be found among the most frequent and abundant species. Species groupings were clearly associated with the seasonal patterns, which were confirmed by the Indval results.

The October-November peak in abundance corresponded to the sardine peak. These larvae are in fact the most abundant species along the Portuguese coast (Ré 1984, Afonso and Lopes 1994; Lopes and Afonso 1995); it is known that sardine spawns along the shelf, with two peaks, one in the Autumn-Winter and the other in the Spring (Ré 1999; Ré *et al*.1990;). Spawning of *S. pilchardus* is more intense in the northern part of the Portuguese western coast in the autumn and in the spring in the southern region. Ré (1984) found the highest peak of *S. pilchardus* in the south of the western coast (Sines), to occur in March. However, in our study, larval density was higher in November when compared to the spring months; this pattern was consistent in 1999 and in 2003 and IndVal results clearly associated sardine to October-November, rather than to the spring months. These results agree with those of Lopes and Afonso (1995) that also found sardine larvae to be very abundant in October-November along the Portuguese shelf. The presence of high larval density for this species indicated that sardine spawning was intense in very nearshore waters at the Arrábida Marine Park. The observed peak in November also explained the segregation of sardine from the other two species of the same assemblage, *M. scolopax* and *A. tobianus* that occurred latter, in February. Ré (1984) included these two species in the group of the main winter spawners in the Portuguese coast and Ramos *et al.* (2006) also reported highest abundance values of *A. tobianus* in January- February. *A. tobianus* was much more abundant in 2000 than in 2003. This species is distributed mainly in higher latitudes and has its southern limit of distribution in South Portugal and Spain (Reay 1986). Therefore,

the high NAOI that occurred in February 2000 (Ribeiro *et al*. 2005), with its lowered water temperatures, could have influenced the observed peak of *A. tobianus* in that year.

During March, when few species are breeding, few larvae were present at the Arrábida Marine Park. However *L. trigloides* and *L. pholis* were associated with this month. In fact, the breeding of *L. pholis* at our study area occurs between December-June (Almada *et al*. 1990); Faria *et al.* (1996) referred October/Novembre-May as the breeding season for this species in Portugal and found a peak in the number of nests with eggs for *L. pholis* to occur in Jan- March.

Most of the species whose adults live associated to the rocky substratum had highest values of larval abundance during the April- July period. The IndVal revealed that most larvae associated to this season were from nearshore species living at Arrábida Marine Park, like *P. pilicornis* and *T. delaisi*, These two species were grouped in the same assemblage and in all the years sampled larvae occurred in this same season. Adults of these species are also very common at the Arrábida marine Park (Henriques *et al*. 1999). The results obtained agree with the breeding season of these species that is known to occur between February/March and August/September (Almada *et al*. 1987; Gonçalves 1997). Not so abundant but also present in our study, was the blennid *C. galerita*. Adults of this species live in the intertidal at the study area. Larvae were found between April and October which also agrees with the breeding season for this species that in Portugal extends from February-March to September-October (Almada *et al*. 1983; Almada *et al*. 1996).

We found few or no larvae from some common species that also breed in the Spring-Summer period. It was the case of the clingfishes *L. lepadogaster* or *L. candolei*; however high abundances of these clingfish larvae have been caught using light traps at the Arrábida Marine Park (unpublished data); these larvae have short PLD (Raventós and Macpherson 2001) and larvae may soon be able of active behave and stay near the adults habitats or of avoiding the net. Also larvae from gobies common in the area like *P. pictus* or *Gobius xanthocephalus* had low abundances, but can occur in dense schools near the bottom (unpublished data).

The highest densities of *E. encrasicolus* larvae were found during August-September. This result contrasts with other studies, where maximum spawning was registered in April (Ré 1984). Larval stages of these species are known to occur inside estuaries, where they present higher densities and where retention seems to occur (e.g. Ré 1984; 1990; 1996). The presence of *E. encrasicolus* at the Arrábida Marine Park may reflect the influence of the nearby Sado estuary. The lower Sado estuary seems to act as a coastal lagoon and the spring and summer flow is almost negligible (Martins *et al*. 2001, 2002), but the interaction between the estuary and the coastal zone should be investigated in future studies.

Several factors may influence the temporal occurrence of larvae. Among these are the growth and mortality patterns (Leis and McCormick 2002). These aspects could not be analyzed in the present study because only small larvae were caught. Future studies using other sampling methods should address this issue for the most abundant species. Larval growth and survival are affected by primary production and food

availability (Pérez-Ruzafa *et al*. 2004; Lee *et al*. 2005; Vélez *et al*. 2005). Planktonic larval duration and larval behaviour may also influence larval occurrence nearshore (Gray 1996; Leis and McCormick 2002; Sponaugle *et al*. 2002). On the other hand, several oceanographic features may influence the temporal patterns of larval occurrence. Upwelling events (Hernandez-Miranda, 2003; Vélez *et al*. 2005), water temperature (Walker Jr *et al*.1987; Houde and Zastrow 1993; Harris *et al*. 1999; Sampey *et al*. 2004), circulation (Koutrakis *et al*. 2004) and stratification (Lee *et al*. 2005; Vélez *et al*. 2005) are among the factors that could influence larval survival and distribution. Seasonal patterns of oceanographic conditions may affect both the survival of larvae and the reproduction of adults (Young *et al*. 1986).

Other factors should be understood in order to better explain the seasonal and inter-annual patterns of larval occurrence. These include the interaction between upwelling events and the coastline topography, the influence of the winter flow of the Sado river in productivity and spawning patterns and especially the micro scale circulation patterns at the Arrábida Marine Park.

Despite the possible influence of the above cited factors, from our results, the temporal patterns of variation in larval assemblages seemed to reflect the adults spawning patterns. The fact that most larvae were in the pre-flexion stage is indicative that these larvae were locally produced and that the high abundance found for some species is not just explained by the shallow nature of the study area. These results prove that the Arrábida Marine Park seems to function as a spawning area for most of these species.

There are several possible explanations for the absence of bigger larvae and for the low abundances obtained for some of the common species at Arrábida Marine Park: 1) larvae produced at the Arrábida Marine Park are dispersing and growing offshore; 2) bigger larvae are able of avoiding nets; 3) bigger larvae are deeper in the water column. Probably the three situations can occur and the extent to which each of them influences the pattern observed must be species specific and must be investigated. However, for some of these species hatching from benthic eggs spawned nearshore, bigger larvae have been caught near the bottom with bottom trawling, within different size classes (unpublished data); also, preliminary results from night trawling seem to indicate that most larvae caught in night samples using a bigger net, were pre-flexion stage larvae. This seems to indicate that at least visual avoidance of the net is unlikely. So, for some species, there are probably vertical ontogenetic patterns of distribution and for others offshore dispersal should be the expected pattern.

In conclusion, the Arrábida Marine Park has a high abundance and diversity of larval fishes probably reflecting the high diversity and breeding patterns of adult populations living in the area. Larval assemblages showed a temporal succession, with some species clearly associated to seasons. The temporal patterns reflected well the adults spawning patterns. Most of the larvae were small and in the pre-flexion stage, indicating that the Arrábida Marine Park is a spawning location for a diverse number of species. From these results, we propose that the presence of newly hatched larvae could be used as a good indicator of the presence and of the extent of the breeding seasons of adult populations.

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III - HORIZONTAL SPATIAL PATTERNS OF DISTRIBUTION OF NEARSHORE LARVAL FISH ASSEMBLAGES AT A TEMPERATE ROCKY SHORE

Rita Borges, Radhouan Ben-Hamadou, M. Alexandra Chícharo, Pedro Ré &

Emanuel J. Gonçalves (*submitted*).

ABSTRACT

In the Portuguese coast there are no previous studies on the composition of nearshore larval assemblages. We aimed at investigating composition and horizontal distribution patterns of larval fish assemblages and their temporal dynamics at the very-nearshore (depths shallower than 13 m) and at two miles from shore, and along transects perpendicular to the shore line. Most larvae belonged to coastal species associated to rocky reefs. Total larval abundance and diversity were higher from May to July, which agrees with the adults spawning activity. Diversity and total larval abundance decreased significantly with increasing distance from shore, both in the inshore/offshore comparison and in the transects. This decrease was evident at a very small spatial scale. Species assemblages differed in the pattern of distribution, with most species clearly associated to the extreme nearshore. The distribution patterns obtained were independent of the spawning mode of species. Results are discussed in the light of the possible physical mechanisms that can potentially act at the Arrábida Marine Park to facilitate larvae retention and the role of larval behaviour.

Key-words: larval fishes; horizontal distribution; retention; nearshore; temperate reefs

INTRODUCTION

Recruitment of reef fish populations is variable and can be strongly affected by patterns of larval supply (Victor, 1986; Doherty, 1991; Milicich *et al*., 1992; Meekan *et al*. 1993; Sponaugle and Cowen, 1996; Jenkins *et al*. 1998; Valles *et al*., 2001; Cowen, 2002; Leis and McCormick, 2002). Larval dispersal influences connectivity between reef fish populations, affecting their dynamics (Cowen, 2002; Leis, 2002; Mora and Sale, 2002; Sponaugle *et al*., 2002; Swearer *et al*., 2002; Irisson *et al*, 2004; Sale, 2004). Populations can thus be more opened or closed, depending on the scale considered (Caley *et al*. 1996; Cowen, 2002; Leis, 2002). To best understand this issue, which can have strong impact in the management of fisheries and marine protected areas (Planes *et al*., 2000; Stobutzki, 2001; Caselle *et al*., 2003; Leis, 2003; Shanks *et al*., 2003; Miller and Shanks, 2004), it is necessary to investigate the patterns of larval distribution and their temporal variation at ecologically relevant scales (Warner and Cowen, 2002). Given that local scale processes may affect dispersal of reef fish larvae (Pineda, 2000; Cowen, 2002; Sponaugle *et al*., 2002; Largier, 2003), small scale spatial studies on larval distributions can give important information about possible retention mechanisms near the adults' habitat (Cowen, 2002). Moreover, the study of small scale temporal patterns of variation in the composition of assemblages can be used to determine duration of breeding seasons and dynamics of recruitment patterns of coastal species.

There is a growing body of evidence showing that some reef fish populations may have a degree of self-recruitment higher than previously expected, leading to more closed populations at ecologically relevant scales (Jones *et al*., 1999, 2005; Swearer *et*

al., 1999; Leis and McCormick, 2002; Swearer *et al*., 2002; Taylor and Hellberg, 2003; Miller and Shanks, 2004; Paris and Cowen, 2004). Reef fish larvae must find a suitable habitat to settle after the end of the pelagic phase and remaining close to reefs may be advantageous (Leis 1991; Swearer *et al*., 1999).

Recent in situ and laboratorial studies have shown that fish larvae can have strong swimming capabilities in coral reef systems (e.g. Leis and Stobutzki, 1999, Fisher and Bellwood, 2002, 2003; Fisher, 2005), but also in temperate waters (Dudley *et al*., 2000; Leis *et al*., 2006). These larvae seem to react to different environmental factors (reviewed by Montgomery *et al*., 2001; Kingsford *et al*., 2002; Myrberg and Fuiman, 2002), including reef sounds (Stobutzki and Bellwood, 1998; Tolimieri *et al*., 2000, 2004; Leis *et al*., 2002, 2003; Simpson *et al*., 2004, 2005; Leis and Lockett, 2005) and chemical cues (Atema *et al*., 2002). These swimming capabilities can allow larvae to regulate their horizontal and vertical position in the water column, potentially affecting their retention near reefs (Fisher, 2005; Leis *et al*., 2006). In fact, the ability of larval fishes to vertically migrate is well documented for offshore waters (reviewed by Neilson and Perry, 1990), but also for the estuarine environment (reviewed by Norcross and Shaw, 1984; Boehlert and Mundy, 1988). Vertical migrations seem to allow larvae to actively select the appropriate currents for transport (Paris and Cowen 2004). Larval behaviour and other biological factors such as planktonic larval durations, size at hatching, and spawning mode of adults, can interact with physical factors, affecting dispersal in nearshore environments (Cowen and Sponaugle, 1997; Sponaugle and Cowen, 1997). For instance, particular oceanographic features such as the interaction between tidal flow and bottom topography, fronts, eddies and internal bores, can facilitate retention of planktonic organisms (Pineda, 2000; Cowen, 2002; Largier, 2003).

Several studies found cross-shelf gradients in the structure of larval assemblages (e.g. Gray, 1993; John and Ré, 1993). However most of these studies were performed at large spatial scales which miss smaller patterns that may be relevant to population dynamics. Small scale spatial and temporal patterns in the composition of larval assemblages have been a focus of attention in coral reefs (see reviews by Leis, 1991; Cowen, 2002; Leis and McCormick, 2002). If larval retention occurs near reefs, the expected horizontal patterns of distribution will be a decrease of reef fish larval abundance with increasing distances from shore, while species that spawn offshore should show the opposite trend. This was clearly described by Leis and Miller (1976) in Hawaii who found that the patterns of larval distribution were visibly associated with the mode of spawning. The inshore assemblage was mainly composed by reef fish larvae hatching from benthic eggs, while offshore larvae were mainly from species which lay pelagic eggs. Several other studies showed evidence of reef fish larvae being retained nearshore (reviews by Cowen, 2002, Leis and McCormick, 2002 and Swearer *et al*., 2002; Sponaugle *et al*., 2003; Paris and Cowen, 2004). However, the patterns obtained in these studies were found to be quite variable and species specific (Cowen, 2002).

In temperate rocky reefs, studies of nearshore larval fish assemblages' composition and dynamics are scarce. However, differences between the composition of those assemblages and the ones found offshore have been described (Marliave, 1986; Kingsford and Choat, 1989; Suthers and Frank, 1991; Brogan, 1994; Tilney *et al*., 1996; Sabatés *et al*., 2003). Some of these studies suggest that larvae from inshore species spawning demersal eggs dominate the shallow water assemblages, being more abundant

than in offshore waters (e.g. Marliave, 1986; Suthers and Frank, 1991), which indicates that larval retention is also possible in temperate reefs.

There are no previous studies on the composition of nearshore larval assemblages in the Portuguese coast. The Arrábida Marine Park has good conditions to sample and study these assemblages. A highly diverse adult reef fish community has been documented in this area (Henriques *et al*., 1999; Gonçalves *et al*., 2003), but nothing is known on the distribution of their larval stages and recruitment processes. With the general goal of studying larval dispersal patterns of reef fishes living at the Arrábida Marine Park, in this work we investigate: i) the composition and temporal patterns of larval assemblages at the very nearshore and at two miles from shore; and ii) the spatial patterns of distribution of larval assemblages with increasing distance from shore.

MATERIALS AND METHODS

The study area is located on the west Portuguese shore, at the Arrábida Marine Park (8º58'40" – 9º04'20"'W and 38º26' – 38º27'N). This area faces south (Figure 1) and is protected from the prevailing north and north-west winds by the adjacent mountain chain of Arrábida. The rocky subtidal habitat is very shallow (maximum depth about 13 m) and heterogeneous due to different sized boulders resulting from erosion of the calcareous cliffs. The rocky subtidal extends offshore for only some tens

of meters. Calm conditions exist almost all year round and wave action is negligible, allowing sampling at the very nearshore (< 50 m from shore).

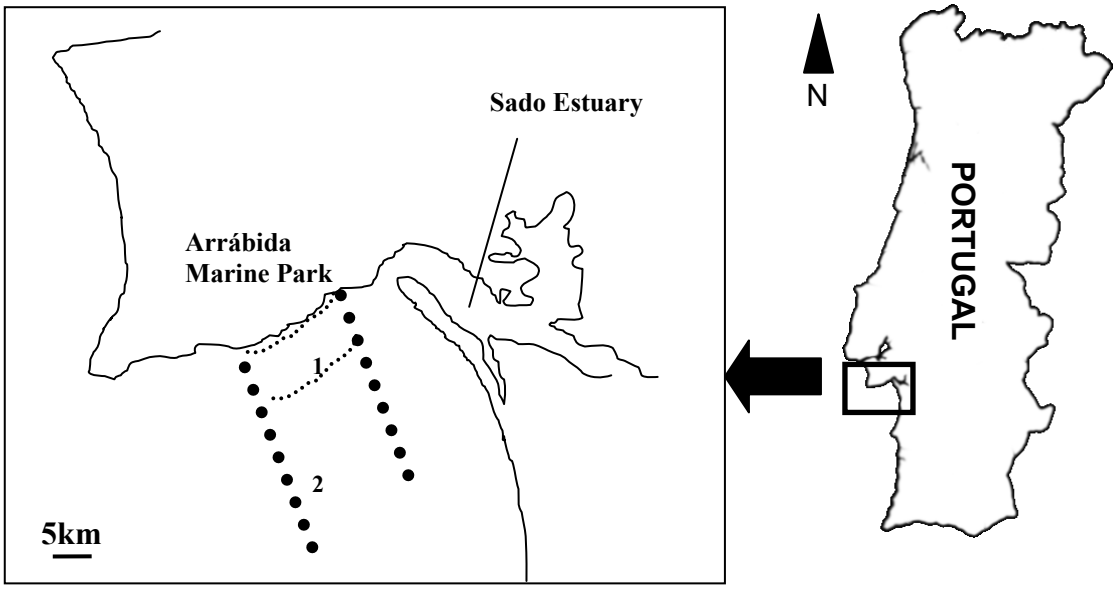


Figure 1 - Study area and the sampling stations. Small dots (1) represent the alongshore inshore vs. offshore sampling (at 0 miles and at 2 miles); larger dots (2) represent the two outer perpendicular transects.

A 350 μm mesh plankton net with 0.30 m mouth diameter and a 1:5 mouth diameter: net length ratio was towed by a small 4.6 m semi-rigid inflatable boat at a distance of 20 m from the boat, and at a speed of approximately 1.5 knots. One Hydrobios flowmeter was attached to the net to estimate the sampled volumes. Filtered volumes, and number of larvae caught are shown in Table 1. Samples were preserved in 4% saline formalin buffered with sodium borate, for at least one month before larvae were sorted and identified to the lowest possible taxonomic level under a stereomicroscope equipped with a digital camera. The developmental stage of each individual was classified as: pre-flexion, incomplete flexion or complete flexion,

according to Leis and Carson-Ewart (2000). Body length (BL) was measured to the nearest 0.01 mm, under a stereomicroscope. This measurement corresponded to the length of the notochord in pre-flexion or incomplete flexion stage larvae and to standard length in post-flexion larvae. All larval stages from hatching were considered, including yolk-sac larvae. For sardine larvae, only individuals bigger than 2.75 mm BL were considered as larvae, since hatching occurs at 3.0-4.0 mm (Ré, 1999). Smaller individuals were considered as free embryos. For the other species with larvae hatching from pelagic eggs this distinction was not possible and every individual caught was considered as a larva.

Inshore/offshore comparison

Sampling Procedure

All samples were collected between May and October 2000 (Table 1). This period corresponds to the breeding season of most reef species occurring in the study area (Henriques *et al*., 1999). Monthly sampling was performed with at least 11 samples taken each month in the extreme nearshore and at two miles offshore. Samples were collected through five minute sub-superficial tows (1 m depth), parallel to the shoreline (see Figure 1). All samples were taken during the day and at all tidal phases.

Table 1 - Sampling periods, volumes filtered and number of larvae caught in the inshore/offshore sampling and in the transects perpendicular to the shore.

				Volume		Nº larvae		
Distance	**Month**		**N**	**Mean**	**SD**	**Mean**	**SD**	**Total**
Inshore/offshore								
very-nearshore	May	03 May	15	**28.96**	2.39	**16.80**	7.66	252
(0 miles)	Jun	31May;08 jun	15	**29.87**	7.71	**9.93**	5.04	149
	Jul	11-Jul	15	**26.87**	5.43	**21.33**	10.24	320
	Aug	01 Aug	15	**28.73**	3.52	**5.13**	3.44	77
	Sep	04;06 Sep	29	**28.90**	3.20	**0.34**	0.72	10
	Oct	10;18 Oct	15	**29.66**	5.04	**2.33**	2.06	35
two miles	May	03 May	15	**31.73**	3.14	**2.93**	2.28	44
	Jun	31May;08 jun	15	**29.86**	2.86	**3.87**	2.67	58
	Jul	11-Jul	15	**23.72**	2.63	**0.47**	0.74	7
	Aug	01 Aug	14	**30.42**	3.41	**2.86**	3.23	40
	Sep	04;06 Sep	30	**33.13**	2.06	**0.00**		0
	Oct	10;18 Oct	11	**23.80**	9.79	**2.64**	1.57	29
Total			204	**29.22**	5.05	**5.00**	7.59	1021
Perpendicular transects								
very-nearshore (0 Miles)			30	**27.80**	4.59	**13.23**	11.14	397
1st Mile			17	**30.19**	5.75	**6.00**	5.57	102
2nd Mile			17	**29.32**	4.21	**2.59**	2.21	44
3rd Mile			18	**28.41**	5.24	**0.94**	0.73	17
4th Mile			17	**30.43**	2.85	**0.59**	1.00	10
5th Mile			17	**29.96**	3.50	**0.59**	0.87	10
6th Mile			17	**31.66**	3.85	**0.47**	0.62	8
7th Mile			17	**31.14**	2.54	**0.76**	0.90	13
8th Mile			17	**30.37**	3.38	**0.47**	1.01	8
9th Mile			17	**30.62**	3.01	**0.41**	0.94	7
10th Mile			16	**31.49**	3.24	**0.63**	1.31	10
Total			200	**29.96**	4.12	**3.13**	6.48	626

Data analysis

An overview of the overall analysis is shown in Figure 2. Detailed procedures are described below.

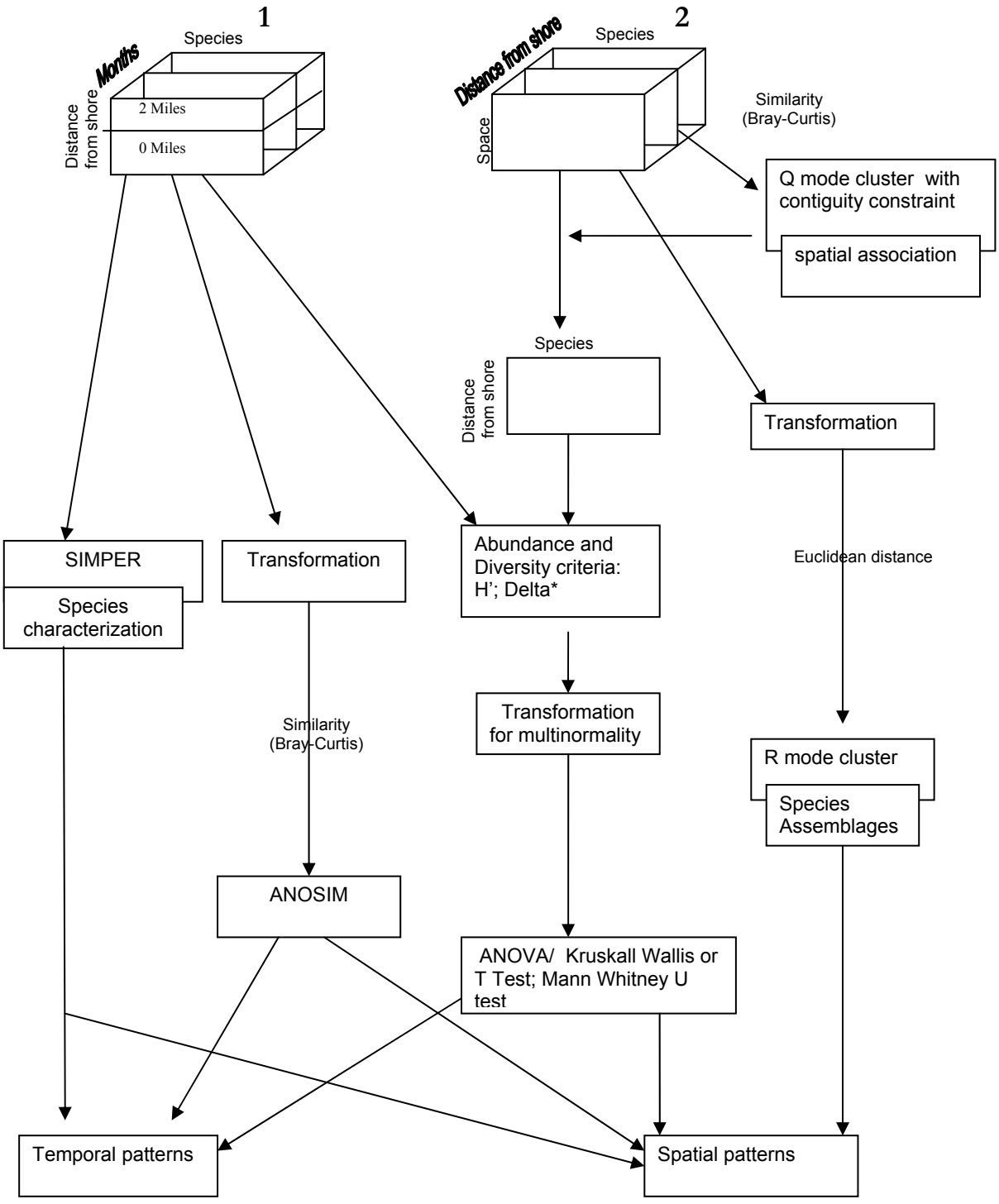


Figure 2 - Resume of the statistical analysis used in the inshore/offshore comparison (1) and in the spatial analysis of transects (2).

Total abundance and Diversity

Larval abundance is expressed as the number of larvae per 1000 m^3. Two biodiversity indices were calculated for each sample: the Shannon Diversity Index (H') using the natural logarithm in its formulation; and the Average Taxonomic Distinctness Index (Delta*) which reflects the taxonomic spread of species among samples (Clarke and Warwick, 2001). Delta* is based in the taxonomic distances between every pair of individuals. High Delta* values (maximum=100) reflect high taxonomic diversity in the assemblage (Clarke and Warwick, 2001). Four taxonomic levels were used, from species to order, considering equal step-lengths between each taxonomic level. Mean values and standard deviations of total abundance and of diversity indices were calculated for each month at each distance from shore.

In order to investigate the spatial and temporal patterns of variation in H', possible interactions between months and distance from shore were tested using a factorial ANOVA, considering months and distance from shore as factors. September was excluded from this comparison since no larvae were caught at two miles (Table 1). The Newman-Keuls test was used for post-hoc comparisons. Total abundance and Delta* at each distance from shore were tested with a Kruskall-Wallis ANOVA given that the heteroscedascity assumption was violated even after log (x+1) transformation of the data. Post-hoc comparisons were performed with the Dunn's test. Using the same criteria, t-student or Mann-Whitney U tests were used for the comparisons between inshore and offshore samples for each month.

Assemblage Structure

Samples were classified in 11 groups at each distance from shore, corresponding to each month. Abundance of each species in each sample was used to calculate a triangular matrix of Bray-Curtis similarities after a log (x+1) transformation. Non-identifiable larvae totalled 13.5%, all in pre-flexion stage and were not considered in the analysis. Differences in assemblage structure were tested with a one-way analysis of similarities (ANOSIM). High R values indicate differences between groups (Clarke and Warwick, 2001). Similarity percentages analysis (SIMPER) was used to determine the species contribution to each group, assuming a cut off at 95%.

Spatial comparison of transects

Sampling Procedure

Sampling was made along 17 transects perpendicular to the shoreline (Figure 1) between 11 July and 1 August 2000. We chose this period since July is the summer month with the highest larval abundance (see results). In each transect samples were taken within each mile from the nearshore (1st mile) to the 10th mile (Table 1). Each transect was performed along a different longitude, covering the area of the Arrábida Marine Park between the two lines shown in Figure 1). Samples collected in July and August in the very-nearshore for the inshore/offshore comparison were used as the zero mile sampling point for this comparison. The reason for this is the fact that larval density decreases abruptly during the first mile and therefore a small-scale analysis seemed appropriate (see results). The zero mile sampling point differed from the others since sampling was done alongshore instead of perpendicularly to the shore line.

Data Analysis

Total abundance and Diversity

The same abundance and diversity criteria defined in the inshore/offshore comparison were used. To evaluate grouping patterns between transect stations, a Q-mode clustering (Legendre & Legendre, 1998) with contiguity constraint for each transect was calculated, based on the Bray-Curtis similarity index, and using total abundances. Since the data are multivariate we used a pooled variance-covariance matrix to compute overall variances. The Pillai-Bartlett trace criterion was applied, because of its robustness versus the Wilk's lambda test (Olson, 1976). Diversity indices were tested across the cluster groups defined with a one-way ANOVA and total abundance was tested across the groups of miles with a Kruskall-Wallis ANOVA, since heteroscedascity assumptions were not met even after a log (x+1) transformation. For post-hoc comparisons the Newman-Keuls and the Dunn's test were used, respectively. Contour mapping was utilised to present results of the spatial patterns of total abundance and diversity, excluding four transects where there was no sampling at the zero mile and one transect without sampling at the 10^{th} mile. The resulting matrix had 11 stations (from 0 to 10 miles) x 12 transects. The SURFER software was used to display the maps. Interpolation was made with the Kriging method (Legendre & Legendre, 1998).

Assemblage Structure

The species assemblages were defined using an R-mode clustering (after Legendre & Legendre, 1998), based on euclidean distances and after normalization of the abundance values. Overall variances were also calculated with the Pillai-Bartlett

trace criterion. Total larval abundance for each group of species defined by the cluster analysis was also mapped using the Surfer Software. For those species belonging to the nearshore group (0-2 miles), small scale patterns of distribution were investigated within the group by comparing the first three stations (0, 1 and 2 miles) with a Kruskall-Wallis ANOVA or Mann Whitney U tests, since parametric assumptions were violated even after log (x+1) transformation.

RESULTS

Inshore/offshore comparison

A total of 61 taxa were identified. From these, 57 taxa occurred at the very-nearshore, comprising 27 families with 35 genera and at least 40 recognizable species. At two miles from shore, the number of taxa was lower (29) belonging to 17 families with 19 genera and at least 15 identified species (Table 2).

Diversity and Abundance

The Shannon diversity index (H') revealed a temporal ($F(4, N=121) = 6.98$, $P< 0.001$) and spatial ($F(1, N=121) = 56.22$ $P< 0.001$) pattern of variation with a significant interaction ($F(4, N=121) = 6.46$, $P< 0.001$) between these factors (Figure 3a). A significantly higher diversity was found in nearshore samples in May and July than in August ($P< 0.05$) and in May, June and July than in October ($P< 0.001$). Only June did not differ from August. At two miles from shore the only significant fluctuation was an increase in diversity from July to August ($P< 0.05$). In May, June and July diversity was significantly higher ($P< 0.001$) very-nearshore than at two miles (Figure 3a).

Table 2 - Species composition and abundance (expressed as number of larvae 1000m-3) of the very-nearshore (0 miles) and offshore (two miles) assemblages.

		0 miles		2 miles	
Family	**Species**	**Mean**	**SD**	**Mean**	**SD**
Ammodytidae	*Ammodytes tobianus*			**0.97**	9.67
	Ammodytidae n.i.			**1.05**	7.45
	Gymnammodytes semisquamatus	**0.97**	5.65		
Atherinidae	*Atherina presbyter*	**1.77**	7.97	**0.32**	3.25
Belonidae	*Belone* spp.	**2.05**	10.86	**2.70**	12.69
Blenniidae	*Coryphoblennius galerita*	**12.28**	27.61		
	Parablennius gattorugine	**3.27**	15.71		
	Parablennius tilicornis	**62.52**	131.00	**6.38**	22.15
Bothidae	*Arnoglossus* spp.	**5.44**	18.63	**0.61**	4.31
Callionymidae	*Callionymus* spp.	**3.30**	12.13	**0.32**	3.25
Caproidae	*Capros aper*	**0.83**	6.10	**0.45**	4.53
Carangidae	*Caranxidae* spx	**0.41**	4.18		
	Trachurus mediterraneus	**0.37**	3.80		
	Trachurus spp.	**0.34**	3.46	**0.28**	2.85
	Trachurus trachurus	**1.74**	7.82		
Clupeidae	*Sardina pilchardus*	**28.86**	61.13	**1.45**	7.21
Engraulidae	*Engraulis encrasicolus*	**1.03**	6.03	**0.51**	5.08
Gobiesocidae	*Lepadogaster lepadogaster*	**0.34**	3.44		
Gobiidae	Gobiidae A	**0.29**	2.92		
	Gobiidae n.i.	**1.35**	6.81	**0.66**	4.67
	Gobius niger type	**9.99**	41.28	**6.28**	17.96
	Gobius spp.	**0.29**	2.91	**0.29**	2.92
	Gobius cruentatus type	**0.33**	0.03		
	Gobiusculus flavescens	**0.37**	3.74		
	Pomatoschistus pictus	**3.36**	10.53	**0.32**	3.24
	Pomatoschistus spp.	**0.86**	6.27		
Labridae	*Coris julis*	**8.29**	24.18	**1.54**	7.84
	Ctenolabrus rupestris	**1.04**	6.05		
	Symphodus melops type	**3.21**	11.71	**0.42**	4.20
	Symphodus roissali	**5.64**	18.47		
	Symphodus spp.	**0.33**	3.32		
Lotidae	*Gaidropsarus mediterraneus*	**0.35**	3.61		
Macrorhamphosidae	*Macrorhamphosus scolopax*	**0.28**	2.89		
Mugilidae	*Liza ramada*	**1.23**	7.25		
	Mugilidae n.i.	**2.53**	12.10	**4.73**	19.28
Mullidae	*Mullus surmuletus*	**4.12**	16.75	**1.41**	6.94
Myctophidae	Myctophidae n.i.	**0.29**	2.94		
Scombridae	*Scomber japonicus*	**0.29**	2.91		
Scorpaenidae	*Scorpaena porcus*	**0.37**	3.74		
Serranidae	*Serranus atricauda*	**0.32**	3.27		
	Serranus cabrilla	**0.30**	3.04	**1.01**	7.12
	Serranus spp.	**24.26**	65.22	**1.61**	7.08
Soleidae	*Solea lascaris*	**0.36**	3.65		
	Solea spp.	**0.37**	3.74	**0.32**	3.25
	Solea vulgaris	**0.29**	2.94		
	Soleidae n.i.	**0.73**	5.28	**0.30**	3.00
Sparidae	*Boops boops*	**2.69**	18.06		
	Diplodus spp.	**7.49**	24.45	**1.92**	9.88
	Pagellus sp1	**5.51**	14.64	**0.67**	4.68
	Pagellus spp.			**0.66**	4.72
	Sparidae n.i.	**3.94**	12.52	**1.75**	9.49
	Sparidae sp1	**27.78**	49.19	**5.62**	18.01
	Sparidae sp2	**4.11**	17.73		
	Sparidae sp3			**2.54**	11.69
	Spondyliosoma cantharus	**0.35**	3.56		
Syngnathidae	*Entelurus aequoreus*	**0.34**	3.50		
	Hippocampus ramulosus	**0.75**	5.41		
Trachinidae	*Echiichthys vipera*	**0.38**	3.87		
	Trachinus draco	**2.44**	12.22		
Triglidae	Triglidae n.i.	**0.88**	6.41		
Trigterygiidae	*Tripterygion delaisi*	**7.71**	27.37		
n.i.	n.i.	**32.49**	54.74	**14.89**	33.44

The average taxonomic index (Delta *) had a similar temporal pattern of variation (Figure 3b). However, at two miles no significant variation was found (H (4, N=50) = 4.74, *n.s.*) while at the very-nearshore significant temporal differences occurred (H (5, N= 78)= 16.77, P< 0.01) with a decrease in taxonomic diversity from May to September (P< 0.001) and from August to September (P< 0.05). Delta * was

also always higher at the very-nearshore than at two miles with significant differences in May and July (Figure 3b, Table 3).

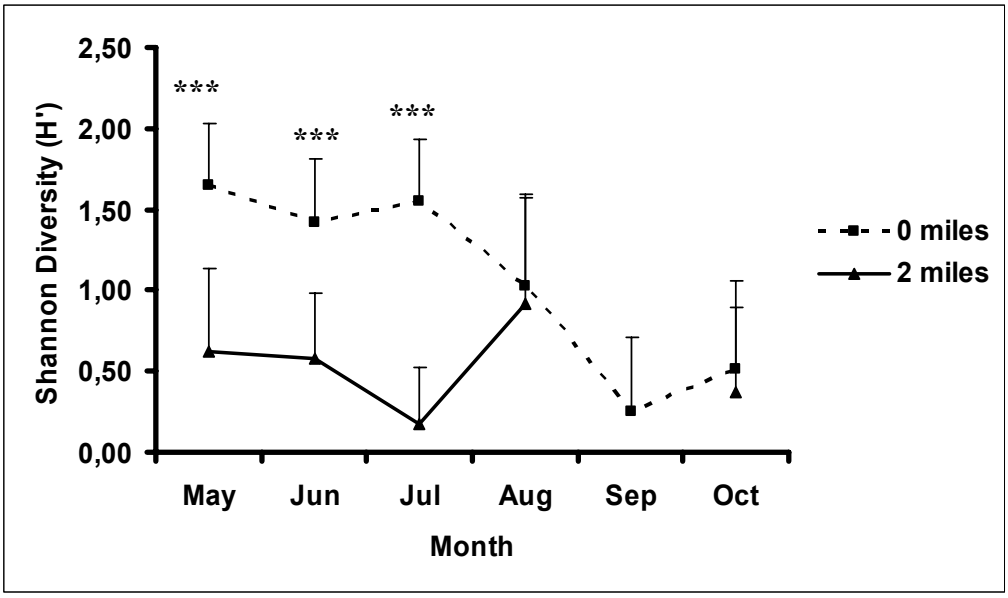


Figure 3 a) - Temporal variation of mean Shannon Diversity Index (H') at both distances from shore. *** represent significant differences (at P< 0.001, NK test) in H' between 0 and 2-miles, for each month. Error bars are standard deviations.

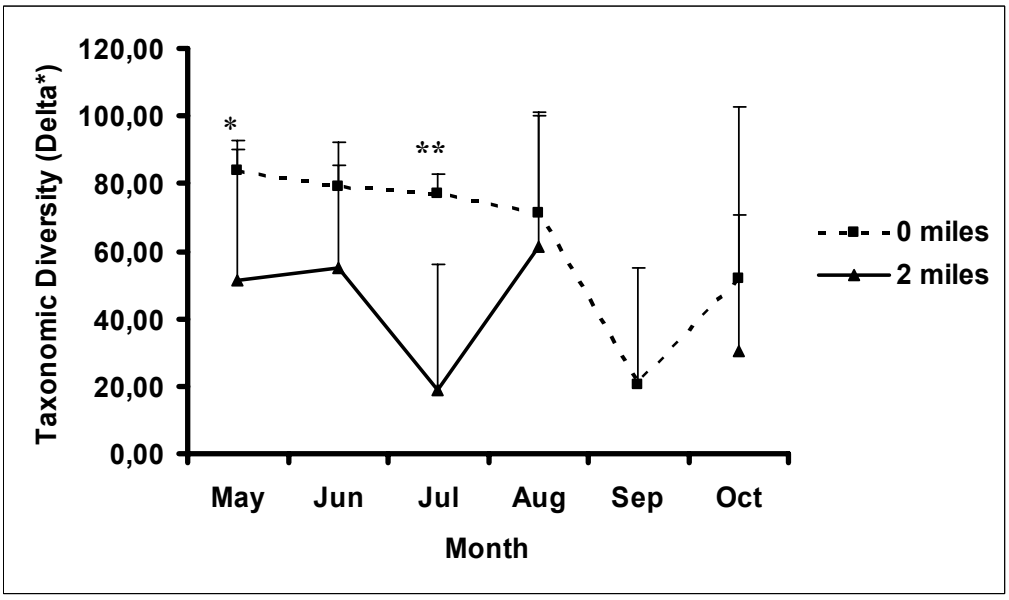


Figure 3 b) - Temporal variation of mean Taxonomic Diversity Index (Delta*) at both distances from shore. *, ** represent significant differences between inshore and offshore samples, for each month, at P< 0.05 and at P< 0.01, respectively (statistics results shown in Table 3). Error bars are standard deviations.

Abundance changed significantly through time, both at the very-nearshore (H (5, N= 104) = 86.58 P< 0.01) and at two miles (H (5, N=100)= 58.88, P< 0.01). Very-

nearshore samples had higher values of abundance in May, June and July (when they reach the maximum) (Figure 4). From August onwards a significant decrease occurred with significantly lower values than in July ($P< 0.05$) and higher than in September ($P< 0.01$). September and October samples also differed significantly from those of May, June and July ($P< 0.001$), except for June vs. October ($P< 0.05$). At two miles, the pattern was somewhat different. June was the month with the highest abundance, and a significant decrease from June to July ($P< 0.01$) and again from August to September ($P< 0.01$) occurred, followed by a significant increase in October ($P< 0.001$). September differed significantly from May and June ($P< 0.001$) and October presented higher abundances than July ($P< 0.05$). From May to August, abundance was significantly higher nearshore than at two miles (Figure 4, Table 3).

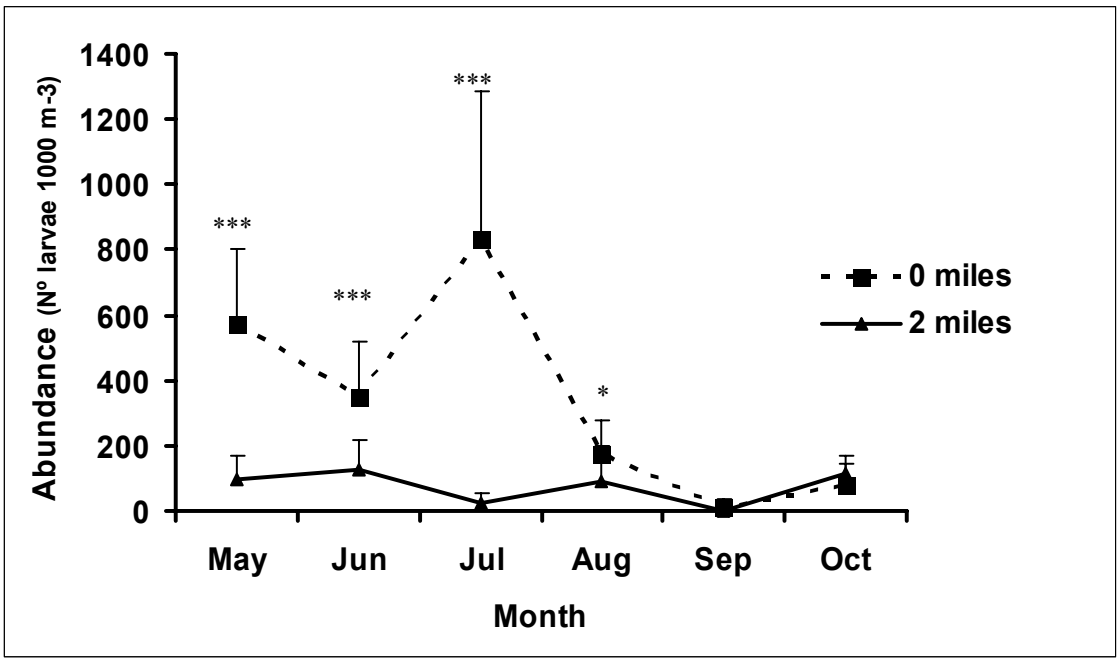


Figure 4 - Temporal variation of mean larval abundance at both distances from shore. *, *** represent significant differences between inshore and offshore samples, for each month, (at $P< 0.05$ and at $P< 0.001$, respectively (statistics results shown in Table 3); error bars are standard deviations

Table 3 - Statistic results of the comparison between very-nearshore (0 miles) and offshore (2 miles) values of Delta* and total abundance in each sampled month. T = value of the t-test for independent samples; Z = value of the Mann-Whitney U test; *ns* not significant *P< 0.05, ** P< 0.01, *** P< 0.001.

	Delta *			Abundance		
Month	N (0 Miles)	N (2 Miles)	Statistics	N (0 Miles)	N (2 Miles)	Statistics
May	**15**	**14**	**Z=-2.28 ***	**15**	**15**	**Z= 4.58*** **
June	**15**	**14**	**Z= -1.90 *ns***	**15**	**15**	**T= 4.93 *** **
July	**15**	**4**	**Z= -2.45 ** **	**15**	**15**	**Z=-4.67 *** **
August	**15**	**8**	**t= -0.65 *ns***	**15**	**14**	**T=2.15 ***
September	**--**	**--**	**--**	**29**	**30**	**Z= 1.59 *ns***
October	**11**	**10**	**t= -0.76 *ns***	**15**	**11**	**T=-1.32 *ns***

Assemblages Structure

Considering the similarities between the 11 groups (each group being constituted by the samples of each month at each distance), global R was low (Table 4). Nevertheless, some differences were detected in the pair-wise comparisons. At the very-nearshore, high R values were obtained in the comparison of assemblage structure between July and the other months. R values close to 0.5 could be found when comparing July with the preceding months. Stronger differences were detected when contrasting the May, June and July assemblages with those of September and October. At two miles, the highest R values were obtained in the June-July and July-August comparisons. Within each month July was the only with a clear spatial structure, with a strong difference between the inshore and offshore assemblages (Table 4).

Table 4 - Summary of one-way analysis of similarity (ANOSIM) with pair-wise comparisons of larval assemblages between months at each distance to shore. 999 permutations were used for each test. The value of the R statistic is shown with R > 0.5 values in bold. All the values are significant at P< 0.001, except those values signalled in italic, that are significant at P< 0.01.*ns*, non significant.

ANOSIM			0 miles						2 miles				
Global R=0.39			May	Jun	Jul	Aug	Sep	Oct	May	Jun	Jul	Aug	Oct
0 miles	**Group 1**	May		0.32	0.49	0.40	**0.65**	**0.52**	0.37				
	Group 2	Jun			0.44	0.28	0.48	0.49		*0.18*			
	Group 3	Jul				0.35	**0.67**	**0.71**			***0.92***		
	Group 4	Aug					0.36	*0.25*				0.29	
	Group 5	Sept						0.31					
	Group 6	Oct											*ns*
2 miles	**Group 7**	May								0.24	*0.38*	*ns*	0.34
	Group 8	Jun									*0.47*	0.38	0.42
	Group 9	Jul										*0.45*	*ns*
	Group 10	Aug											*0.27*
	Group 11	Oct											

Table 5 lists the species that contributed more to explain the similarities between samples of the same group, from the SIMPER results. Within each group, the average similarity between samples was generally low. Nonetheless, these results are informative in what concerns the composition of the assemblages. At the very-nearshore the number of contributing taxa was always higher than at two miles for each month. Species whose adults live and spawn nearshore like *T. delaisi*, *C. galerita*, *P. gattorugine* or *Symphodus* spp. were unimportant at two miles, but contributed to similarities among nearshore samples. *P. pilicornis* was the main species explaining similarities between May samples at both distances and also contributed to similarities among nearshore samples in June, July and September.

Table 5 - Similarity percentages analysis (SIMPER) results for each month at the very-nearshore (0 miles) and at two miles from shore. Average similarity values, average abundance and percentage contribution of the most representative species to the average similarity within each group are shown. Cut off for low contributions = 95%

SIMPER		0 miles					2 miles			
May		**Average similarity: 32.93**					**Average similarity: 13.97**			
	Species Group1	**Av.Abund**	**Sim/SD**	**Contrib%**	**Cum.%**	**Species Group 7**	**Av.Abund**	**Sim/SD**	**Contrib%**	**Cum.%**
	Parablennius pilicornis	120.02	1.05	41.49	41.49	*Parablennius pilicornis*	20.6	0.39	34	34
	Sardina pilchardus	114.86	1.08	27.9	69.39	*Diplodus* spp.	13.69	0.26	19.43	53.43
	Sparidae sp1	65.57	0.6	12.28	81.67	*Gobius niger* type	13.02	0.26	12.12	65.56
	Diplodus spp.	41.73	0.52	7.22	88.89	*Mullus surmuletus*	7.37	0.17	10.9	76.46
	Sparidae n.i.	13.67	0.31	1.69	90.58	*Belone* spp.	14.05	0.26	10.4	86.86
	Tripterygion delaisi	26.61	0.21	1.61	92.19	Sparidae sp3	13.89	0.26	10.05	96.91
	Parablennius gattorugine	18.86	0.22	1.19	93.38					
	Symphodus melops type	11.49	0.24	1.13	94.5					
	Arnoglossus spp.	12.69	0.23	0.96	95.47					
Jun		**Average similarity: 23.26**					**Average similarity: 24.82**			
	Species Group 2	**Av.Abund**	**Sim/SD**	**Contrib%**	**Cum.%**	**Species Group 8**	**Av.Abund**	**Sim/SD**	**Contrib%**	**Cum.%**
	Sparidae sp1	59.16	1	48.59	48.59	Sparidae sp1	32.72	0.6	49.91	49.91
	Gobius niger type	56.38	0.42	11.9	60.5	*Gobius niger* type	26.49	0.62	45.57	95.48
	Coryphoblennius galerita	20.98	0.46	10.16	70.66					
	Symphodus roissali	22.01	0.35	7.55	78.21					
	Tripterygion delaisi	24.37	0.39	6.02	84.23					
	Parablennius pilicornis	27.02	0.29	5.61	89.84					
	Sardina pilchardus	14.73	0.3	4.45	94.29					
	Coris julis	11.1	0.24	1.92	96.21					
Jul		**Average similarity:37.75**					**Average similarity: 9.82**			
	Species Group3	**Av.Abund**	**Sim/SD**	**Contrib%**	**Cum.%**	**Species Group 9**	**Av.Abund**	**Sim/SD**	**Contrib%**	**Cum.%**
	Parablennius pilicornis	270.92	1.4	50.22	50.22	*Serranus cabrilla*	25.19	0.41	100	100
	Serranus spp.	142.13	1.28	27.6	77.82					
	Sparidae sp1	59.17	0.7	8.92	86.74					
	Coris julis	37.55	0.45	3.43	90.16					
	Coryphoblennius galerita	32.56	0.35	2.62	92.79					
	Pagellus sp1	15.22	0.28	1.61	94.39					
	Symphodus roissali	17.1	0.29	1.34	95.74					
Aug		**Average similarity: 16.66**					**Average similarity: 16.94**			
	Species Group 4	**Av.Abund**	**Sim/SD**	**Contrib%**	**Cum.%**	**Species Group 10**	**Av.Abund**	**Sim/SD**	**Contrib%**	**Cum.%**
	Coryphoblennius galerita	15.81	0.39	27.3	27.3	*Parablennius pilicornis*	39.73	0.96	69.82	69.82
	Serranus spp.	21.72	0.46	26.67	53.97	*Serranus* spp.	12.09	0.33	12.74	82.56
	Sardina pilchardus	30.03	0.39	20.57	74.54	*Mugilidae* n.i.	7.76	0.19	6.87	89.43
	Trachurus trachurus	9.73	0.22	5.99	80.53	*Sparidae* sp3	7.41	0.19	5.99	95.41
	Sparidae sp1	8.72	0.23	5.88	86.4					
	Ctenolabrus rupestris	7.18	0.17	5.57	91.97					
	Trachinus draco	11.45	0.17	3.4	95.37					
Sep		**Average similarity: 16.37**								
	Species Group5	**Av.Abund**	**Sim/SD**	**Contrib%**	**Cum.%**					
	Parablennius pilicornis	19.33	0.58	100	100					
Oct		**Average similarity: 16.22**					**Average similarity: 12.72**			
	Species Group 6	**Av.Abund**	**Sim/SD**	**Contrib%**	**Cum.%**	**Species Group 11**	**Av.Abund**	**Sim/SD**	**Contrib%**	**Cum.%**
	Sardina pilchardus	37.49	0.53	63.1	63.1	**Mugilidae n.i.**	33.46	0.52	57.66	57.66
	Liza ramada	11.63	0.23	15.21	78.32	*Sardina pilchardus*	11.49	0.25	31.93	89.59
	Pagellus sp1	13.73	0.34	15.15	93.47	Ammodytidae n.i.	10.55	0.15	6.52	96.11
	Mugilidae n.i.	11.42	0.13	6.53	100					

In June, the only two species (Sparidae sp1 and *Gobius niger* type) contributing to similarities among samples at two miles, were also present nearshore with higher abundances. July presented the highest similarities among nearshore samples and the lowest at two miles, explained by only one species (*S. cabrilla*).

Developmental stage

For the most abundant taxa (more than 20 individuals) BL and larval developmental stage is presented in Table 6. At both inshore and offshore locations most larvae were small and undeveloped (pre-flexion stage). A few species however presented more developed larvae. Nearshore, 33.3% of *Gobius niger* type larvae were in the incomplete flexion stage and 6.7% in the post-flexion stage, while at two miles flexion-stage larvae corresponded to 36.8%. In *C. galerita*, only one incomplete flexion and one post-flexion larva occurred nearshore and in *P. pilicornis* only one post-flexion stage larva was collected both nearshore and at two miles. Larvae of species hatching from pelagic eggs, like *Diplodus* spp., *S. pilchardus* and *Serranus* spp., were present nearshore at incomplete flexion-stage with respectively 21.7%, 6.9% and 1.6 % of occurrences.

Table 6 - Body length (in mm) for larvae of the species with more than 20 individuals present in the very-nearshore (0 miles) and offshore (2 miles) samples. Symbols represent the occurrence of larvae in each developmental stage: ▲ Pre-flexion; • Flexion; ■ Post-flexion.

	0 miles					2 miles				
Species	**Dev. Stage**	**N**	**Mean**	**SD**	**Range**	**Dev. Stage**	**N**	**Mean**	**SD**	**Range**
Coris julis	▲	21	**2.30**	0.50	1.58 - 3.35	▲	4	**2.44**	0.91	1.72 - 3.70
Coryphoblennius galerita	▲•■	37	**3.48**	1.10	2.80 - 9.80					-
Diplodus spp.	▲•	23	**3.88**	1.22	2.65 - 6.90	▲	6	**3.90**	0.47	3.25 - 4.50
Gobius niger type	▲•■	30	**3.08**	0.81	1.95 - 5.05	▲•	19	**3.00**	0.50	2.25 - 3.90
Parablennius pilicornis	▲ ■	175	**2.77**	0.57	1.85 - 9.80	▲•	20	**2.96**	0.60	2.15 - 4.45
Sardina pichardus	▲•	86	**5.53**	1.51	3.00 - 14.50	▲	3	**4.62**	1.27	3.50 - 6.00
Serranus spp.	▲•	61	**2.19**	0.29	1.52 - 2.85	▲	4	**2.01**	0.36	1.50 - 2.35
Sparidae sp1	▲	83	**2.21**	0.78	1.25 - 4.60	▲	17	**2.20**	0.90	1.32 - 4.00
n.i.	▲	83	**1.73**	0.41	1.10 - 2.75	▲	33	**1.78**	0.51	1.10 - 2.95

Spatial comparison of transects

An evaluation of the grouping patterns of stations within transects showed a significant spatial segregation of stations in four distinct groups (Pillai's trace = 0.735, P< 0.01) (Figure 5). Group 1 contained stations 0 to 2 with the very nearshore (mile 0) separated from miles 1 and 2. Group 2 gathered stations 3 to 6 and Group 3 stations 7 to 9. The fourth Group contained only station 10. Overall, 51 taxa were identified, comprising 22 families with 31 genera and 33 species.

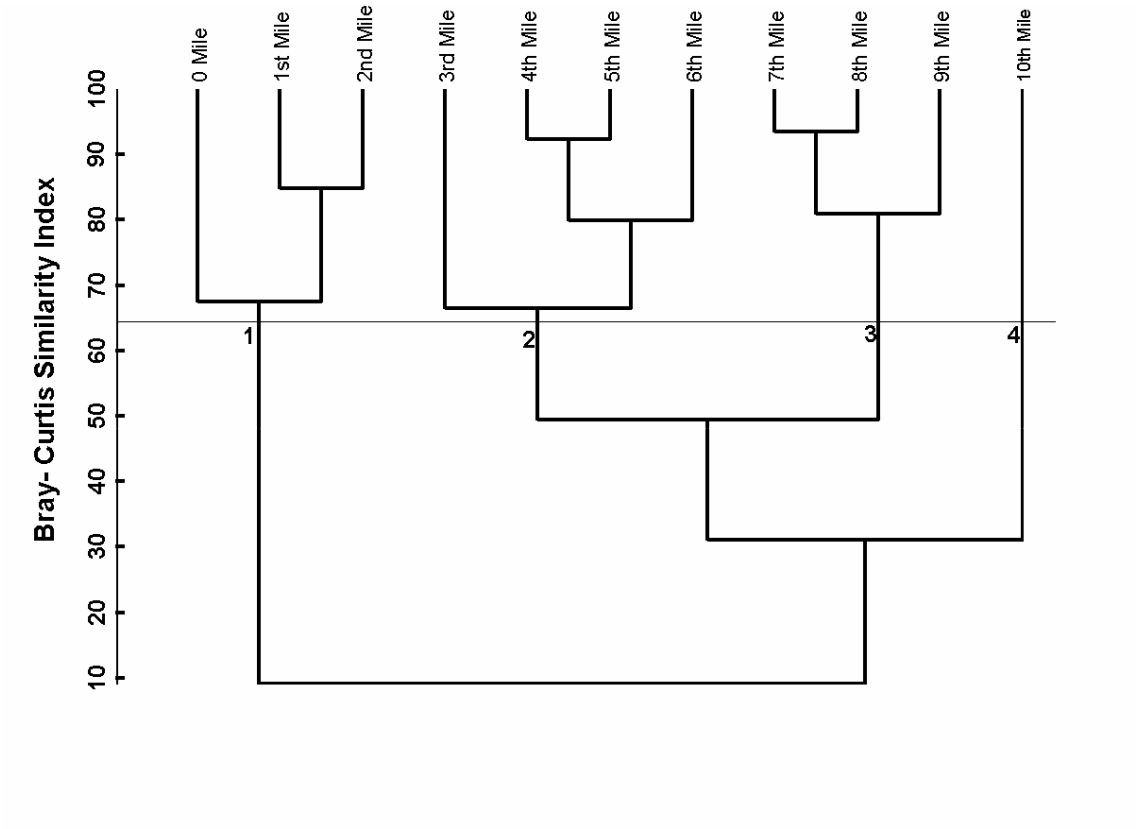


Figure 5 - Groups of Miles (1-4) as defined by the Q- mode Clustering with contiguity constraint.

Diversity and Abundance

Spatial patterns of diversity found along transects are shown in Figure 6. Diversity was significantly higher (P< 0.01) nearshore (Group 1) when compared with the other groups of stations for both H' and Delta* (Figure 7). Larval abundance followed a similar trend with maximum values occurring nearshore and decreasing with increasing distance from shore (Figure 8a). Total abundance was significantly higher in Group 1 (Figure 9) when compared to the other three groups.

Assemblages Structure and Composition

R-mode clustering of larval composition and density defined four distinct assemblages of species (Pillai's trace = 0.53, P< 0.05). Species composition and mean densities for each group of stations defined above are expressed in Table 7. For each group of species, spatial variation along transects is shown in Figure 8.

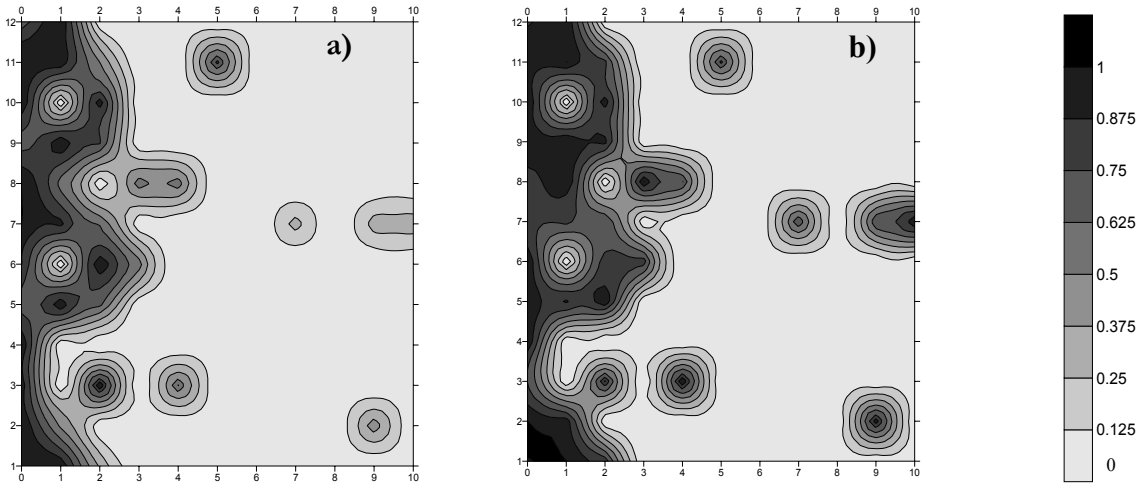


Figure 6- Spatial distribution of normalized values of diversity indices along the 12 transects. Stations are shown from left to right (respectively from the extreme nearshore to the 10th Mile). a) Shannon diversity (H'); b) Average taxonomic index (Delta*).

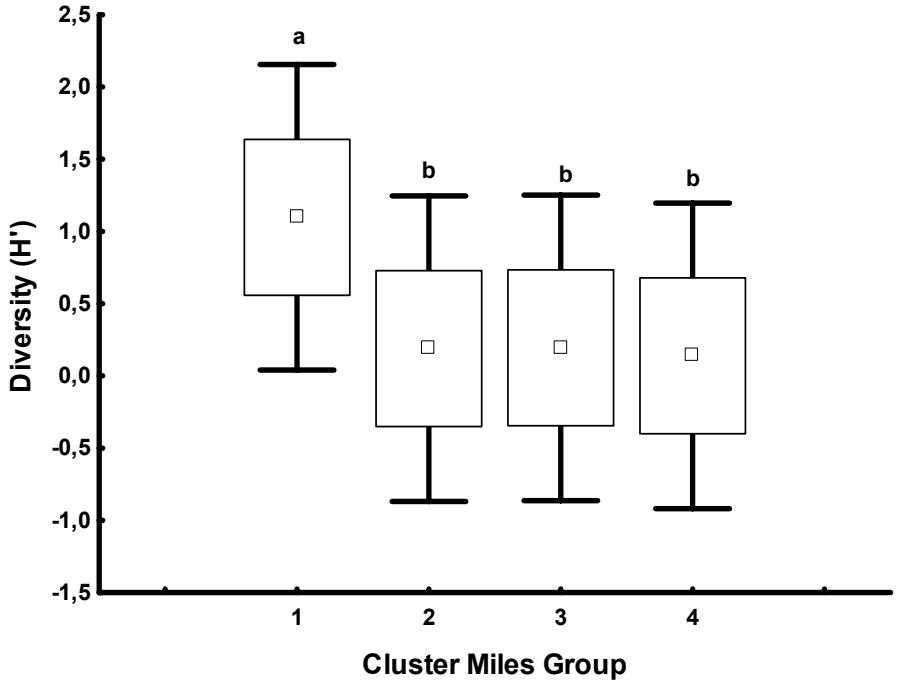


Figure 7 a) – Shannon diversity (H') at each group of miles. ANOVA F (3 , N= 108) = 25.38, P < 0.001*** a,b, represent differences among groups of miles at P< 0.001. Central square = Mean; large rectangle = Mean ± S.D. Whiskers = Mean±1.96 S.D.

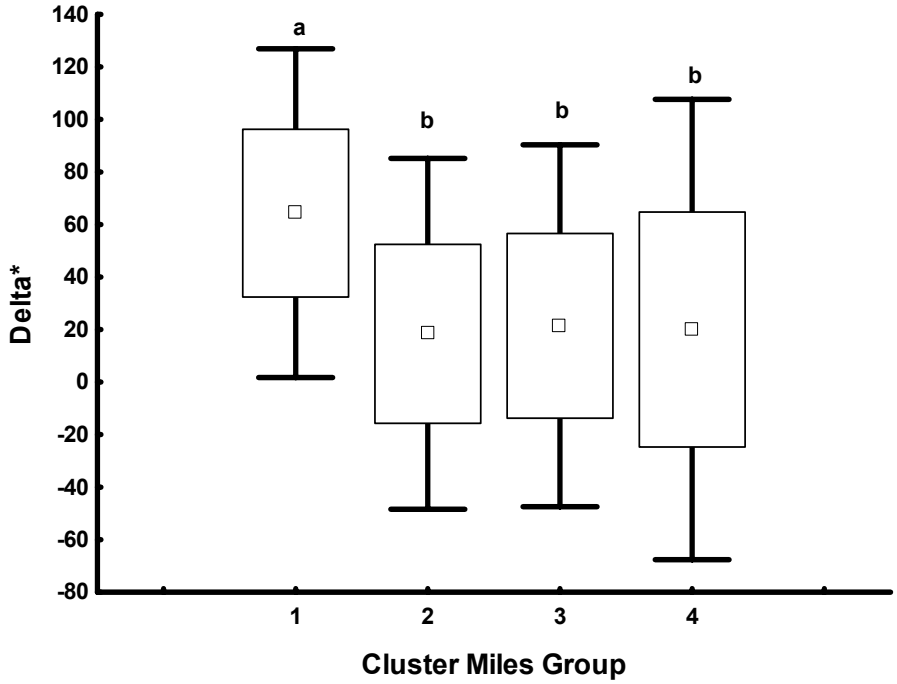
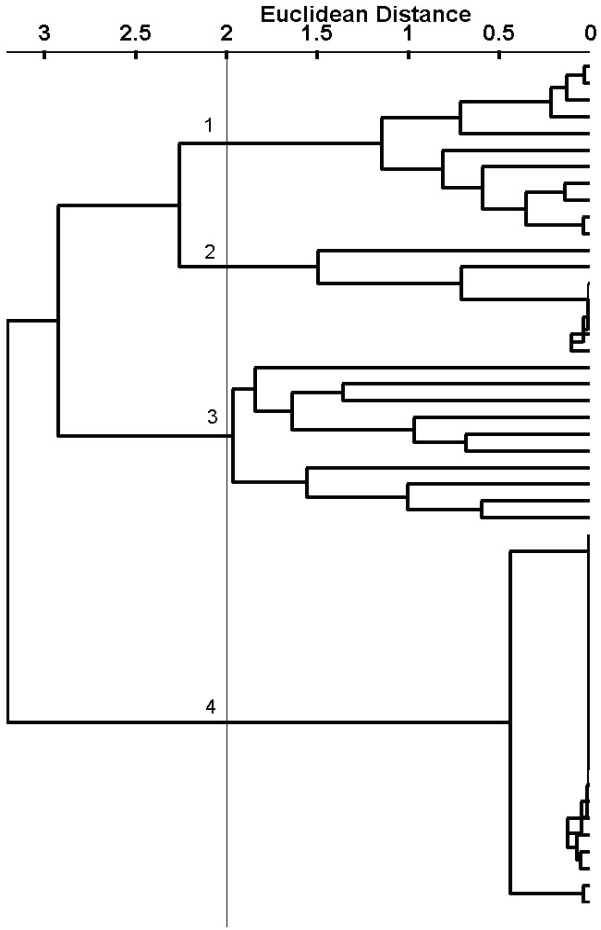


Figure 7 b) – Spatial variation of Average taxonomic index (Delta*) by groups of miles. F= (3 , N=108)= 16.06 P< 0.001 ; a,b, represent differences among groups of miles at P< 0.001. Central square = Mean; large rectangle = Mean ± S.D. Whiskers = Mean±1.96 S.D.

The first assemblage was composed by species that appeared only in Group 1 of stations (0-2 miles) (e.g. *C. rupestris*, *B. boops* and *P. pictus*) and by some species that,

although having higher abundances nearshore, were also present in more offshore waters, like *S. pilchardus* and *Symphodus melops* type (Table 7, Figure 8b). The second assemblage, contained species that were present within the first two groups of miles (e.g. *P. gattorugine*) or exclusively nearshore (Table 7, Figure 8c). Species from the third assemblage presented a broader range of distribution (Figure 8d). For example, Mugilidae n.i. and *Gobius niger* type were present in all groups. *G. niger* type was more abundant in the first and third groups of miles and Mugilidae n.i. had higher abundances in the second group (3-6 Miles). *M. surmuletus* was the only species with a higher abundance offshore (Group 4) (Table 7). Finally, the fourth assemblage (Table 7, Figure 8e) comprised 45% of the total taxa and showed the strongest association in the cluster analysis. Six species were benthic spawners (*T. delaisi*, *G. flavescens*, *A. presbyter*, *P. pilicornis*, *C. galerita*, *S. roissali*) and the remaining live associated to the rocky habitat or in shelf waters. All species of this assemblage occurred exclusively within the first two miles from shore. The only exception was *P. pilicornis* (the most abundant species nearshore) which was also found in Group 3 (7-9 Miles). A more detailed analysis of their distribution within the first two miles is shown in Table 8. Only five out of 23 species were found outside the very-nearshore (0 mile) stations and they were all more abundant there.

Table 7 - Composition of the assemblages as defined by the R-mode clustering (vertical line represents the chosen distance for group separation), and abundances (expressed as number of larvae $1000m^{-3}$) at each Group of miles defined by the Q-mode clustering. Body length range (in mm) is expressed for each species. Symbols represent the occurrence of larvae in each developmental stage: ▲ Pre-flexion; • Incomplete flexion; ■ Post-flexion



	Group 1 Very-nearshore-2nd mile		Group 2 3th-6th mile		Group 3 7th -9th mile		Group4 10th mile			
Species	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	Size range	Developmental stage
Ctenolabrus rupestris	**3.12**	11.11							2.05-4.45	▲•
Boops boops	**1.01**	5.70							5.5-8.30	•
Sardina pilchardus	**17.93**	42.14	**0.49**	4.08			**2.12**	8.48	3.3-10.00	▲•
Sparidae sp2	**2.30**	9.14							1.6-3.25	▲
Symphodus melops type	**1.97**	9.71					**1.89**	7.57	2.8-3.45	▲•
Pagellus sp1	**6.10**	14.92	**1.43**	6.79					2.55-4.95	▲•
Trachinus draco	**6.01**	17.79			**0.67**	4.78			2.05-3.35	▲
Sparidae sp1	**22.73**	40.13							1.32-4.6	▲
Coris julis	**13.50**	30.18			**0.67**	4.78			1.62-3.35	▲
Soleidae n.i.	**1.67**	7.65							1.48-1.72	▲
Pomatoschistus pictus	**4.32**	12.62							1.98-6.9	▲ ■
Parablennius gattorugine	**0.40**	3.17	**0.43**	3.54					4.8-17.84	• ■
Serranus hepatus	**1.06**	8.46	**0.45**	3.76					2.5-4.15	▲
Triglidae n.i.	**0.55**	4.41							3.8	▲
Solea senegalensis	**0.40**	3.17							2.9	▲
Pagrus pagrus	**0.53**	4.23							3.6	▲
Trachurus spp.	**11.99**	54.52							1.65-3.4	▲
Hippocampus hippocampus	**0.41**	3.25							10.1	
Sparus aurata					**0.67**	4.81			4.9	•
Mugilidae n.i.	**6.14**	17.57	**8.60**	21.57	**1.29**	6.49	**2.12**	8.48	1.75-4.6	▲•■
Gobius niger type	**9.40**	22.29	**5.32**	12.79	**9.62**	24.03	**1.91**	7.66	2.12-5.2	▲•■
Sparidae sp3	**1.03**	5.82							3.70-4.30	▲•
Sparidae n.i.	**4.59**	16.55	**0.99**	5.78					2-3.2	▲
Echiichthys vipera	**0.56**	4.45							2.35	▲
Mullus surmuletus	**7.27**	21.32	**1.54**	7.31	**0.60**	4.27	**11.70**	38.42	2.12-8.4	▲•■
Callionymus spp.	**4.48**	13.79	**0.94**	5.48					1.54-1.9	▲
Diplodus spp.	**1.04**	5.90	**0.49**	4.07					3-3.24	▲
Belone spp.	**2.66**	12.82	**0.47**	3.89					7.9-12.31	■
Triptergyion delaisi	**0.58**	4.65							4.25	▲
Trachurus trachurus	**2.28**	8.97							3.3-3.7	▲
Trachurus mediterraneus	**0.61**	4.84							2.65	▲
Solea spp.	**0.60**	4.77							1.88	▲
Solea lascaris	**0.58**	4.65							---	▲
Serranus cabrilla	**0.48**	3.87							1.46	▲
Serranus atricauda	**0.52**	4.16							3.55	▲
Scorpaena porcus	**0.60**	4.77							2.19	▲
Scomber japonicus	**0.46**	3.71							4.00	▲
Hippocampus ramulosus	**0.61**	4.88							9.5	
Gobiusculus flavescens	**0.60**	4.77							2.47	▲
Gobius spp.	**0.46**	3.71							2.8	▲
Gobiidae n.i.	**1.12**	6.28							2.2-2.41	▲
Entelurus aequoreus	**0.56**	4.46							17.07	
Engraulis encrasicolus	**0.52**	4.13							4.00	▲
Carangidae spx	**0.67**	5.32							2.95	▲
Capros aper	**0.52**	4.16							2.47	▲
Atherina presbyter	**1.28**	7.17							5.81-6.8	▲
Serranus spp.	**41.32**	79.91							1.52-2.85	▲•
Parablennius pilicornis	**71.16**	153.98			**1.85**	7.47			1.85-9.80	▲•■
Coryphoblennius galerita	**12.01**	31.06							2.8-5.8	▲•
Symphodus roissali	**4.46**	14.89							2.75-3.45	▲
Arnoglossus spp.	**6.16**	20.78							1.65-2.8	▲

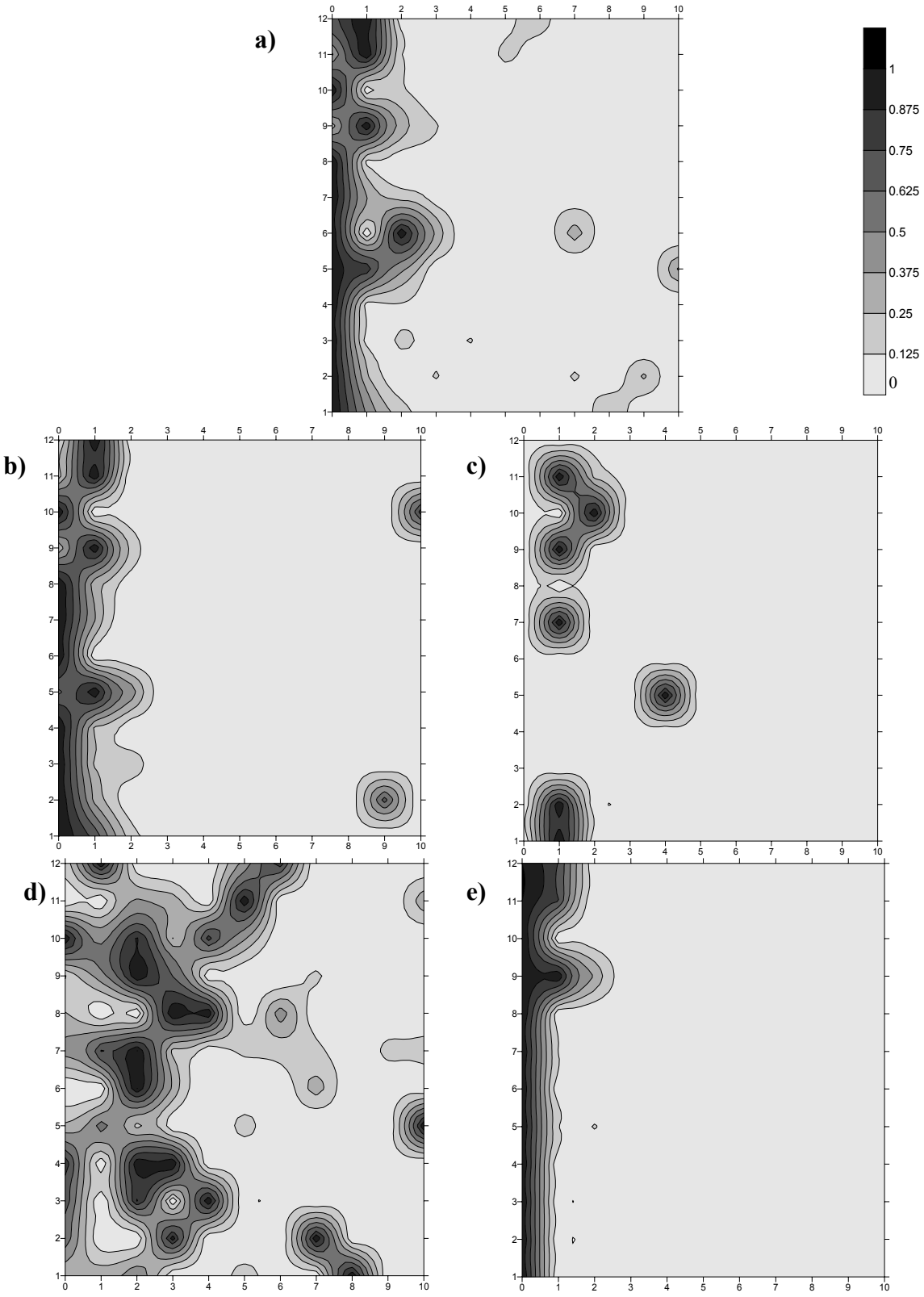


Figure 8 - Spatial distribution of normalized values of larval abundance along the 12 transects. Stations are shown from left to right (respectively from the very-nearshore to the 10th Mile). a) Total abundance; b) Larval abundance in the first species assemblage ; c) Larval abundance in the second species assemblage; d) larval abundance in the third species assemblage; e) larval abundance in the fourth species assemblage.

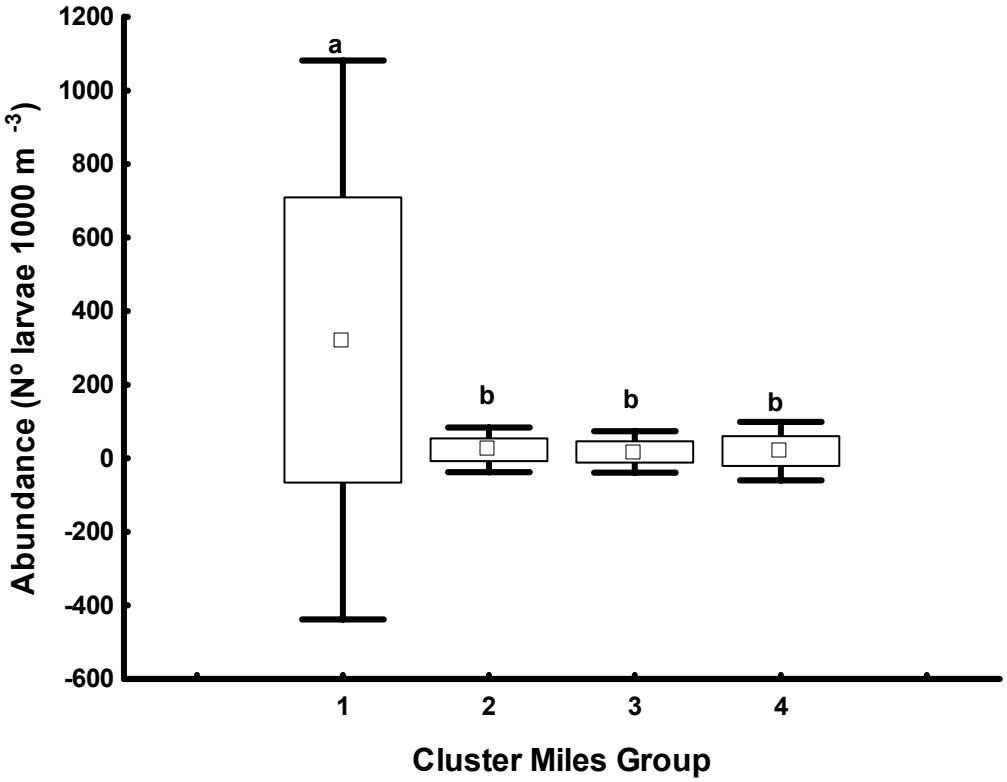


Figure 9 – Total larval abundance at each group of Miles. Kruskal-Wallis H (3, N= 200) =94.42, P < 0.001 .a,b, represent differences among groups of miles at P< 0.001 . Central square = Mean; large rectangle = Mean± S.D. Whiskers = Mean±1.96 S.D.

Developmental stage

As in the inshore/offshore comparison, only larvae in the pre-flexion stage were found for most species (Table 7), and only in six species more advanced post-flexion larvae were collected.

Table 8 - Fine scale distribution of the fourth assemblage of species. Abundance (expressed as the number of larvae 1000 m^{-3}) is represented for each mile within the 1st Group of miles defined by the Q-mode clustering. H = value of Kruskal-Wallis ANOVA; Z= value of Mann-Whitney U test; *ns* not significant. ** P< 0.01, *** P< 0.001

	0 Miles		1st Mile		2nd Mile		Statistics	Dunn
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**		
Tripterygion delaisi	**1.24**	6.79						
Trachurus trachurus	**4.86**	12.71						
Trachurus mediterraneus	**1.29**	7.07						
Solea spp.	**1.27**	6.97						
Solea lascaris	**1.24**	6.79						
Serranus cabrilla	**1.03**	5.65						
Serranus atricauda	**1.11**	6.08						
Scorpaena porcus	**1.27**	6.97						
Scomber japonicus	**0.99**	5.42						
Hippocampus ramulosus	**1.30**	7.13						
Gobiusculus flavescens	**1.27**	6.97						
Gobius spp.	**0.99**	5.42						
Gobiidae n.i.	**2.38**	9.09						
Entelurus aequoreus	**1.19**	6.51						
Engraulis encrasicolus	**1.10**	6.03						
Carangidae spx	**1.42**	7.78						
Capros aper	**1.11**	6.08						
Atherina presbyter	**2.72**	10.37						
Serranus spp.	**81.93**	101.10	**5.77**	23.80	**5.19**	15.05	H(2. N=64)=24.33 ***	(0>1***; 0>2**)
Parablennius pilicornis	**138.70**	204.38	**17.46**	36.32	**5.68**	12.65	H(2. N=64)=10.92 **	(0>2 *)
Coryphoblennius galerita	**24.18**	41.77	**2.54**	10.45			Z=1.88 *ns*	
Symphodus roissali	**8.55**	20.52	**1.71**	7.03			Z=0.66 ns	
Arnoglossus spp.	**11.58**	28.41	**2.76**	11.38			Z= 0.77 ns	

DISCUSSION

Several factors may influence the temporal changes in larval composition of coastal larval assemblages: temperature (Walker Jr. *et al*., 1987; Houde and Zastrow, 1993); upwelling and wind forcing (Pitts, 1999; Hernández-Miranda *et al*., 2003); current patterns, among others (reviewed by Pineda, 2000; Cowen, 2002; Sponaugle *et al*., 2002). Some studies have however shown that temporal changes in composition and abundance may be mostly related to the spawning patterns of adult fishes rather than other biological and physical factors (Sampey *et al*., 2004). In our study most larvae belonged to coastal species associated with shallow water rocky reefs. Monthly patterns of variation of this nearshore assemblage were clearly detected, with diversity and total larval abundance higher from May to July. This agrees with the breeding season for most coastal species occurring at the Arrábida Marine Park (Henriques *et al*., 1999; Gonçalves *et al*., 2003). In August, larval abundance and diversity decreased abruptly, which is in accordance with the end of the spawning activity for most species. Other temperate nearshore studies also refer that the spring and summer are periods of high larval abundances where the highest diversity values occur (Palomera and Olivar, 1996; Sabatés *et al*., 2003). However, in some cases, in spite of the higher abundances larvae were also found closer to shore than offshore and the distribution patterns of larval assemblages were many times weakly related to the spawning mode of adults (Kingsford and Choat, 1989; Gray, 1993; Brogan, 1994; Hickford and Schiel, 2003).

Clear temporal patterns of variation in diversity, abundance and structure of the assemblages at the extreme nearshore were found but these were not so evident at two miles from shore. Nevertheless, diversity and total larval abundance decreased with increasing distance from shore, both in the inshore/offshore comparison and in

transects. This decrease was evident at a small spatial scale, immediately after the first mile. Moreover, all taxa present in transects also occurred and were in general more abundant in nearshore waters, indicating a probable inshore origin. Four different assemblages could be identified, with the strongest association corresponding to species which were collected almost exclusively nearshore. The absence of larvae from oceanic species could reflect an offshore displacement of surface waters caused by upwelling events, which take place very close to our study area during summer months (Fiúza, 1984).

While Leis and Miller (1976) described contrasting patterns of distribution for species hatching from pelagic and benthic eggs with distance to shore, our results do not follow this trend. There was no clear distinction on the distribution of larvae from demersal and pelagic spawners with distance to shore. Nearshore larvae from demersal spawners included *T. delaisi*, *P. gattorugine*, *C. galerita* and *Symphodus*. Offshore larvae were both from demersal spawners like *G. niger* type, *P. pilicornis* and *Belone* spp. and from pelagic spawners like *S. pilchardus*, *M. surmuletus* and several sparids. Among the species that showed a more "dispersive" pattern of distribution, no clear pattern was also found between pelagic and demersal spawners. Among the pelagic spawners, *S. pilchardus* is the most abundant species spawning off the Portuguese coast (Ré *et al*., 1990) and high densities of larvae have been found over shelf waters (Lopes and Afonso, 1995). Mullets are coastal species frequently found in estuaries but spawning at sea (Ben-Tuvia, 1986), and were also abundant. *M. surmuletus* is a benthic species inhabiting shallow waters, but with a clear offshore dispersive pattern. For this species, our observations agree with those of Russell (1973) off Plymouth and Deudero (2002) in the Mediterranean, with more larvae found in the more offshore group of

miles in transects. *Coris julis* is a reef associated species that also hatch from pelagic eggs and has been described to disperse in shelf waters (Sabatés *et al*., 2003) Considering the "dispersive" demersal spawners, *G. niger* is a nearshore species often found in estuaries and lagoons (Miller, 1986). However, larvae of this species can be abundant in more offshore waters (Fives and O'Brien, 1976; Tully and O'Ceidigh, 1989; Acevedo *et al*., 2002; Koutrakis *et al*., 2004). *P. gatorugine* and *Symphodus melops* type larvae have also been recorded offshore by Russell (1973), Fives and O'Brien (1976), Tully and O'Ceidigh (1989), Riley *et al.* (1986), Koutrakis *et al*. (2004), Lee at al. (2005), and off the Portuguese coast by Afonso (1995). Species like *L. pholis, L. lepadogaster, G. flavescens, C. galerita, P. pictus* and *Atherina* have also been recorded offshore (Russell, 1973; Fives and O'Brien, 1976; Riley *et al*. 1986; Acevedo *et al*., 2002; Lee *et al*., 2005). From these only *L. pholis* was absent from our samples, and the other species were only recorded in the extreme nearshore.

Larval retention near reefs depends on complex interactions between biological and physical factors, with some nearshore environments having particular oceanographic features that can facilitate larval retention (Harris *et al*., 1999; Pineda, 2000; Sanvicente-Añorve *et al*., 2000; Sponaugle *et al*., 2002; Largier, 2003). At our study area, the mix of factors possibly affecting dispersal must be further investigated. Although nothing is known on micro scale circulation patterns in this area, there are however a number of features which could potentially act as retention mechanisms. A possible interaction between shallow depths, bottom complexity and the prevailing alongshore currents exists, which may create layers of flows with different directions (Largier, 2003). In these conditions, it is known that water flow is often slowed near the epibenthic boundary layer, increasing the potential retention of larvae that stay near the

bottom (Breitburg *et al*., 1995). Moreover, sampling in the leeward and windward locations in islands has shown differences in larval distribution patterns (Leis, 1991). A high degree of self-recruitment has also been found in sheltered assemblages in the lee side of islands (Jones *et al*., 1999; Swearer *et al*., 1999; Jones *et al*., 2005). Wind forcing is therefore another factor that can have a strong influence over dispersal (Cowen, 2002). At the Arrábida Marine Park this force is greatly reduced due to the geomorphology of the coastline. In addition, in the nearby coastal area, upwelling events occur frequently in the spring/summer months (Fiúza, 1984) with known relaxation episodes related to the shadow effect of the coastline (Moita *et al.*, 2003). This phenomena has been described as an important retention mechanism for planktonic organisms in other coastal systems (Cowen, 2002; Hernandéz-Miranda *et al*., 2003; Roughan *et al.*, 2005a,b). Finally, the interaction between slope topography and tidal flow creates vertical eddies at the mouth of the nearby Sado river (Martins *et al*., 2001, 2002). The extent to which these eddies influence the hydrodynamics of the nearshore area is not known. Internal tides also occur in the adjacent coastal area (J. Silva, personal communication) and have the potential to promote the shoreward transport of organisms. This can also be an important mechanism has been associated with recruitment peaks for some coastal species with a planktonic larva (Lamb, 1997; Pineda, 1994; Pineda 2000).

The fact that a high abundance of larvae from pelagic eggs was found inshore can be indicative that such passive retention of planktonic organisms may be occurring at the Arrábida Marine Park. The described physical mechanisms could retain eggs and recently hatched larvae nearshore during the first days of development, while sensory abilities develop. Some larvae could then actively behave in response of those

environmental features, regulating their position in the water column, thus avoiding dispersal. The onset of these behavioural capabilities should influence the dispersal patterns differently, depending on the species. This could explain the different patterns found between species with similar life histories and spawning modes. The growing evidence of larval strong swimming abilities in some coral reef species (reviewed by Cowen, 2002; Leis and McCormick, 2002; Sponaugle *et al*., 2002; Fisher, 2005) as well as in temperate rocky reefs (Dudley *et al*., 2000; Leis *et al*. 2006), gives evidence that dispersal patterns of reef fish species can be strongly influenced by specific larval behaviours.

Nearshore retention could also be facilitated for some species hatching from benthic eggs and having small planktonic larval durations. For instance, *T. delaisi* larvae were only found inshore. Although Hickford and Schiel (2003) found Tripterygiidae larvae of all size classes away from shore, this is the taxon that most consistently has been referred to be retained near reefs (Marliave, 1986; Kingsford and Choat, 1989; Tilney *et al*., 1996; Sabatés *et al*., 2003). Other taxa often associated to nearshore environments are the Gobiidae (Leis 1991). In spite *G. niger* type larvae were found occurring offshore, these and other Gobiidae larvae are present near the rocky bottom at the very-nearshore within all size classes (unpublished data). In addition, there is also some evidence of retention for species from the family Gobiesocidae which lay large benthic eggs and have small planktonic larval durations (Marliave, 1986; Tilney *et al*., 1996; Sabatés *et al*., 2003). Although *L. lepadogaster* have also been found offshore (e.g. Lee *et al*., 2005), we have caught large numbers of gobiesocid larvae of all size classes with light traps in the very-nearshore (unpublished data).

From our results it is however premature to make definitive conclusions on the mechanisms explaining the patterns of nearshore larval distribution at the Arrábida Marine Park. In fact, the reduced offshore larval densities found do not necessarily reflect larval retention. The sampling method used in this study only sampled the surface water layer and therefore did not include the vertical profile of larval distribution. These vertical profiles have been described for a number of species occurring in shelf waters (reviewed by Neilson and Perry, 1990). Other studies collecting larvae with oblique trawls along Portuguese shelf waters have also obtained higher larval abundances (e.g. Lopes and Afonso, 1995).

Nevertheless, the fact that most larvae were in the pre-flexion stage suggests a high degree of local production at our study area. The Arrábida Marine Park is therefore a spawning ground for coastal fish species. Our results also show that, at least in surface waters, late stage larvae were absent or rare. Therefore, to better understand the specific dispersal patterns and retention mechanisms at this site, it is crucial to have information on the distribution patterns of all size class larvae. One possible explanation for the absence of bigger larvae in our samples could be net avoidance by the larvae. However it seems unlikely that all taxa found would present the same degree of avoidance and some more advanced larvae were found for some species. Offshore dispersal is another possibility for some species. For others, we have found that there is a fine scale vertical distribution of larvae at the very-nearshore with bigger larvae occurring near the substrate (unpublished data). Knowing the extent to which the physical processes operating in the area affect larval dispersal and the detailed ontogeny of behaviours and sensory skills of larvae will further help understand factors influencing the dispersal patterns of the different species in these nearshore assemblages.

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IV. VERTICAL STRUCTURE OF VERY NEARSHORE LARVAL FISH ASSEMBLAGES IN A TEMPERATE ROCKY COAST

Rita Borges, Ricardo Beldade & Emanuel J. Gonçalves (*submitted*).

ABSTRACT

A very nearshore larval fish assemblage was studied at two depth strata: surface and bottom. A total of 4590 larvae (2016 from surface samples and 2574 from bottom samples) belonging to 62 taxa included in 22 families was collected. Most larvae belonged to coastal species. Although inter-annual variations in larval density could be found, total larval abundance was always higher near the bottom whereas diversity was higher at the surface. A marked distinction between the structure of surface and bottom assemblages was found. Surface assemblage contained 22 taxa which explained 95% of the similarity among groups. Larvae which contributed most to this similarity included species like clupeiformes, sparids and serranids, and also blenniids, tripterygiids and some labrids. In the bottom samples fewer species were present with only 9 taxa contributing to 95% of the similarity between samples being almost exclusively from species which lay benthic eggs. Larvae present at the surface were significantly smaller than at the bottom. For some of the most abundant species caught in the bottom samples, only small larvae occurred at the surface while the whole range of sizes was present at the bottom. Possible nearshore retention and dispersal patterns of coastal species are discussed.

INTRODUCTION

In temperate waters extensive work has been done on ichthyoplankton composition and vertical distribution in oceanic or shelf waters (e.g. Kendall and Naplin 1981, Southward and Barret 1983, Conway *et al*. 1997, Olivar and Sabatés 1997, Gray 1998, Somarakis *et al*. 2002, Sabatés 2004). Some studies showed evidence of vertical migration patterns for some species (for a review see Neilson and Perry 1990).

Traditional sampling methods for ichthyoplankton studies are difficult to use in nearshore waters due to shallower depths, complex bottom topography (Smith *et al*. 1987) and wave action. This resulted in a poorer knowledge of coastal ichthyoplankton communities and their distribution patterns at small spatial scales. Several studies on larval assemblage composition and spatial distribution patterns nearshore have however been conducted in recent years on coral reefs (Smith *et al*. 1987, Kobayashi 1989, Sponaugle and Cowen 1996, Kingsford and Finn 1997, Hendriks *et al*. 2001, Kingsford 2001, Wilson 2001, Sponaugle *et al*. 2003). In these environments, evidence is growing on the ability of larvae to actively modify their position in the water column which can result in larval retention in the vicinity of the reefs (Leis 1991a,b, Jones *et al*. 1999, Swearer *et al*. 1999, Cowen 2002, Leis and McCormick 2002, Taylor and Hellberg 2003). Depth stratified sampling with plankton nets and light traps used in shallow waters directly over reefs (Hendriks *et al*. 2001; reviewed by Cowen 2002 and Leis and McCormick 2002) have identified vertical distribution patterns sometimes with a clear daily or ontogenetic basis (Leis 1986, 1991a,b, Sponaugle and Cowen 1996, Sponaugle *et al*. 2003). In situ behavioural studies also revealed species-specific behaviours and showed that larvae of coral reef fish exhibit directional swimming capabilities and

regulate their vertical position at a fine scale (Leis and Carson-Ewart 1999, 2000a, Leis and McCormick 2002).

In nearshore temperate waters little is known on the spatial distribution of fish larvae. Some studies on micro-scale distribution of larval fish have focused on only one species. Marliave (1981) found vertical migration patterns in *Gibertidia sigalutes* (Cottiidae) larvae within the first 3m layer, in Vancouver Island. Jenkins *et al*. (1998, 1999) reported diurnal vertical migrations of *Sillaginodes punctata* (Sillaginidae) in nearshore waters. Breitburg (1989) studied in situ behaviour of *Gobiosoma bosci* (Gobiidae) in an oyster reef and suggested that pre-settlement schooling may be a common behaviour among temperate benthic fish species. Breitburg *et al*. (1995) performed field studies to examine the relationship between these aggregations and water flow and suggested that larvae actively respond to water flow patterns near reefs and that this may be determinant to understand the fine scale spatial patterns of distribution at settlement.

Boehlert *et al*. (1985), Tilney *et al*. (1996) and Gray and Miskiewicz (2000) found differences in larval assemblages between inshore and offshore samples and also that, in some occasions, they were depth stratified. Brewer and Kleppel (1986) detected clear vertical patterns in the densities and length frequency of neritic fish larvae (below the 40 m isobath) and suggested that these diel vertical positions, with clear ontogenetic patterns, could contribute to their retention in nearshore waters. There is also some evidence of micro-scale larval distribution patterns. Marliave (1986) sampled the extreme nearshore over rocky reefs and found that larvae of intertidal fishes occurred more frequently along rocky shores than in adjacent sandy beaches. This author suggested that intertidal fish larvae are able of resisting offshore and alongshore dispersal and could prefer more turbulent waters or avoid more laminar velocity

gradients along sand or mud shores. Tilney *et al*. (1996) also suggested larval retention nearshore for some rock associated species present in the Tsitsikamma National Park Marine Reserve, South Africa.

More recently, Sabatés *et al*. (2003) found differences in patterns of larval distribution among species from a nearshore rocky fish assemblage in the northwest Mediterranean. Vélez *et al*. (2005) described distinct vertical assemblages of nearshore fish larvae at Independencia Bay, Peru. These authors compared the larval composition at the surface and at 10 m depth. These assemblages were distinct even though a strong vertical mixing was present. However the bottom assemblages were not sampled (the bottom at the sampling stations was at 22-25 m). For several species of this inshore assemblage, larvae were present at different developmental stages, suggesting retention in nearshore waters.

In this paper we describe the nearshore larval assemblages present at the Arrábida Marine Park (west coast of Portugal) where we have observed dense schools of larvae near the reefs at shallow depths (less than 15m) during SCUBA diving. Our aims are: (1) to investigate the composition and annual variation of the coastal larval fish assemblages present during the Spring-Summer period; (2) to compare the structure of the assemblage and larval density at the surface and bottom depth strata; (3) to search for possible ontogenetic vertical distribution patterns.

MATERIALS AND METHODS

Study Area

This study was carried out at the Arrábida Marine Park, between Sesimbra and Portinho da Arrábida, 30 Km South of Lisbon (9º00'15'' – 9º03'48''W and 38º26' – 38º27'N) (Fig. 1). Although located on the Portuguese west coast, the study site faces south, being protected from the prevailing north and north-west winds and waves. Relatively calm conditions exists throughout the year, allowing sampling in the very-nearshore where wave action is negligible. Tidal currents parallel to the shore-line prevail. The nearby Sado estuary has little influence over this coastal area. The adjacent mountain chain of Arrábida is characterized by high vertical calcareous cliffs. Boulders of many different sizes, resulting from the disintegration of these cliffs, originate a highly heterogeneous rocky subtidal habitat where many benthic fish species occur (Gonçalves *et al*. 2003). In the extreme nearshore, the rocky substratum extends offshore only for some tens of meters and depths are very shallow (maximum around 13 m).

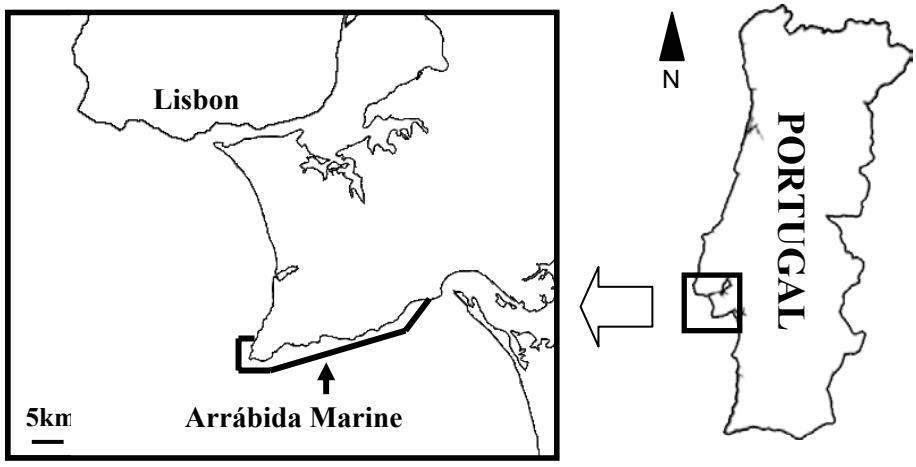


Fig. 1. Study site location

Sampling Procedure

Sampling was performed in the extreme nearshore in the Spring-Summer period, when most coastal species breed. The surface larval assemblage was sampled with sub-superficial trawls in 1999 (N = 48) and 2000 (N = 30). The bottom assemblage was sampled in 2001 (N = 48) and 2002 (N = 54). In 2003, both depth strata were sampled (N = 27 surface tows and N = 24 bottom tows). Due to logistic constraints all samples were taken during the day between 9h and 18h at all tidal phases.

Surface samples consisted of five minute sub-superficial (1 m depth) trawls using a standard plankton net with a 350 μm mesh size, 0.30 m mouth diameter and a mouth diameter: net length ratio of 1:5. A small 4.6 m semi-rigid inflatable boat towed the net at a distance of 20 m from the boat, and a speed of approximately 1.5 knots. Bottom sampling was performed with a plankton net attached to an underwater scooter. This net was similar to the one used at the surface trawls, but the mouth diameter: net length ratio was 1:3 due to manoeuvrability reasons. The plankton trawls were undertaken at a distance of approximately 0.50 m from the rocky substrate. After reaching the bottom the diver opened the net and begun the trawl following a direction parallel to the shoreline, contouring obstacles when needed. Five minutes later the diver would close the net and slowly ascend to the surface. Sampling speed was approximately 1.5 knots. All samples were performed over the whole extent of the rocky bottom, from 4 to 13 m. In each bottom sample we followed the bottom contour. The average difference between maximum and minimum depths per bottom sample was 1.92 m (SD = 0.86). Hydrobios flowmeters were attached to both nets. Filtered volumes, sampling periods and number of larvae caught are shown in Table 1.

Table 1. Sampling periods, volume filtered and number of larvae caught at the surface and bottom samples in each year

				Volume filtered (m^3)			Number of larvae		
Depth	**Year**	**Sampling period**	**N**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Total**
Surface	1999	26 May - 30 Aug	48	25.44	6.45	14.12-42.58	22.29	24.17	1070
	2000	31 May - 21 Aug	30	28.67	4.20	15.12-35.52	12.87	9.63	386
	2003	11 Jun - 21 Aug	27	28.93	6.09	16.31-42.75	20.74	15.22	560
Bottom	2001	26 Jun - 09 Aug	48	6.87	1.32	4.54-9.10	20.19	29.23	969
	2002	02 Jul - 25 Jul	54	7.22	2.15	3.07-11.34	13.98	19.04	755
	2003	19 May - 07 Aug	24	11.15	1.78	7.44-13.91	35.38	31.58	849

All samples were preserved in 4% saline formalin buffered with sodium borate, for at least one month before larvae were sorted and identified under a stereomicroscope to the lowest possible taxonomic level (species level when possible). We identified 94% of the larvae to the family level (99% in the bottom samples and 88% in the surface samples), 86% to the genus level (97% in the bottom samples and 71% in the surface samples) and 83% to the species level (95% in the bottom samples and 69% in the surface samples).

Photographs were made to help in the identifications, using a digital camera attached to a stereomicroscope. Body length (BL), corresponding to the notochord length in pre-flexion larvae or to the standard length in post-flexion larvae, was measured to the nearest 0.01 mm using a micrometer scale. For larvae larger than 15.00 mm measurements were made using a calliper. A total of 14.5% of larvae in the surface samples and 5.1% in the bottom samples were in bad condition and were not measured.

Data analysis

Composition and annual patterns of larval assemblages

Larval abundances were calculated for every taxa identified in each sample and are expressed as the number of larvae per 1000 m^3. Two biodiversity indices were calculated for each sample, the Shannon Diversity Index (H') using the natural logarithm in its formulation and the Average Taxonomic Distinctness Index (Delta*) which reflects the taxonomic spread of species among samples (Clarke and Warwick 2001). This index is based not just on the species abundances but also in the taxonomic distances between every pair of individuals; high Delta* values (maximum=100) reflect high taxonomic diversity in the assemblage (Clarke and Warwick 2001). Equal step-lengths were assumed between each taxonomic level. Four taxonomic levels were used, from species to order. Mean values and standard deviation of these indices were calculated for each year at each depth strata.

Annual differences in total larval abundances and diversity indices were tested with One-way ANOVA and Student-Newman-Keuls tests for post-hoc comparisons, when heteroscedascity assumptions were met. If needed, variables were log (x+1) transformed. When variances were heterogeneous a Kruskall-Wallis ANOVA was used and post-hoc comparisons were performed with the Dunn's test. Using the same criteria, T-student tests or Mann-Whitney U tests were used for the comparisons of overall abundance and diversity indices between the surface and bottom samples.

Given that tide may have an effect on larval distribution (Neilson and Perry 1990; Cowen, 2002) and as it was randomized in this study, we tested for possible interaction between tides and depth on the larval abundance. To do so we used a

factorial ANOVA considering the tidal phase and the depth strata as factors. Prior to the analysis, data were transformed following a log(log (x+1)+1) transformation to meet parametric assumptions.

Differences in larval assemblages between depth strata

Using the relative abundance of each species, differences between the structure of surface and bottom assemblages were graphically displayed with a non-metric multidimensional scaling (MDS) two-dimensional plot. The ordination was based on a triangular matrix of Bray-Curtis similarities after a log (x+1) data transformation.

Samples in plots that are closer together are less distinct and a stress coefficient determines the relationship among samples from distinct groups (Clarke and Warwick 2001). Larvae which could not be identified were not considered in the analysis: 11.1% of the larvae present in surface samples (from which 87% were in the pre-flexion stage) and 0.58% of the larvae from the bottom samples (98% of which were in the pre-flexion stage). Six groups were considered in the analysis, corresponding to the different years sampled at each depth. In order to test for differences between groups a One-way Analysis of Similarities (ANOSIM) was performed. High R values indicate differences between groups (Clarke and Warwick 2001). Similarity percentages analysis (SIMPER) was used to determine the species contribution to each group after log (x+1) transformation of the data, assuming a cut off at 95%. As the MDS stress level was higher than 0.1 (Clarke and Warwick 2001), we performed a cluster analysis based on the Bray-Curtis similarities matrix with log (x+1) transformed data. To simplify the cluster graphical interpretation we used the average similarity contribution of each species to the average similarity within each group, according to the SIMPER results.

Ontogenetic vertical distribution patterns

To access possible ontogenetic differences in the distribution of larvae between depth strata, the length of larvae of the most representative species was compared between the surface and bottom samples with T-student tests (the log x transformation was used when needed) or Mann-Whitney U tests (if variances were heterogeneous even after transformation). Developmental stage of each larva was categorized in: pre-flexion, incomplete flexion and post-flexion stages following Leis and Carson-Ewart (2000b). We considered all larval stages from hatching, including yolk-sac larvae.

The PRIMER 5 programme was used for the calculation of diversity indices and multivariate analyses. STATISTICA 7 (StatSoft, Inc. 2004) was used for all other statistics.

RESULTS

Composition and annual patterns of larval assemblages

A total of 4589 larvae (2016 from the surface samples and 2573 from the bottom samples) were collected belonging to 62 identifiable taxa included in 22 families (Table 2). Most larvae caught belonged to species whose adults live in nearshore waters laying benthic eggs (e.g. Blenniidae, Gobiidae, Tripterygiidae, and some Labridae). However there were also a few coastal larvae hatching from pelagic eggs (e.g. Sparidae, Serranidae) and species whose adults live and spawn in coastal and shelf waters like *Sardina pilchardus*, *Trachurus trachurus* and *Engraulis encrasicolus*.

Although variation in total larval abundance among years was apparent for both depth strata (Fig. 2), total larval abundance was always higher at the bottom than at the surface (surface samples: mean = 735.51 larvae 1000 m^{-3}, SD = 646.83, N = 105; bottom samples: mean = 2632.83 larvae 1000 m^{-3}, SD = 3334.12, N = 126; Z = 6.214, P < 0.001).

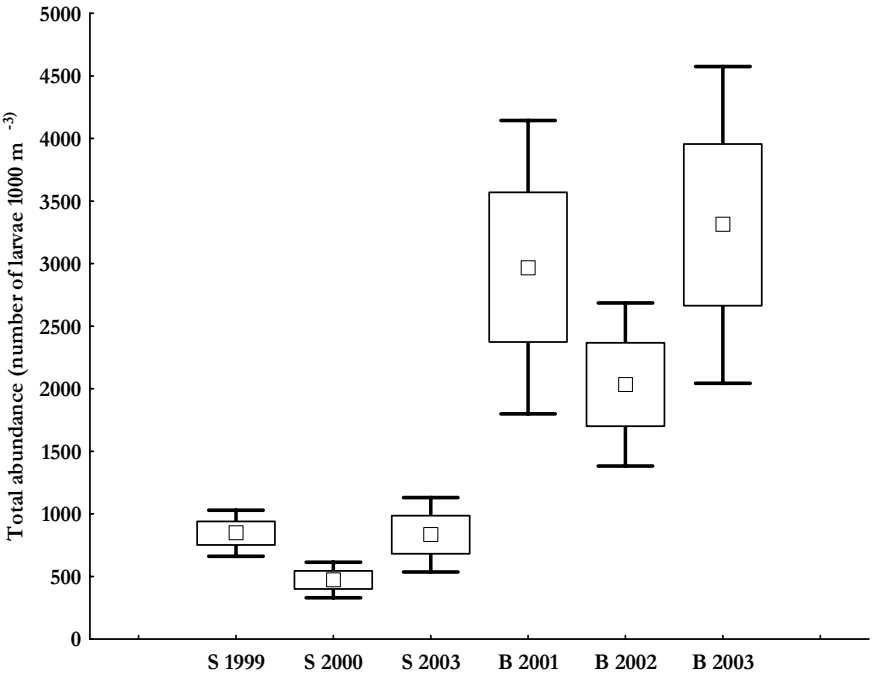


Fig. 2. Total larval abundance at each depth strata and in each year sampled. (S = surface; B = bottom). Central square = mean; large rectangle = mean ± S.E.; whiskers = Mean ± 1.96 S.E.

Table 2. Species composition and abundance (expressed as number of larvae 1000 m^{-3}) of the surface and bottom assemblages

		SURFACE						BOTTOM					
		1999 (N=48)		2000 (N=30)		2003 (N=27)		2001 (N=48)		2002 (N=54)		2003 (N=24)	
Family	**Species**	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD
Atherinidae	*Atherina presbyter*	**3.93**	12.45	**3.93**	12.01	**9.49**	18.72	**----**	**----**	**----**	**----**	**----**	**----**
Belonidae	*Belone belone*	**----**	**----**	**2.20**	12.07	**1.15**	5.98	**----**	**----**	**----**	**----**	**----**	**----**
Blenniidae	*Coryphoblennius galerita*	**6.82**	19.67	**24.17**	30.58	**5.32**	13.18	**----**	**----**	**----**	**----**	**----**	**----**
	Lipophrys pholis	**----**	**----**	**----**	**----**	**1.15**	5.98	**----**	**----**	**----**	**----**	**----**	**----**
	Parablennius gattorugine	**4.72**	16.79	**1.89**	7.19	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**
	Parablennius pilicornis	**40.60**	78.04	**118.76**	183.87	**201.35**	435.76	**4.04**	28.01	**5.59**	29.15	**3.78**	18.51
Bothidae	*Arnoglossus thori*	**13.84**	28.47	**5.74**	13.21	**6.23**	18.25	**----**	**----**	**19.48**	101.26	**----**	**----**
Callionymidae	*Callionymus reticulates*	**2.97**	10.48	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**4.24**	20.79
	Callionymus spp.	**24.98**	92.46	**4.78**	15.96	**11.33**	26.15	**----**	**----**	**28.82**	76.63	**----**	**----**
Carangidae	*Trachurus* spp.	**3.51**	11.97	**10.02**	49.81	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**
	Trachurus mediterraneus	**----**	**----**	**1.11**	6.06	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**
	Trachurus trachurus	**8.88**	24.36	**3.43**	10.48	**8.48**	18.29	**----**	**----**	**2.76**	20.31	**5.31**	26.02
Clupeidae	*Sardina pilchardus*	**114.06**	161.06	**15.83**	27.55	**13.27**	27.08	**11.68**	59.57	**69.64**	169.51	**----**	**----**
Engraulidae	*Engraulis encrasicolus*	**86.93**	144.40	**----**	**----**	**22.85**	37.12	**----**	**----**	**4.37**	32.09	**----**	**----**
Gobiesocidae	*Lepadogaster candolii*	**----**	**----**	**----**	**----**	**----**	**----**	**12.14**	42.75	**8.39**	49.74	**10.19**	34.70
	Lepadogaster lepadogaster	**5.03**	15.95	**----**	**----**	**1.07**	5.56	**----**	**----**	**2.37**	17.39	**----**	**----**
Gobiidae	*Aphia minuta*	**----**	**----**	**----**	**----**	**----**	**----**	**2.32**	16.09	**----**	**----**	**----**	**----**
	Gobius niger	**27.16**	54.71	**21.10**	67.16	**20.47**	37.70	**71.88**	432.43	**----**	**----**	**----**	**----**
	Gobius xanthocephalus	**----**	**----**	**----**	**----**	**2.26**	8.14	**1226.83**	2824.89	**244.16**	577.83	**977.30**	2637.92
	Gobius spp.	**0.54**	3.71	**----**	**----**	**3.23**	16.79	**2.88**	19.96	**3.25**	23.86	**----**	**----**
	Gobiusculus flavescens	**3.13**	18.55	**1.27**	6.96	**12.02**	28.63	**5.92**	41.00	**3.89**	20.08	**157.24**	427.62
	Gobiidae spp.	**3.86**	13.58	**3.53**	10.79	**5.63**	14.07	**30.58**	127.40	**10.57**	55.95	**47.99**	102.12
	Pomatoschistus spp.	**1.43**	6.92	**2.48**	9.49	**1.21**	6.29	**20.43**	64.67	**----**	**----**	**30.23**	148.09
	Pomatoschistus microps	**----**	**----**	**----**	**----**	**----**	**----**	**2.52**	17.50	**4.18**	30.70	**----**	**----**
	Pomatoschistus pictus	**6.02**	17.35	**1.51**	8.29	**24.32**	38.77	**975.93**	2159.22	**1111.54**	1855.59	**1662.32**	1416.36
Labridae	*Centrolabrus exoletus*	**----**	**----**	**----**	**----**	**----**	**----**	**16.76**	45.20	**36.32**	106.86	**----**	**----**
	Coris julis	**39.04**	60.13	**24.02**	39.60	**16.94**	30.92	**----**	**----**	**6.89**	36.76	**----**	**----**
	Ctenolabrus rupestris	**0.49**	3.39	**2.34**	8.92	**----**	**----**	**3.52**	24.40	**16.09**	58.22	**----**	**----**
	Labridae spp.	**----**	**----**	**----**	**----**	**----**	**----**	**3.15**	21.83	**----**	**----**	**----**	**----**
	Symphodus spp.	**7.22**	26.33	**5.38**	15.05	**62.16**	107.79	**105.73**	218.56	**51.18**	93.67	**----**	**----**
	Symphodus bailloni	**0.72**	4.95	**----**	**----**	**----**	**----**	**18.20**	66.15	**24.86**	65.20	**----**	**----**
	Symphodus melops	**----**		**----**	**----**	**----**	**----**	**209.62**	477.68	**106.36**	159.76	**15.52**	36.02
	Symphodus roissali	**5.30**	24.28	**9.72**	21.75	**3.36**	12.92	**22.46**	69.35	**20.61**	75.96	**3.00**	14.72

Table 2. (cont.)

		SURFACE						BOTTOM					
		1999 (N=48)		2000 (N=30)		2003 (N=27)		2001 (N=48)		2002 (N=54)		2003 (N=24)	
Family	**Species**	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD
Mugilidae	*Mugil cephalus*	**4.49**	15.31	**----**	**----**	**4.97**	12.17	**----**	**----**	**----**	**----**	**----**	**----**
	Mugilidae spp.	**1.82**	8.89	**1.15**	6.29	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**
Mullidae	*Mullus surmuletus*	**3.08**	10.81	**4.09**	12.49	**1.15**	5.96	**----**	**----**	**----**	**----**	**----**	**----**
No ident.	*Gadoide no ident*	**----**	**----**	**----**	**----**	**1.62**	8.40	**----**	**----**	**----**	**----**	**----**	**----**
	no ident	**93.27**	89.36	**62.19**	52.54	**74.58**	87.13	**6.37**	30.89	**34.32**	76.38	**5.31**	26.02
	no ident sp.1	**2.06**	10.02	**2.10**	11.50	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**
	no ident sp.2	**8.93**	25.90	**1.11**	6.08	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**
Phycidae	*Gaidropsarus mediterraneus*	**----**	**----**	**----**	**----**	**1.62**	8.40	**----**	**----**	**----**	**----**	**----**	**----**
Scorpaenidae	*Scorpaena porcus*	**----**	**----**	**1.27**	6.96	**0.87**	4.50	**----**	**----**	**----**	**----**	**----**	**----**
Serranidae	*Serranus* spp.	**75.30**	94.30	**58.72**	88.70	**31.42**	53.62	**----**	**----**	**10.54**	45.22	**----**	**----**
	Serranus atricauda	**----**	**----**	**1.11**	6.08	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**
	Serranus cabrilla	**3.37**	23.34	**1.03**	5.65	**3.08**	11.38	**----**	**----**	**----**	**----**	**----**	**----**
	Serranus hepatus	**1.81**	12.56	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**
Soleidae	*Microchirus variegatus*	**0.81**	5.63	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**
	Soleidae spp.	**11.49**	29.35	**2.53**	9.70	**3.20**	11.72	**----**	**----**	**9.15**	39.19	**----**	**----**
	Solea spp.	**----**	**----**	**1.27**	6.96	**----**	**----**	**----**	**----**	**----**	**----**	**4.24**	20.79
	Solea lascaris	**1.66**	8.13	**----**	**----**	**1.87**	9.73	**----**	**----**	**2.42**	17.82	**----**	**----**
	Solea senegalensis	**4.67**	19.27	**----**	**----**	**3.26**	11.90	**----**	**----**	**----**	**----**	**----**	**----**
Sparidae	*Boops boops*	**----**	**----**	**1.12**	6.07	**12.18**	42.95	**137.12**	244.09	**72.52**	150.25	**----**	**----**
	Diplodus spp.	**15.52**	34.34	**2.24**	8.64	**4.68**	14.27	**----**	**----**	**----**	**----**	**----**	**----**
	Sparidae spp.	**36.99**	59.60	**6.01**	16.28	**17.44**	34.44	**7.06**	34.35	**29.62**	125.23	**----**	**----**
	Sparidae sp.1	**92.90**	129.14	**44.07**	52.32	**134.17**	177.93	**----**	**----**	**34.39**	119.59	**3.98**	19.48
	Pagellus sp.1	**5.96**	21.26	**4.79**	15.20	**6.82**	23.59	**----**	**----**	**----**	**----**	**----**	**----**
Syngnathidae	*Entelurus aequoreus*	**----**	**----**	**1.12**	6.07	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**
	Hippocampus hippocampus	**----**	**----**	**----**	**----**	**1.14**	5.95	**----**	**----**	**----**	**----**	**----**	**----**
	Hippocampus ramulosus	**1.05**	7.26	**1.30**	7.13	**----**	**----**	**----**	**----**	**----**	**----**	**----**	**----**
Trachinidae	*Echiichthys vipera*	**0.98**	6.78	**----**	**----**	**1.20**	6.22	**----**	**----**	**----**	**----**	**----**	**----**
	Trachinus draco	**4.21**	14.11	**3.75**	11.49	**12.91**	33.69	**----**	**----**	**----**	**----**	**----**	**----**
Triglidae	*Trigla* spp.	**----**	**----**	**----**	**----**	**3.44**	13.13	**----**	**----**	**----**	**----**	**----**	**----**
Tripterygiidae	*Tripterygion delaisi*	**63.71**	168.98	**7.65**	27.38	**78.52**	166.78	**71.07**	202.83	**59.79**	101.39	**378.66**	919.31

Factorial ANOVA results showed that this difference was significant (F=6.46, d.f. = 1 $P < 0.05$), that there was a tidal effect over larval abundance (F=3.14, d.f. = 3, $P<0.05$), but there was no interaction between tide and depth (F = 0.19, d.f. = 3, P = 0.90.). The inter-annual variation in larval density in the bottom samples was not significantly different (H = 4.26, d.f. = 2, P = 0.12), but at the surface significant variations between years were found (F = 3.673, d.f. = 2, $P < 0.05$) with larval densities observed in 2000 lower than both in 1999 ($P < 0.05$) and 2003 ($P < 0.05$).

Table 3. Shannon diversity index (H') and average taxonomic distinctness index (Δ*) in each depth strata and year sampled. F = value of One-Way ANOVA (Newman-Keuls post-hoc test); t = value of t-test for independent samples; Z = value of Mann-Whitney U test; *ns* not significant * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Depth	Year	N	Mean H'	SD H'	Statistics	Post-hoc	Mean Δ*	SD Δ*	Statistics	Post-hoc
Surface	1999	47	1.65	0.53	F = 4.44	99-00 *;	83.06	18.61	F = 1.72	
	2000	30	1.34	0.48	*	00-03 *	76.33	16.29	*ns*	
	2003	26	1.65	0.35			80.96	4.68		
Bottom	2001	43	0.88	0.53	F = 5.72	01-03 *;	53.24	25.86	F = 5.38	01-02 *;
	2002	53	1.03	0.46	**	02-03 **	67.14	20.64	**	02-03 *
	2003	24	0.62	0.46			53.27	22.64		
Surface x Bottom		103	1.56	0.50	t = -9.81		80.57	15.66	Z = -9.44	
		120	0.90	0.51	***		59.39	23.86	***	

Diversity was significantly higher in the surface samples (Table 3). Annual variation in diversity was significant at the bottom samples for both the Shannon Diversity Index and the Average Taxonomic Distinctness Index, with a decrease in the overall diversity in 2003 and an increase in taxonomic diversity in 2002. On the

contrary, no significant changes in taxonomic diversity were found in the surface samples, but overall diversity exhibited a significant decrease in 2000 (Table 3).

Differences in larval assemblages between depth strata

The MDS graphical representation showed a clear distinction between the structure of surface and bottom assemblages (Fig. 3). A similar result was obtained with the cluster analysis (Fig. 4). One-way analysis of similarity (ANOSIM) revealed that these differences were significant (Global R = 0.46, p = 0.001; Table 4). There were low R values in every pair-wise comparison between years in the same depth strata, but all comparisons between any surface layer group with any bottom layer group revealed high values of R (above 0.55), showing significant differences between surface and bottom assemblages (Clarke and Warwick 2001).

Table 4. Summary of one-way analysis of similarity (ANOSIM) with par-wise comparisons of larval assemblages between years and depth strata. 999 permutations were used for each test. The value of the R statistic and its significance are shown. Numbers in bold represent statistically significant comparisons. S = Surface; B = Bottom

		R	Significance
	Global R	**0.46**	0.001
Surface	S1999 vs S2000	0.24	0.001
	S1999 vs S2003	0.18	0.001
	S2000 vs S2003	0.16	0.001
Bottom	B2001 vs B2002	0.04	0.02
	B2001 vs B2003	0.09	0.04
	B2002 vs B2003	0.03	0.23
Surface x Bottom	S1999 vs B2001	**0.75**	0.001
	S1999 vs B2002	**0.68**	0.001
	S1999 vs B2003	**0.81**	0.001
	S2000 vs B2001	**0.69**	0.001
	S2000 vs B2002	**0.71**	0.001
	S2000 vs B2003	**0.78**	0.001
	S2003 vs B2001	**0.55**	0.001
	S2003 vs B2002	**0.55**	0.001
	S2003 vs B2003	**0.65**	0.001

The similarity percentages analysis (SIMPER) showed that surface assemblages included 22 taxa which explained 95% of the similarity among groups (Table 5). Larvae which contribute most to this similarity include clupeiformes (*Sardina pilchardus* and *Engraulis encrasicolus*), sparids, serranids (*Serranus* sp.), blenniids (*Parablennius pilicornis* and *Coryphoblennius galerita*), tripterygiids (*Tripterygion delaisi*) and the labrids *Symphodus* spp.. In the bottom samples fewer species were present with only 9 taxa contributing to 95% of the similarity between samples.

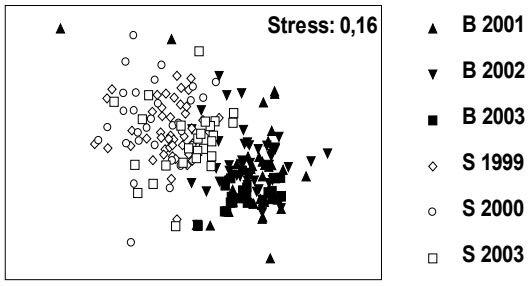


Fig. 3. Non-metric multidimensional scaling (MDS) two-dimensional plot for each year and depth strata (B = bottom samples; S = surface samples)

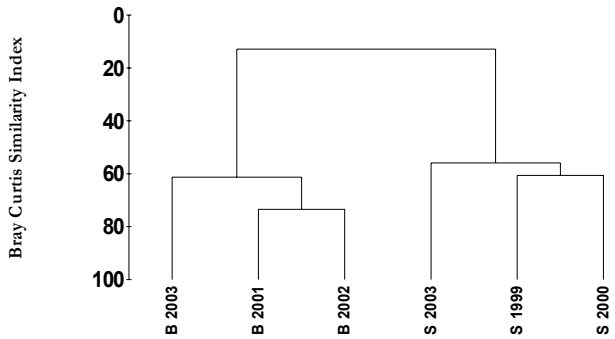


Fig. 4. Cluster analysis on log (x+1) transformed data based on a Bray-Curtis similarity matrix for the different years and depth strata (B = bottom samples; S = surface samples)

Table 5. Similarity percentages analysis (SIMPER) results for the surface and bottom assemblages in the years sampled. Average similarity values and percentage contribution of the most representative species to the average similarity within each group, after log (x+1) transformation of abundance data, are shown. Cut off for low contributions = 95%

Taxa	Average similarity	Contribution (%)	Cumulative %
Surface 1999	32.05		
Sardina pilchardus	7.52	23.46	23.46
Serranus spp.	5.43	16.95	40.42
Sparidae sp.1	4.45	13.87	54.29
Engraulis encrasicolus	3.02	9.43	63.72
Coris julis	2.32	7.24	70.96
Parablennius pilicornis	2.00	6.24	77.20
Tripterygion delaisi	1.78	5.56	82.76
Sparidae spp.	1.73	5.41	88.17
Gobius niger	0.87	2.73	90.90
Arnoglossus thori	0.48	1.51	92.40
Diplodus spp.	0.47	1.46	93.87
Soleidae spp.	0.30	0.94	94.81
Callionymus spp.	0.22	0.69	95.50
Surface 2000	25.21		
Parablennius pilicornis	5.23	20.75	20.75
Serranus spp.	4.79	19.01	39.76
Coryphoblennius galerita	4.75	18.86	58.62
Sparidae sp.1	4.73	18.77	77.40
Coris julis	1.74	6.91	84.30
Sardina pilchardus	1.39	5.51	89.81
Gobius niger	0.52	2.08	91.89
Symphodus roissali	0.45	1.77	93.66
Arnoglossus thori	0.30	1.21	94.87
Trachurus trachurus	0.22	0.85	95.73
Surface 2003	27.29		
Sparidae sp.1	5.79	21.22	21.22
Parablennius pilicornis	4.08	14.94	36.16
Tripterygion delaisi	3.53	12.92	49.08
Symphodus spp.	2.75	10.07	59.15
Serranus spp.	1.74	6.37	65.52
Engraulis encrasicolus	1.53	5.60	71.12
Pomatoschistus pictus	1.33	4.86	75.98
Gobius niger	0.98	3.58	79.56
Sparidae spp.	0.95	3.49	83.05
Coris julis	0.92	3.38	86.43
Sardina pilchardus	0.56	2.05	88.47
Trachurus trachurus	0.53	1.95	90.42
Atherina presbyter	0.45	1.66	92.08
Mugil cephalus	0.36	1.31	93.39
Trachinus draco	0.32	1.16	94.55
Gobiusculus flavescens	0.28	1.03	95.58

Taxa	Average similarity	Contribution (%)	Cumulative %
Bottom 2001	33.74		
Pomatoschistus pictus	15.11	44.77	44.77
Gobius xanthocephalus	8.90	26.39	71.16
Boops boops	4.11	12.19	83.35
Symphodus spp.	2.09	6.18	89.53
Symphodus melops	1.40	4.16	93.69
Tripterygion delaisi	0.96	2.84	96.53
Bottom 2002	33.52		
Pomatoschistus pictus	18.23	54.38	54.38
Symphodus melops	4.42	13.20	67.58
Gobius xanthocephalus	3.37	10.04	77.62
Tripterygion delaisi	1.90	5.67	83.29
Symphodus spp.	1.55	4.63	87.92
Boops boops	1.37	4.10	92.02
Sardina pilchardus	0.89	2.65	94.67
Callionymus spp.	0.36	1.07	95.74
Bottom 2003	49.38		
Pomatoschistus pictus	30.89	62.56	62.56
Tripterygion delaisi	8.57	17.36	79.92
Gobius xanthocephalus	6.80	13.77	93.69
Gobiusculus flavescens	1.68	3.40	97.09

Larvae which contribute most to the similarity are almost exclusively from coastal species which lay benthic eggs. The only exception was the sparid *Boops boops* which lays pelagic eggs but also breeds in nearshore waters. Gobiids dominate this assemblage with only two species, *Pomatoschistus pictus* and *Gobius xanthocephalus*, explaining together 71.16%, 64.42% and 76.33% of the similarity among groups in 2001, 2002 and 2003, respectively (Table 5).

Ontogenetic vertical distribution patterns

Larvae present at the surface were significantly smaller than at the bottom (surface: mean = 2.99 mm, SD = 1.39, range = 1.07 – 17.07, N = 1724; bottom: mean = 7.24 mm, SD = 2.36, range = 1.10 – 23.00, N = 2442; Z = 48.62, $p < 0.001$). This overall pattern was found for most species present at the bottom; exceptions were *Callionymus* spp., Sparidae sp1 and *Tripterygion delaisi* (Table 6). Most larvae caught at the surface were small and undeveloped (83.0% of the larvae were less than 4 mm BL and 92.3% were in the pre-flexion stage).

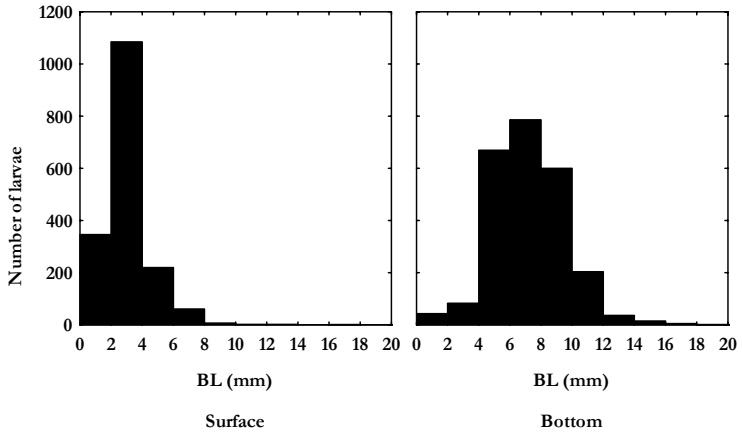


Fig. 5. Size class distribution of larvae caught at the surface and bottom samples.

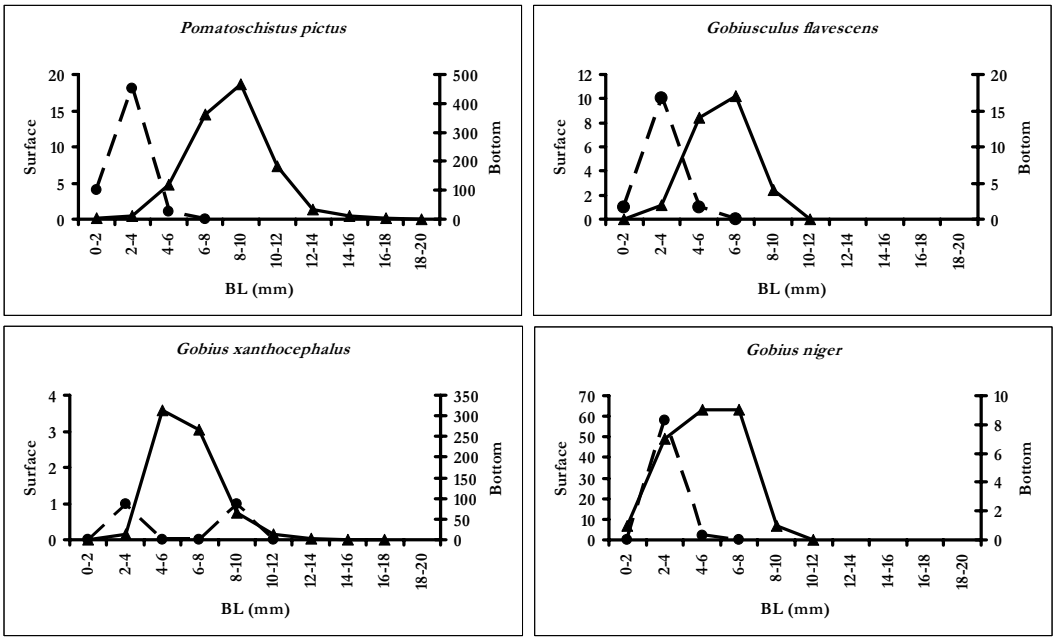
On the contrary, larvae caught at the bottom were larger (94.8% >4 mm, see Fig. 5) and more developed (90.3% were in the flexion or post-flexion stages).

Analysing the size distribution of the most representative species according to the SIMPER analysis, an interesting pattern emerges for those species which were present at both depths. In most cases, only small larvae occurred at the surface whereas all size classes were present at the bottom (Fig. 6). For species which were abundant at the bottom, larvae from 4 mm to the 10-12 mm or to the 18-20 mm size classes (depending on the species considered) were present. These patterns of small larvae at the surface and different size-classes at the bottom could be observed in the gobiids *Pomatoschistus pictus*, *Gobiusculus flavescens* and *Gobius niger*; the sparid *Boops boops* and labrids from the genus *Symphodus*. *Symphodus melops* larvae were also present at the bottom in different size classes, although no larvae of this species occurred at the surface. Small *Symphodus* larvae (2-4 mm size class) present at the surface and included in the category *Symphodus* spp., could belong to either *S. melops* or *Symphodus cinereus*, since adults of both species are common at the study site and both larvae have similar pigmentation patterns when newly hatched (Quignard 1967, 1968, Fives 1976). For *Gobius xanthocephalus*, only two larvae were caught at the surface, but different size classes were captured near the bottom. Finally, *Tripterygion delaisi,* the third most abundant species at the bottom, represents an exception to this pattern with size-class distribution of larvae caught at the surface and at the bottom overlapping, although slightly bigger larvae were caught at the surface (Fig. 6).

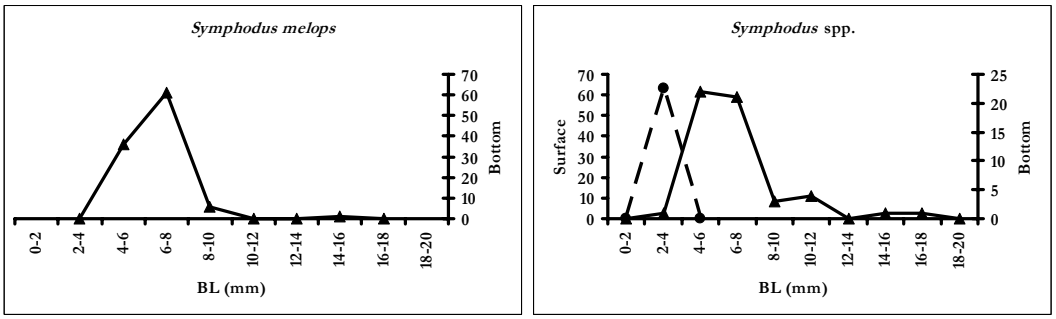
Table 6. Body lengths (in mm) for larvae of the most abundant species present in the surface and bottom samples. Statistical tests were computed for species with at least five individuals at both depth strata. t = t-test for independent samples; Z = Mann-Whitney U test. *ns* not significant * P < 0.05, ** P < 0.01, *** P < 0.001.

		Surface			Bottom			
Family	Species	Mean	SD	N	Mean	SD	N	Statistics
Atherinidae	*Atherina presbyter*	6.26	0.44	14	----	----	----	
Blenniidae	*Coryphoblennius galerita*	3.23	0.22	27	----	----	----	
	Parablennius pilicornis	2.66	0.16	229	2.45	0.18	3	
Bothidae	*Arnoglossus thori*	2.35	0.34	21	2.82	0.33	5	t =2.78 *
Callionymidae	*Callionymus* spp.	1.70	0.20	34	1.54	0.15	9	t = 2.27 *
Carangidae	*Trachurus* spp.	1.81	0.60	11	----	----	----	
	Trachurus trachurus	3.31	0.97	19	2.30	0.06	2	
Clupeidae	*Sardina pilchardus*	4.56	1.21	156	4.68	1.62	22	t = -0.44 ns
Engraulidae	*Engraulis enchrasicolus*	3.53	0.64	103	3.20	----	1	
Gobiesocidae	*Lepadogaster candollii*	----	----	----	4.71	0.60	10	
Gobiidae	*Gobius niger*	2.83	0.56	60	5.23	1.54	27	Z = 6.49 ***
	Gobius xanthocephalus	5.55	3.75	2	6.38	1.50	674	
	Gobiusculus flavescens	2.73	1.07	12	6.37	1.28	37	t = 8.87 ***
	Pomatoschistus spp.	2.96	0.88	4	6.77	1.51	14	
	Pomatoschistus pictus	2.47	0.61	23	8.44	2.10	1191	t = 21.67 ***
Labridae	*Centrolabrus exoletus*	----	----	----	7.39	1.19	19	
	Coris julis	2.28	0.50	61	2.16	0.34	2	
	Ctenolabrus rupestris	2.55	0.23	3	8.48	5.56	7	
	Symphodus spp.	2.83	0.17	63	6.97	2.39	53	Z = 8.90 ***
	Symphodus bailloni	----	----	----	5.36	0.82	14	
	Symphodus melops	----	----	----	6.47	1.18	104	
	Symphodus roissali	2.86	0.19	13	5.42	1.77	14	Z = 3.62 ***
Serranidae	*Serranus* spp.	2.15	0.39	135	1.97	0.32	3	
Soleidae	*Soleidae* spp.	2.13	0.35	12	1.47	0.13	3	
Sparidae	*Boops boops*	5.91	1.17	9	9.05	2.13	62	t = 4.31, ***
	Diplodus spp.	2.90	0.30	20	----	----	----	
	Sparidae spp.	2.47	0.46	59	2.74	1.42	11	t = 0.54 ns
	Sparidae sp.1	2.31	0.68	222	1.79	0.35	12	Z = 2.34 *
	Pagellus sp.1	2.95	0.30	14	----	----	----	
Trachinidae	*Trachinus draco*	2.52	0.34	16	----	----	----	
Tripterygiidae	*Tripterygion delaisi*	5.39	1.15	127	4.67	0.62	119	Z = 5.06 ***

Gobiidae



Labridae



Sparidae **Tripterygiidae**

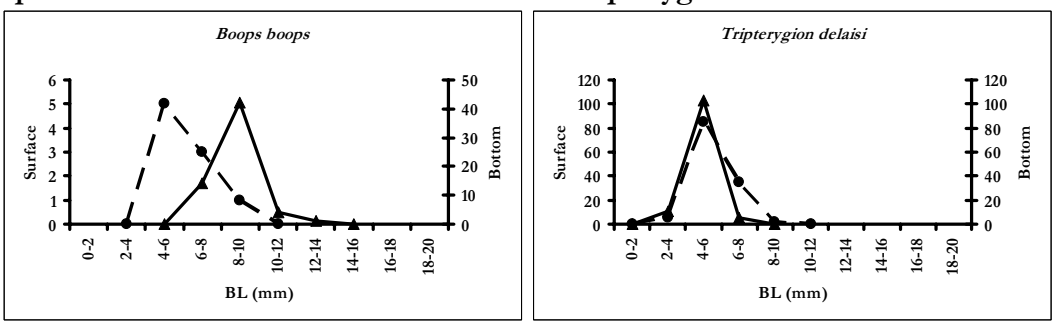


Fig. 6. Size-class distribution at the surface and bottom samples for species that occur with > 25 individuals at the bottom samples. Dashed line = surface samples; Solid line = bottom samples.

DISCUSSION AND CONCLUSIONS

The very nearshore larval fish assemblages studied in the present work were exclusively composed by shore or shelf-dwelling species. Larvae from shore fish species included sparids, serranids, blenniids, gobiids, tripterygiids and labrids, reflecting the adult fish assemblage occurring at the study area (Henriques *et al*. 1999). Larvae from shelf-dwelling spawners were mainly clupeids, carangids and engraulids. These results generally agree with Sabatés *et al*. (2003) who found nearshore larval assemblages at a rocky shore in the northwest Mediterranean to be essentially composed by shorefish species (also including gobiids, sparids, labrids, tripterygiids and a few shelf species). Other studies found similar results in other geographic areas: New Zealand (Kingsford and Choat 1989); Gulf of California (Brogan 1994); South Africa (Tilney *et al*. 1996); Peru (Velez *et al*. 2005).

Larvae from slope or oceanic families which are abundant off the Portuguese coast, like myctophids or paralepidids (John and Ré 1993), were not found. Most coastal species known to breed at the Arrábida Marine Park during the spring and summer period (Henriques *et al*. 1999) were present in our samples. However, there were a few exceptions like clingfishes (family Gobiesocidae).

Some authors have shown that clingfish species can be abundant near reefs (e.g. Marliave 1986, Kingsford and Choat 1989, Tilney *et al*. 1996, Sabatés *et al*. 2003). Using light-traps we have been able to confirm this as we caught many clingfish larvae from all size classes in the study area (unpublished data). A possible explanation for the fact that although larvae from this family are present in the area and do not occur in our samples, is the short planktonic larval duration of clingfishes (15 days for *Apletodon dentatus* and 13 days for *Lepadogaster candolii*; Raventós and Macpherson 2001). Moreover, these species hatch at a large size with an advanced stage of development

and are probably able to actively swim and find shelter (for instances hide among algal tufts) very early in life. This could explain why they were caught using light-traps, but absent from the bottom and surface sampling.

Larval assemblages at the surface and at the bottom were clearly distinct, indicating that this very nearshore larval fish assemblage is vertically structured at a small scale (a few meters). The surface assemblage was much more diverse, being composed by coastal larvae hatching from both pelagic and benthic eggs. The bottom assemblage was composed by a small number of exclusively nearshore reef-associated species laying benthic eggs (like gobiids, labrids and tripterygiids), with the exception of the sparid *Boops boops*, which is abundant in the study area (Henriques *et al*. 1999) and lays pelagic eggs but also breeds nearshore.

Despite some inter-annual fluctuations overall larval density was much higher at the bottom than at the surface. This result indicates that larvae school near the substrate at high densities for some species. The gobies *Pomatoschistus pictus* and *Gobius xanthocephalus* dominated this assemblage. Several studies have documented the presence of Gobiidae larvae nearshore (Leis 1986, Smith *et al*. 1987, Kingsford and Choat 1989, Kobayashi 1989, Gray 1993, Brogan 1994, Gray and Miskiewicz 2000, Kingsford 2001, Sabatés *et al*. 2003, Sponaugle *et al*. 2003), but little is known on the small-scale distribution patterns near the substrate in very nearshore waters. Some gobies are present nearshore at all size classes of their planktonic life in different environments. Leis *et al*. (1998) found larvae of all size classes for gobies occurring in shallow waters at Taiaro Atoll and concluded that they completed their entire planktonic life cycle near the reefs. The same result was obtained by Leis *et al*. (2003) for several fish families (including Gobiidae) in four lagoons at two Atolls and one island in the French Polynesia. In temperate waters, Beyst (1999) sampled the hyperbenthos at a

maximum depth of 10 m, in subtidal and tidal marshes at the Dutch Delta, and found *Pomatoschistus microps* and *Pomatoschistus lozanoi* larvae within the full range of developmental sizes (3 to 20mm). Drake and Arias (1991) sampled larvae in a shallow coastal inlet at southwest Spain and described that *P. microps* was the most abundant species with larvae ranging from 5 to 13 mm; *Gobius paganellus* ranged from 7 to 13 mm. Brogan (1994) also found larvae of reef-associated species to be present in all size classes near reefs at the Gulf of California.

Larvae from the surface assemblage were mostly small and undeveloped. This indicates that these larvae are essentially newly hatched, which is in accordance with the presence of spawning grounds for most of these species in the study area. For some of the most abundant species occurring at the bottom, our results provide evidence of depth-related ontogenetic distribution patterns, with smaller larvae, mostly newly-hatched, at the surface and larger and more developed larvae at the bottom. This is true for *Pomatoschistus pictus*, *Gobiusculus flavescens, Gobius niger*, *Boops boops* and probably for *Symphodus melops*. Moreover, larvae of these species were present at the bottom in the whole size range of their planktonic phase. At settlement, size varies with the species considered: at least 17-18 mm for *P. pictus*; 12 mm for *G. flavescens*; and 9 mm for *G. niger* (Petersen 1919, Russel 1976). For *Gobius xanthocephalus* size at settlement is unknown, but larvae were present in the bottom samples at up to the 14-16 mm size-class, indicating that this species is also completing its planktonic life nearshore. In the case of *B. boops*, larvae settle within 16-18 days with a TL of 12 mm (Raventós and Macpherson 2001).

Retention of larvae near reefs has been documented in recent years in different systems and is presently identified as an important mechanism of self-recruitment for some coral reef populations (e.g. Jones *et al*. 1999, Swearer *et al*. 1999, Taylor and

Hellberg 2003). One of the advantages of nearshore retention for coastal species is the ability to find a suitable habitat to settle. Dispersion may increase mortality since oceanographic processes influencing larval transport are variable, both temporally and spatially, and if larvae are not transported to an adequate habitat they can be lost (Hickford and Schiel 2003). Length of larval life has been proposed as one of the primary determinants of dispersal ability (Thresher *et al*. 1989). Larvae with a small planktonic larval duration (PLD) would have more difficulty in returning to coastal habitats after pelagic dispersal in the ocean and in choosing the right habitat to settle. The data available on PLDs for some of the main species found at our study site show that for *Boops boops* and *Symphodus melops* PLD is less than 19 days (Raventós and Macpherson 2001). Larval durations for *Pomatoschistus pictus* and *Gobius xanthocephalus* are not known but for other gobies which occur at our study area somewhat longer times are described (for *Gobius paganellus* and *Gobius cobitis* is respectively 25 and 22 days; Gil *et al*. 1997, Borges *et al*. 2003).

When compared to larvae hatching from pelagic eggs, shorefish larvae hatching from benthic eggs are larger and typically have functional eyes, fins and guts, and better swimming abilities (Thresher 1984, Hickford and Schiel 2003). Therefore retention is more likely to occur in these kind of larvae. However, larvae from other species which lay benthic eggs seem to disperse. For the most abundant blenny at our site, *Parablennius pilicornis*, small larvae were very abundant in surface samples but almost no larvae were caught at the bottom. Drake and Arias (1991) also found only small *Parablennius* sp. larvae inshore (3 to 5 mm). Blenniids have been suggested to disperse away from reefs (Brogan, 1994). The long PLD (over 70 days at controlled conditions, C. Faria, personal communication) and well developed pectoral fins of *P. pilicornis* makes them good candidates for dispersal. For the tripterygiid *Tripterygion delaisi*

although larvae are abundant at both depth strata, they were smaller at the bottom. Moreover, the bigger larvae were not found in our nearshore samples, indicating that they also probably disperse.

Although tidal effects were not specifically addressed and tidal phase was randomised in our sampling design, a preliminary analysis showed that tidal phase influences larval abundance at this nearshore assemblage. Samples collected at low and ebbing tides contained higher larval abundances, especially at the bottom. No interaction was detected between the tide and depth.

The vertical patterns described were found during the day. They could be different at night due to nocturnal ascent of the larger larvae, the commonest patterns of diel vertical migration of larval fishes (Leis 1991 a). However we have preliminary observations, based in night trawling at surface, that seem to indicate that, for the species considered, the pattern found during the day is maintained at night (unpublished data).

Our results seem to indicate that although length of larval life, size and development characteristics at hatching can be important in determining larval ability to remain near the adults' habitat in coastal species, other factors like larval swimming and sensory abilities and orientation capabilities may also have a strong impact in dispersal patterns (Leis and Carson-Ewart 1999, 2000a, Victor and Wellington 2000, Cowen 2002, Fisher and Bellwood 2002, Leis and McCormick 2002, Mora and Sale 2002, Myrberg and Fuiman 2002, Fisher and Wilson 2004).

The data presented above indicate that, for some of the most abundant species which occurred at the study site, larvae can complete all their planktonic phase in the vicinity of the adults' habitats. Moreover, larvae of these species seem to be able to

actively choose bottom habitats very early in their pelagic phase and not just in the pre-settlement stage. We hypothesise that, for many of these species, larvae are able to remain near the bottom as soon as their swimming and sensory abilities develop. The observed vertical distribution patterns combined with other factors could influence horizontal position, promoting retention near the benthic rocky habitats at the study site. The extent to which this could affect self-recruitment of these populations needs further investigation. Larval distribution depends on the interaction between physical oceanographic features and biological factors like the adults' behaviour and ecology, life history traits, planktonic larval duration, larval behaviour and sensory abilities. The relative importance of the different factors which could influence larval distribution is most likely species-specific and requires further investigation. The relative contribution of passive versus active positioning in the water column and horizontal displacement are central issues in the understanding of retention patterns of fish larvae nearshore. Studies focusing on the active behaviour of larvae and their sensory and swimming abilities may further contribute to explain the very nearshore distribution patterns described in this paper.

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V – EARLY LIFE HISTORY ASPECTS OF REEF FISHES

V- A. EMBRYONIC AND LARVAL DEVELOPMENT OF *LIPOPHRYS PHOLIS* (PISCES: BLENNIIDAE)

Cláudia Faria; Rita Borges; Fátima Gil; Vitor C. Almada & Emanuel J. Gonçalves (2003). Embryonic and larval development of L*ipophrys pholis* (Pisces: Blenniidae). *Scientia Marina* 66(1):21-26.

ABSTRACT

Information on the early ontogeny of *Lipophrys pholis* is scattered and incomplete. In this paper we describe for the first time the full developmental sequence from egg to juvenile in controlled conditions. In addition, some notes on the spawning behaviour of adults and the behaviour of larvae are provided. During oviposition, the female follows the male's path suggesting that the male may apply sperm on the nest before spawning. Embryonic development lasted 16 days (17ºC) and larval development to settlement lasted 29 days (15.5-17.5ºC). At hatching, mean larval total length was 5.0 mm. The larvae hatched with the mouth and anus opened, with pigmented eyes and almost no yolk, and they started to feed within one day. They first settled 29 days after hatching (13-14 mm TL) and presented full juvenile pigmentation and behaviour 8 to 9 days later (17-19 mm TL).

INTRODUCTION

Lipophrys pholis (Linnaeus 1758) is a very common rocky intertidal fish species in the north-eastern Atlantic (Zander, 1986). Many papers have been published concerning its reproductive biology and ecology (*e.g*. Qasim, 1956, 1957; Dunne, 1977; Shackley & King, 1977; Laming *et al*., 1982; Milton, 1983; Almada *et al*., 1990a, 1990b, 1992; Faria *et al*., 1996; Gonçalves, 1997).

In Great Britain, *L. pholis* breeds during spring and early summer (Qasim, 1957) while in Portugal the breeding season occurs in the cooler months, from October/November to May (Almada *et al.,* 1990a; Faria *et al*., 1996). During the breeding period the males establish territories in crevices where spawning takes place (Lebour, 1927; Qasim, 1957; Dunne, 1977; Almada *et al*., 1990b). Males defend and ventilate the developing eggs until hatching (Qasim, 1956; Almada *et al*, 1990b). Breeding males guard multiple clutches and exhibit a typical dark coloration pattern (see Qasim, 1956; Almada *et al*., 1990b, 1992). Almada *et al*. (1990b) presented an ethogram of the breeding males of *L. pholis* but never observed a complete courtship sequence and, as spawning occurred inside rock cavities, provided little information on the spawning process. Qasim (1956) described the spawning behaviour of a pair of fishes maintained in captivity.

In spite of all this information, little is known about *L. pholis* developmental biology and the available information is scattered and incomplete. Qasim (1956) described the embryonic development of this species in captivity. Brief descriptions of the eggs and larvae were provided by Hefford (1910) and Lebour (1927). Hefford (1910) presented a brief description of the pigmentation of a larva 4.4mm TL, which is

probably a developing embryo that hatched precociously. Lebour (1927) provided a detailed description of the pigmentation of the newly hatched larvae (5.4mm TL). Ford (1922) presented a brief description of the pigmentation of larvae 5.0mm, 5.5mm, 9mm and 17.5mm TL. Finally, McIntosh (1905) described the pigmentation and morphology of post-settlement individuals (TL > 19mm).

In this paper we present the full developmental sequence of *L. pholis* from egg to juvenile. Some notes on the behaviour of the spawning pair and the behaviour of larvae are also presented.

MATERIAL AND METHODS

Eggs and larvae were obtained from a captive group of 5 fishes (3 females: 8.6 cm, 9.8 cm and 10.5 cm TL; 2 males: 11.3 cm and 13.4 cm TL) maintained since November 1997 at the Vasco da Gama Public Aquarium, Lisbon. Fishes were fed daily with fish and shrimp. The tank was illuminated with fluorescent light (60W) from 09:00 h to 19:00 h. The bottom of the tank was covered with a sand layer and several large flat stones and shells were provided as shelter and breeding sites.

The complete sequence of embryonic development is based on a spawning that occurred on 2 November 1998 (temperature: 17ºC). We used three other batches that, although they did not survive until hatching, allowed replication of the first developmental stages. Eggs were removed from the stone immediately after spawning

by aspiration with a tube. They were maintained in a glass recipient with aeration. To prevent infections methylene blue was added. Eggs were collected daily for description.

Larval development is based on one batch that hatched on 2 March 1999 (temperature range: 15.5-17.5ºC). We used five other incomplete sequences for confirmation. Upon hatching larvae were collected by aspiration from the progenitors' aquarium and were reared in glass 30 l tanks illuminated with fluorescent light (18 W) 24 h per day. A constant flow of seawater was maintained. Larvae were fed three times a day with *Brachionus* sp. enriched with Selco (Artemia Systems), which were gradually replaced by *Artemia* sp. nauplii 14 days after hatching. Larvae were collected daily until the 14th day after hatching. After that, they were collected each two or three days. After being anesthetized (Hypnodil, Janssen Pharmaceutica)**,** both eggs and larvae were observed under a Nikon stereomicroscope, photographed by a Nikon FX-35DX camera and preserved in 5% buffered formalin. The egg capsules were opened and the embryos distended to allow more detailed observations. All larval measurements presented are total length.

The observed spawning was videotape recorded (with a Sony Hi8 CCD-V600 E camera). Behavioural descriptions were made using *ad libitum* and focal observations (sensu Martin & Bateson, 1986).

RESULTS

Spawning

Spawning lasted more than 9 h (time observed). When our observations started (at 9 a.m.), the female was over the nest wall. The male approached, touched the female with the snout and rotated until the genital papilla touched the female's back. After

touching the female, the male performed pectoral fin beatings and high amplitude movements of the tail and posterior part of the body, rubbing the nest wall with the genital papilla. This movement ended with a brief body shaking. This process has a mean duration of 18.0 sec (s.d.=12.1, range 5.0-40.0 sec, n=10). Following the male's path, the female applied the belly to the nest wall and skimmed over the nest surface with slowly pectoral fin movements while quivering the tail. The genital papilla touched the nest wall several times with the eggs being laid one at a time in a single layer (duration of oviposition: mean=45.0 sec, s.d.=10.9, range 25.0-65.0 sec, n=10). This sequence was repeated several times, alternating with resting periods. In general, both fishes alternated their movements over the stone.

During spawning, the male presented a general black coloration with white lips, while the female showed a light coloration, with fins almost transparent. Both sexes had swollen genital papilla.

Embryonic development

Eggs were golden-brown and transparent, with a spherical shape (Figure 1) except at the attachment disk. The diameter was 1.30mm (s.d.=0.04, range: 1.21-1.41 mm, n=52), which is in agreement with published measurements: 1.18-1.60 mm (McIntosh, 1903; Hefford, 1910; Lebour, 1927; Qasim, 1956).

Hatching occurred on the 15th to 16th day after spawning (Figure 2). There were two peaks of hatching, the first in the morning and the second and most intense at the end of the day. This disagrees with Qasim (1956), who observed maximum hatching during the morning. Qasim (1956) described hatching with the embryo emerging tail

first. In our observations of 12 hatching events the larvae always emerged head first, after rapid shaking movements of the body.

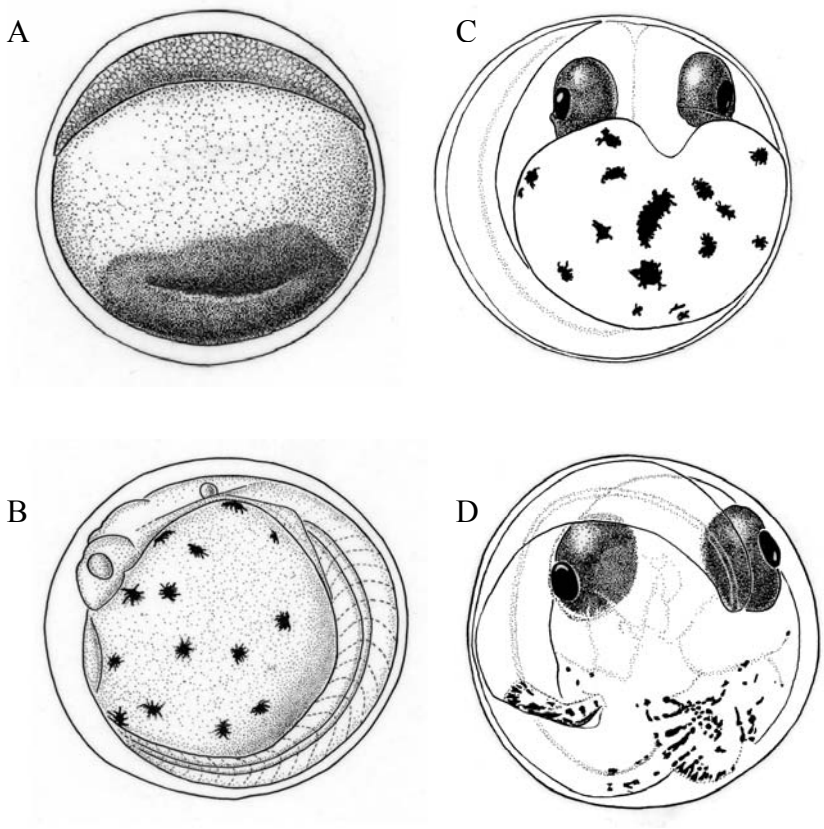

Figure 1. Eggs collected at different developmental stages: (A) Day 1; (B) Day 5: embryo almost reaching the margin of the yolk; (C) Day 8: embryo longer than egg major axis; (D) Day 15: embryo prior to hatching (dorsal view).

Larval development

Newly hatched larvae measured 5.03 mm (s.d.=0.19; range: 4.73-5.33 mm; n=16), which is in agreement with published values presented by Ford (1922), Lebour (1927) and Fives (1986). The anus and mouth were open, with formed lips, teeth and differentiated jaws (Figure 3). The yolk was almost fully absorbed. The liver was developed, the eyes were fully pigmented, and the gas bladder was formed but not completely filled. The pectoral fins were differentiated and all three otoliths were present. The opercula were open with four branchial arches present.

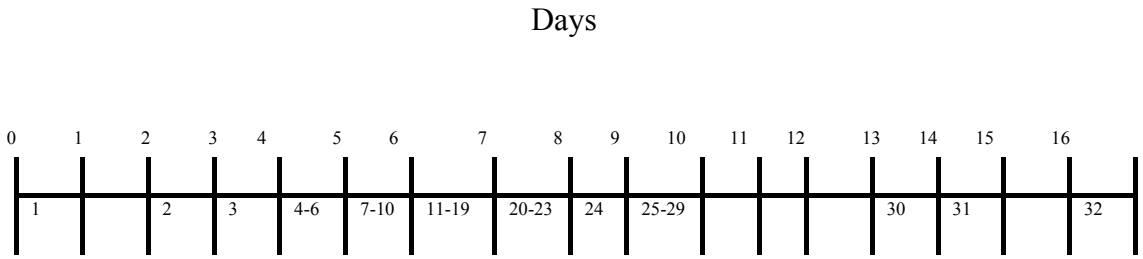

Figure 2. Ontogenetic events of embryonic development of *Lipophrys pholis* in order of first appearance: (1) blastodisc; (2) embryo recognizable; (3) cephalic and caudal dilatation; (4) eye lens; (5) brain; (6) notochord differentiation; (7) brain lobes; (8) notochord; (9) myomeres; (10) heart beatings; (11) pigmented eyes; (12) embryo reaches the margin of the yolk; (13) tail bud free of the yolk; (14) gut differentiation; (15) auditory vesicles; (16) pectoral fin buds; (17) mouth differentiation; (18) median fin fold; (19) hatching glands; (20) anus visible but closed; (21) mouth visible but closed; (22) embryo longer than egg major axis; (23) otoliths; (24) embryo movements; (25) gas bladder; (26) pectoral fins developed; (27) anus opened; (28) mouth opened; (29) mandibles differentiation; (30) eye movements; (31) liver differentiation; (32) hatching.

At hatching the larvae presented peritoneal pigmentation, and twelve rows of melanophores on the pectoral fins. Ventrally, there were 2-4 melanophores on the throat and 7-9 on the last myomeres. Dorsally, there were some sparse melanophores over the brain and the upper lip and there was one melanophore between the inner ear vesicles (Figure 3).

The pigmentation pattern was maintained during development with an increase in the number and intensity of melanophores at the ventral row (from behind the anus to the caudal peduncle), and at the cephalic region, with melanophores extending from between the eyes to the dorsal region (Figure 3).

At day 9 after hatching (6.5-7mm) diffuse yellowish pigmentation, which subsequently extended all over the head, was present. At day 12 after hatching (8mm) there were some melanophores over the midline and the neural tube. Their number and intensity increased and two dorsal and two lateral rows were formed on each side of the

body. Between day 24 and day 30 (13.5-14mm) all fin rays were present (D=XI-XIII +18-20; A=II + 18-20; V=I + 3; P=13, n=20).

After metamorphosis (17-19mm) the fish developed juvenile pigmentation (Figures 3 and 4). A ventral row of melanophores at the base of the anal fin was present and the other fins were also pigmented (less intensely at the caudal fin). The head was extremely pigmented and there was some pigmentation at the throat. Dorsally there were three dark bands (large spots) that extended through the midline, and alternated with three other blotches situated laterally (on each side of the body).

Larval behaviour

After hatching, the larvae immediately swam towards the surface. They avoided sinking by swimming actively until day 4 when the gas bladder was filled. Feeding behaviour began one day after hatching and was characterised by an impulse forward towards the prey item, sometimes preceded by an "S" posture of the posterior part of the body. When two larvae approached, they avoided each other by changing direction with rapid caudal and pectoral fin movements.

Larvae began to settle to the bottom of the tank 29 days after hatching (13-14mm) and 8 to 9 days later they were benthic (15-16mm). However, juvenile behaviour such as turning movements of the head and hiding under objects in close contact with the surfaces by flexing the body against them (tigmotaxis) was observed only at 17–19mm. These results agree with our field observations (unpublished data), since some larvae of this species captured in the plankton measured 16.6 mm (s.d.=1.3, range: 15.0-18.9 mm, n=7) and the smallest fishes found in monthly sampled tidepools (some still lacking juvenile characters) averaged 17.4 mm (s.d.=0.1, range=15.0-19.0mm, n=60). McIntosh (1905) also found 19 mm TL individuals in tidepools.

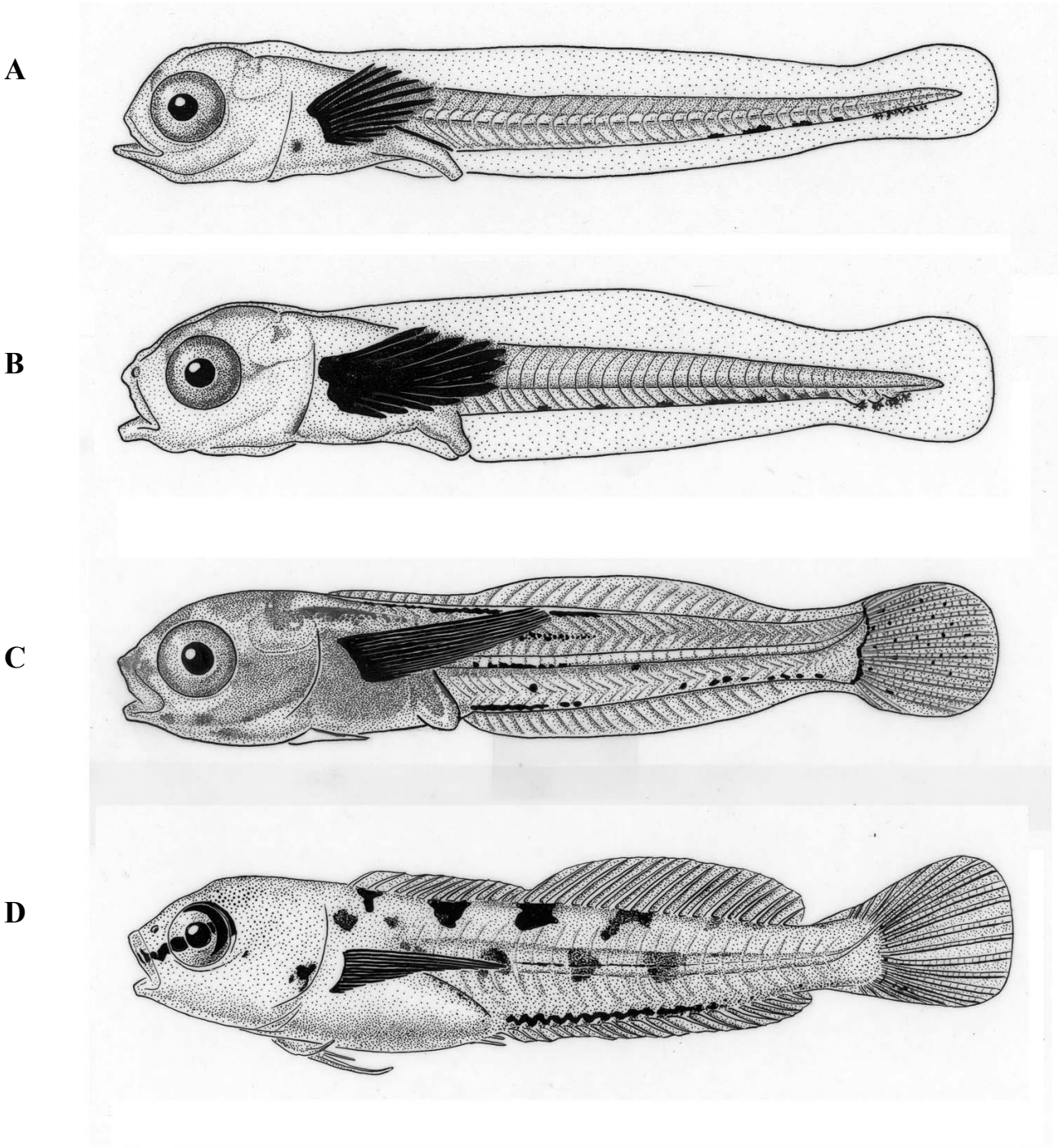

Figure 3. Larvae collected at different developmental stages: (A) Day 1: newly hatched larva (5.5mm TL); (B) Day 5: 6.3mm TL; (C) Day 25: 13.0mm TL; (D) Day 41: juvenile (17.0mm TL).

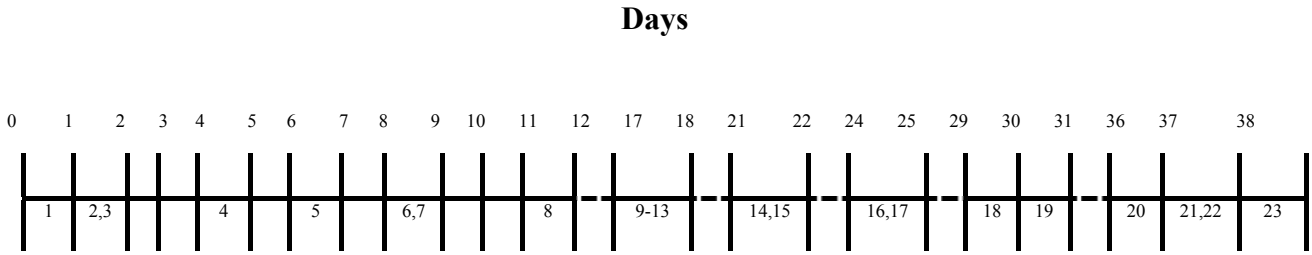

Figure 4. Ontogenetic events of larval development of *Lipophrys pholis* in order of first appearance: (1) nostrils closed; (2) exogenous feeding; (3) filled gas bladder; (4) caudal fin bud; (5) hypurals; (6) caudal fin rays; (7) ventral fin bud; (8) notochord starts to flex; (9) anal fin bud; (10) anal fin rays; (11) 2nd dorsal fin rays; (12) notochord flexion completed; (13) ventral fin rays; (14) segmented caudal fin rays; (15) first dorsal fin rays; (16) median fin fold reabsorption; (17) ossified vertebrae; (18) larvae begun to contact the aquarium bottom; (19) larvae started to settle; (20) nostril tentacles; (21) most larvae settled on the bottom; (22) juvenile behaviours; (23) typical juvenile pigmentation.

DISCUSSION

Our observations of spawning behaviour contrast with that provided by Qasim (1956) in an important detail. While Qasim's description implies that the male fertilises the eggs after attachment, our observations based on videotape recordings point to the contrary. The male first rubs the substratum with the genital papilla and the female follows the male's path while laying eggs, suggesting that the female spawns over a surface that is likely to already contain sperm. Patzner (1984) showed that the micropyle of the eggs of blenniids is in the middle of the adhesion disc and thus faces the substratum when the eggs are attached. Qasim (1956) reported that in the ovary, the position of the eggs is such that they must be extruded with the adhesion disc facing the substratum. This means that it is very likely that contact with sperm must precede attachment, either through the presence of sperm in the water column or by a sperm

layer previously attached to the rocks by the male, as described for some gobiids (Marconato *et al.*, 1996; Ota *et al*., 1996; Faria *et al*., 1998). Our observations suggest that the male probably applies sperm to the rock surface before egg attachment.

The embryonic developmental sequence described here generally agrees with Qasim (1956), except that the timing of events that we observed was much shorter. While Qasim (1956) recognised the differentiation of the embryo at day 8 after hatching, the presence of eye rudiments at day 14, and the formation of myomeres and heart beatings at day 24, we observed these events at day 2, day 4, and day 5, respectively. In our study, embryonic development lasted 16 days at 17ºC, while Qasim reported an embryonic developmental time of 43 days at 11.5-15.0 ºC, and 61 days at 9.5-14ºC. These differences probably are due to the incubation temperature since the decrease of the developmental time with higher temperatures is known for many fish species (Blaxter, 1969). Nevertheless, the difference of almost 50% in the timing of developmental events is remarkable.

The newly hatched larvae of *L. pholis* showed the typical pattern of features characteristic of marine fishes with male parental care (Thresher, 1984). They swum actively immediately after hatching and the onset of exogenous feeding occurred one day after. This pattern is also found in other coastal species with demersal eggs and contrasts with the one generally described for species with pelagic eggs (see e.g. Russel, 1976; Moser *et* al., 1984).

After metamorphosis and settlement the juveniles presented typical behaviours associated with a benthic mode of life, like lateral movements of the head and hiding behaviour, which could be important for survival in a highly irregular substrate.

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V- B. EARLY DEVELOPMENT OF THE RED MOUTHED GOBY, *GOBIUS CRUENTATUS* (PISCES: GOBIIDAE)

Fátima Gil; Rita Borges; Cláudia Faria & Emanuel J. Gonçalves 2002. Early development of the red mouthed goby, *Gobius cruentatus* (Pisces: Gobiidae). *Journal of the Marine Biological Association of the United Kingdom* 82, 161-163.

ABSTRACT

The full developmental embryonic sequence of *Gobius cruentatus* is described for the first time. Embryonic development lasted 13 days (14.0–15.0ºC). The newly hatched larvae (3.3 mm total length) presented pigmented eyes, the yolk is fully absorbed, and the mouth and anus were opened allowing the onset of exogenous feeding almost after hatching.

INTRODUCTION

Gobius cruentatus Gmelin (1789) is an eastern Atlantic goby, occurring from southwest Ireland to Senegal and in the Mediterranean (Miller, 1986). It is found inshore on rocky habitats, sand and sea-grass meadows (Wilkins & Myers, 1992). Although abundant throughout its distributional range, the reproductive biology of this species is virtually unknown. The existing information is concerned mainly with distributional patterns and the use of space (e.g. Miller, 1986, 1990; Minchin, 1987; Wheeler, 1992; Wilkins & Myers, 1992, 1993, 1995).

MATERIALS AND METHODS

Eggs and larvae were obtained from a pair of captive fish maintained since May 1998 at a public aquarium (Aquário Vasco da Gama, Lisbon). Fishes were fed daily with fish and shrimp. The tank was illuminated with fluorescent light (60W) from 09:00 h to 19:00 h. The bottom of the tank was covered with a layer of sand and several large flat stones. Eggs were removed from the spawning stone daily by aspiration with a tube and were observed under a Nikon stereomicroscope, photographed by a Nikon Fx-35DX camera and preserved in buffered 5% formalin.

RESULTS

The complete sequence of embryonic development (temperature: 14–15ºC) was based on a spawning that occurred on 8 December 1998. The breeding male presented a dark colouration with bright red lips. Parental care included fanning and rubbing the

eggs with the dorsal fin or the posterior end of the body as described for other species of the genus *Gobius* (see e.g. Gil *et al.*, 1997; Faria *et al*., 1998).

The eggs were transparent and fusiform (length=2.04 mm, range 1.90–2.10 mm, S.D.=0.08; width=0.56 mm, range 0.50–0.60 mm, S.D.=0.05; N=50) and were attached to the underneath of a horizontal rock by filaments. They were distributed in a single layer within a total area of 121 cm^2 with a density of 176 eggs cm^{-2}. In Figure 1 eggs in different developmental stages and the newly hatched larva are presented. The main ontogenetic events of embryonic development are shown in Figure 2.

Hatching occurred 13 days after fertilization and the egg capsule was disrupted by the lower jaw of the larvae, where hatching glands were visible. The head was the first to emerge after rapid movements of the body. Newly hatched larvae measured 3.30 mm total length (range 3.24–3.34 mm; S.D.=0.03; N=7). The mouth and anus were opened, with formed lips and differentiated jaws. The yolk was fully absorbed. The liver was developed, the eyes were fully pigmented and the gas bladder was filled.

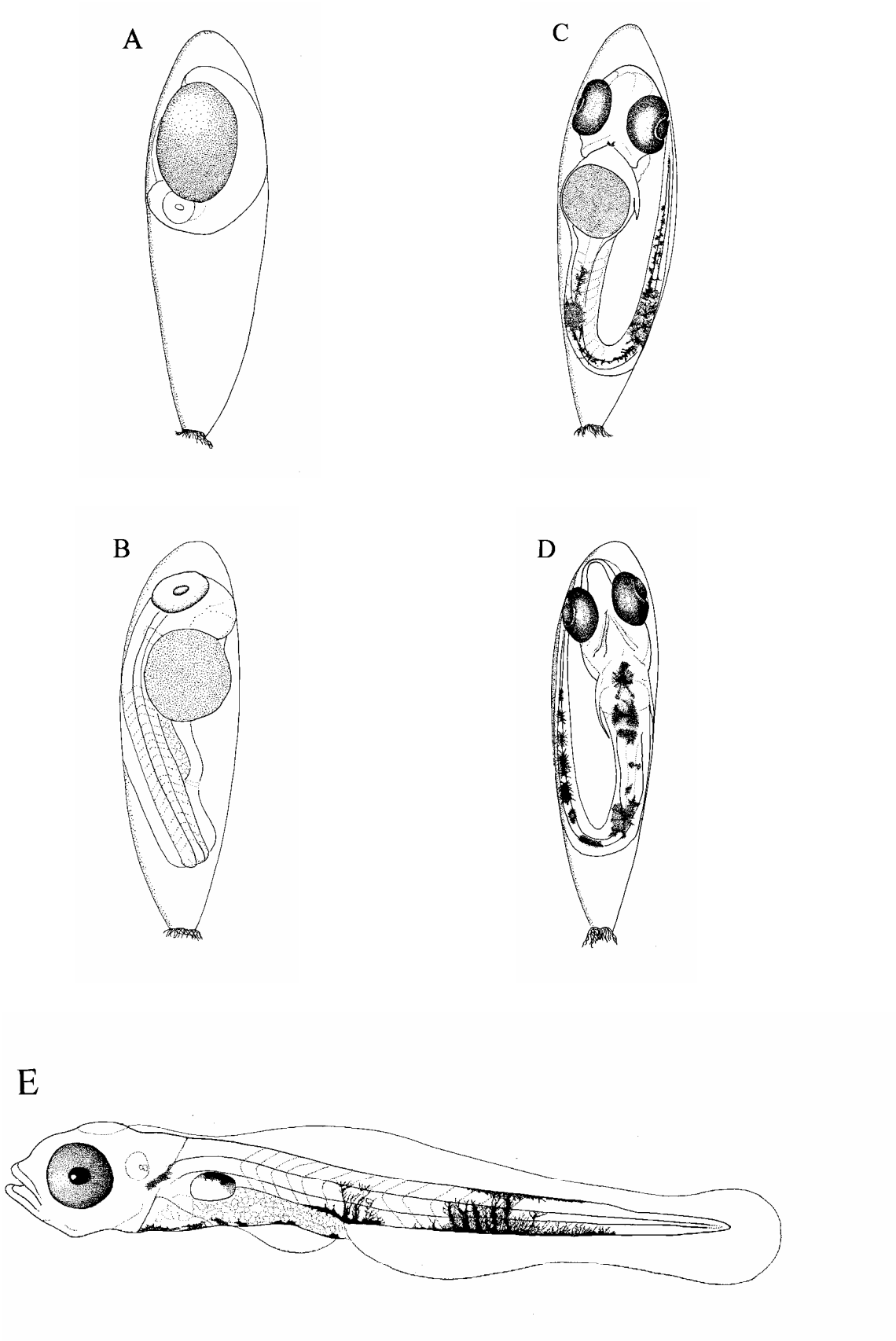

Figure 1. Eggs collected at different developmental stages: (A) Day 1**;** (B) Day 4; (C) Day 8; (D) Day 10; (E) Newly hatched larva (3.3mm TL).

The pectoral fins were differentiated and the inner ear already presented the sagittae and lapilli otoliths. The opercula were opened and the branchial arches were differentiated.

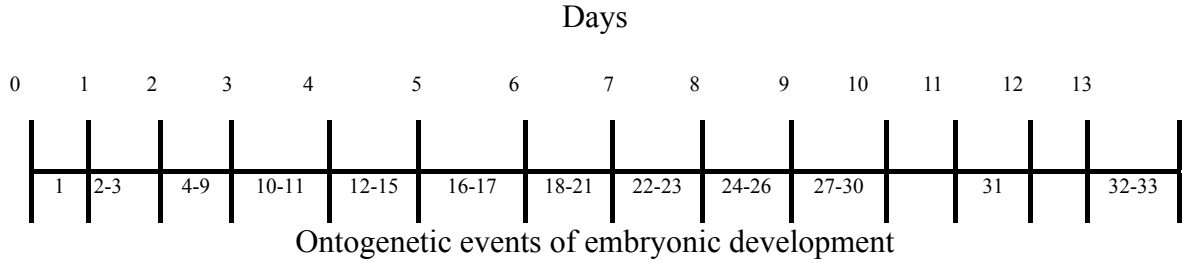

Figure 2. Ontogenetic events of embryonic development of *Gobius cruentatus* in order of first appearance: (1) embryo recognizable; (2) cephalic and caudal dilatation; (3) embryo reaches the margin of the yolk; (4) eye lens; (5) brain; (6) notochord differentiation; (7) tail bud free of the yolk; (8) myomeres; (9) gut differentiation; (10) brain lobes; (11) embryo movements; (12) auditory vesicles; (13) median fin fold; (14) embryo longer than egg major axis; (15) heart beatings; (16) notochord; (17) mouth differentiation; (18) anus visible but closed; (19) pigmented eyes; (20) otoliths; (21) pectoral fin buds; (22) gas bladder; (23) mouth visible but closed; (24) anus opened; (25) hatching glands; (26) opercula visible but closed; (27) mouth opened; (28) liver differentiation; (29) opercula opened; (30) mandibles differentiation; (31) gut movements; (32) eye movements; (33) hatching.

The larvae presented seven to nine pre-anal melanophores ventrally and one above the anus (Figure 1). There was a continuous row of post-anal melanophores with several large and ramified in the middle of this row. Dorsally, there was a melanophore between the brain and the trunk, and a row of ramified melanophores in the direction of the ventral patch. Internally, the dorsal membrane of the gas bladder was fully pigmented and there were one or two ramified melanophores above the gut. There were also yellow pigments along the entire body, being more concentrated in the regions that contained melanophores.

DISCUSSION

The sequence of embryonic development described for *Gobius cruentatus* largely agrees with the known descriptions for other species of the genus *Gobius* (e.g. *Gobius cobitis* Pallas, 1811: Gil *et al*., 1997; *Gobius niger* Linnaeus, 1758: Ballard, 1969; *Gobius paganellus* Linnaeus, 1758: unpublished data). However the incubation periods observed varies between species: *G. niger* also hatch 13 days after spawning, but at a lower temperature – 13ºC (Ballard, 1969); *G. paganellus* hatch 11 days after spawning at 15.5–16.5ºC (unpublished data); *G. cobitis* present the longer incubation period, 22 to 24 days at 12–16ºC (Gil *et al*., 1997). These differences are probably related with size of the newly hatched larvae: 2.5 mm in *G. niger* (Ballard, 1969), 3.3 mm in *G. cruentatus*, 3.5 mm in *G. paganellus* (unpublished data), and 5.5 mm in *G. cobitis* (Gil *et al*., 1997). Additionally, the shorter developmental time described for G. paganellus is probably related with the higher incubation temperature, since the decrease of developmental time with higher temperatures is known for many fish species (Blaxter, 1969). This effect could also explain the similar incubation periods observed for *G. cruentatus* and *G. niger* in spite of the differences in size of the newly hatched larvae. These two factors, size at hatching and incubation temperature, should be clearly differentiated when comparing the early ontogeny of related species.

The area and density of the egg batches are related to species size, since smaller fish tend to have smaller and denser batches (Miller, 1984; Thresher, 1984). This situation was described by (Faria & Almada, 1995) for *G. paganellus* and *G. cobitis*, with the smaller *G. paganellus* presenting smaller eggs in a higher density. *G. cruentatus* which is smaller than *G. cobitis* but larger than *G. paganellus* presented

intermediate egg densities (85 eggs cm^{-2} for *G. cobitis*, 176 eggs cm^{-2} for *G. cruentatus* and 208 eggs cm^{-2} for *G. paganellus*).

Like other coastal species with demersal eggs, the newly hatched larvae of *G. cruentatus* showed the typical pattern of development of marine fishes with male parental care (Thresher, 1984). The eyes and pectoral fins were fully developed at hatching with the larvae immediately swimming in an active way. The mouth and anus were opened, allowing the onset of exogenous feeding almost after hatching.

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This study was supported by Fundação para a Ciência e Tecnologia (FCT) as part of the projects PRAXIS XXI/3/3.2/EMG/1957/95 and the Pluriannual Program (UI&D 331/94). C.F. was also supported by a grant from FCT (PRAXIS XXI/BD/5666/95). We would like to thank the technicians of Aquário Vasco da Gama for the help in the maintenance of tanks and to Maria Dornelas for the illustrations.

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V-C. EMBRYONIC AND LARVAL DEVELOPMENT OF *GOBIUS PAGANELLUS* (PISCES: GOBIIDAE)

Rita Borges; Cláudia Faria; Fátima Gil; Emanuel J. Gonçalves and Vitor C. Almada 2003. Embryonic and larval development of *Gobius paganellus* (Pisces: Gobiidae). *Journal of the Marine Biological Association of the United Kingdom* **83**: 1151-1156.

ABSTRACT

The full developmental sequence from egg to juvenile of *Gobius paganellus* under controlled conditions is described. Embryonic development lasted 9–10 days at 18.5–20.0°C and 10–11 days at 15.0–16.5°C. Newly hatched larvae measured 3.90 mm, had the mouth and anus opened, pigmented eyes and almost no yolk. They first settled 25 days after hatching (10.0–10.5 mm TL) and showed juvenile behaviour and body form 36 days after hatching (14.0 mm TL). However, they only showed full juvenile pigmentation patterns 15 days later (17.0 mm TL) (16.0–16.5°C). In addition, a preliminary differentiation between the newly hatched larvae of the most common *Gobius* species of south-western European shores is presented.

INTRODUCTION

The family Gobiidae is the largest of marine fishes, being a very important element of temperate and tropical reef fish communities (Nelson, 1994). The ecology and biology of many species is well studied (Miller, 1984) but the early ontogenetic development of the Atlantico-Mediterranean species is poorly known with very few descriptions of eggs and larvae (Ruple, 1984).

Gobius paganellus (Linnaeus, 1758) is one of the most abundant gobies found along Portuguese rocky shores (Henriques *et al*., 1999). The distribution of this species ranges from western Scotland to tropical West Africa (Senegal), including the oceanic Islands, Mediterranean and Black Sea, Golf of Eilat and Red Sea (Miller, 1986). Although the biology of this species has been considerably studied (e.g. Lebour, 1919a; Miller, 1961; Faria & Almada, 1995) the information available about its development is scattered and incomplete (see Russel, 1976). Nests and eggs have been described by Holt & Byrne (1898), Lo Bianco (1909), Hefford (1910), Lebour (1919a), Sparta (1934), Miller (1961), Faria & Almada (1995).

In this paper the full developmental sequence of *G. paganellus* from egg to juvenile is described for laboratory reared fish. The identification of fish early developmental stages being one of the main problems in icthyoplankton studies, we have also systematised the available information on the newly hatched larvae of *Gobius* species and other genus commonly found in south-western European shores.

MATERIALS AND METHODS

Eggs and larvae were obtained from a captive pair of fishes (female: 10.6 cm TL; male: 9.4 cm TL) maintained since January 2000 at a public aquarium (Aquário Vasco da Gama, Lisbon). Fishes were fed daily with fish and shrimp. The 110l tank was illuminated with natural light. The bottom of the tank was covered with a sand layer and some large flat stones were provided as shelter and breeding sites.

Five batches were obtained, but the complete sequence of embryonic development described is based only on two of those: 28 February 2000 (mean temperature=19.1°C; SD=0.30; range: 18.5–19.5°C; N=10) and 21 March 2000 (mean temperature=15.6°C; SD=0.45; range: 15.0–16.0°C; N=11). The eggs of a third batch (18 February 2000: mean temperature=19.0°C; SD=0.55; range: 18.5–20.0°C; N=11) were also measured. Eggs were removed daily from the stone guarded by the male by aspiration with a tube.

Full larval development is described from three batches that hatched at: 18 February 2000 (mean temperature=20.1°C; SD=0.80; range: 18.5–21.5°C; N=42), 8 March 2000 (mean temperature=19.0°C; SD=1.38; range: 17.0–21.5°C; N=66) and 20 April 2000 (mean temperature=16.3°C; SD=0.34; range: 16.0–17.0°C; N=96).

Upon hatching larvae were collected by aspiration and were reared in glass 30l tanks illuminated with fluorescent light (18 W) 24 h per day. A constant flow of seawater was maintained. Larvae were fed three times a day with *Brachionus* sp.

enriched with Selco (Artemia Systems) and algae, which were mixed with *Artemia* sp. nauplii 28 days after hatching and replaced by Artemia sp. nauplii by day 41.

Larvae were collected daily until metamorphosis. Eggs and anaesthetized larvae (Hypnodil, Janssen Pharmaceutica) were measured and observed under a Nikon stereomicroscope, photographed with a Nikon FX-35DX camera and preserved in 5% buffered formalin. After preservation the egg capsules were opened and the embryos distended to allow more detailed observations. All larval measurements correspond to total lengths.

RESULTS

Breeding started soon after the male and female were placed together. We recorded and followed five spawnings that occurred at successively longer intervals (Table 1). The female never laid eggs until the previous batch had hatched and in nature we found males of this species guarding eggs in different stages of development (Faria & Almada, 1995). These facts, provide additional evidence that the males of this species guard eggs of different females simultaneously (see also Le Danois, 1913; Vivien, 1939; Miller, 1961; Gibson, 1970).

The fusiform and transparent eggs (Figure 1, Table 2) correspond to the descriptions made by several authors (for a review see Russel, 1976). They were suspended on the underneath of a horizontal rock by filaments and were distributed in a single layer. Egg measurements for three batches are presented in Table 2. Egg size

decreased significantly with successive broods (analysis of variance: F=6.437; df = 2; P< 0.01; Tukey honestly significant differences test: P<0.05 in every comparison).

Table 1. Time of embryonic development at different temperatures and spawning intervals between batches.

Batch	Developmental time (days)	Spawning intervals (days)	Temperature range (ºC)
1	10	–	18.5–20.0
2	9	0	18.5–19.5
3	9	2	18.5–19.5
4	10	2	15.0–16.0
5	11	9	16.0–16.5

The ontogenetic events of embryonic development at different temperatures are shown in Table 3. Hatching occurred at day 9/10 after spawning at 18.5–19.5°C (three batches) and at day 10–11 after spawning at 15.0–16.0°C (two batches) (Table 1).

The hatching event was observed in one batch. Almost all eggs hatched in less than a minute. Immediately after hatching, all larvae swam with spiral movements to the surface, with successive swimming impulses, where they seemed to gulp air, likely to fill the gas bladder. This behaviour was performed repeatedly. About one hour later, the larvae stabilized their position and dispersed in the water column.

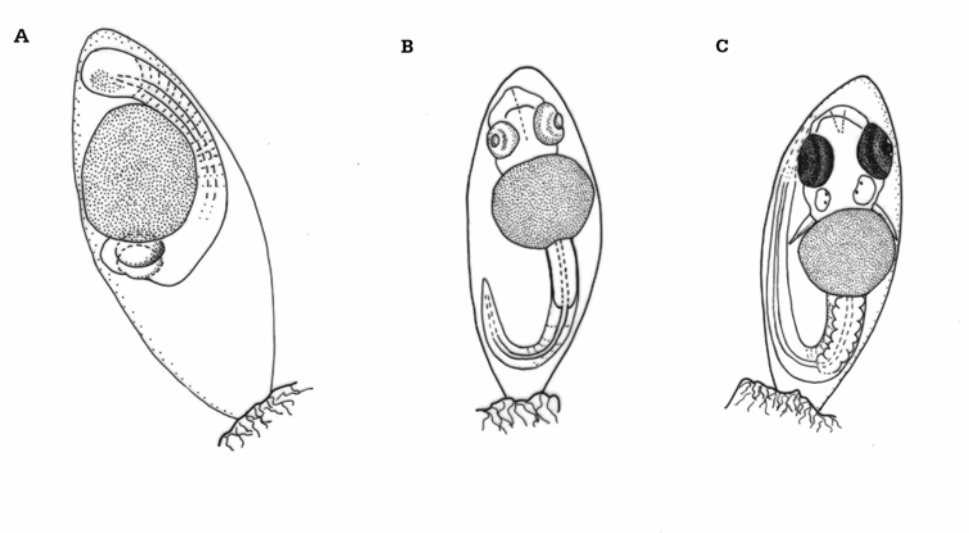

Figure 1. Eggs collected at different developmental stages: (A) embryo differentiation (day 1); (B) embryo as long as major axis (day 3); (C) embryo prior to hatching (day 10).

Figure 2 presents larvae collected at different developmental stages. The ontogenetic events of larval development at different temperatures are shown in Table 4. The newly hatched larvae measured 3.90 mm (SD=0.09; range: 3.78–3.99 mm; N=30, temperature=16.0–17.0°C). The anus and mouth were opened, with formed lips and differentiated jaws (Figure 2). The yolk was almost fully absorbed. The liver was developed, the eyes were fully pigmented, and the gas bladder was formed but not completely filled. The pectoral fins were differentiated and the sagittae and lapilli otoliths were visible. The opercula were opened with four branchial arches present. The dorsal membrane of the gas bladder was fully pigmented. Ventrally there were some dispersed melanophores under the liver, the gut, and the anus, a large one over the anus and a series of ventral post-anal melanophores, with the middle one strongly ramified and a punctate pigment near the caudal tip. Dorsally, over the larger ventral pigment there was a ramified melanophore.

Table 2. Egg Height and width for three batches (data from eggs preserved in 5% buffered formalin).

Batch		Average (mm)	SD	Range (mm)	N	Temperature range
1	Height	1.99	0.18	1.60–2.20	14	18.5–20°C
	Width	0.86	0.07	0.80–0.90	14	
2	Height	1.83	0.17	1.60–2.10	31	18.5–19.5°C
	Width	0.80	0.09	0.70–0.90	31	
4	Height	1.81	0.17	1.60–2.10	40	15.0–16.0°C
	Width	0.80	0.14	0.70–0.90	40	

The pigmentation pattern changed with larval development. At day 13 after hatching (7.5–8.0 mm), when the larvae had already all the anal and 2nd dorsal fin rays (A=I+11–12 (10–13); D2=I+13–14 (12–15)), a small melanophore was visible in the otic vesicle. Throughout development there was an increase in the number and intensity of pigment cells in this area. Ventrally, the large ramified melanophore disappeared and the number of melanophores increased until a row was visible from the throat to near the caudal peduncle. At day 25 (10.0–10.5 mm), two rows of melanophores were visible at the insertion of the anal fin. By this time, there were melanophores on the base of the ventral rays of the caudal fin that tended to spread to the base of all the caudal fin rays. Around day 30 (11.0–11.5 mm) a melanophore appeared over the tip of the upper jaw and the ramification of the dorsal post-anal melanophore was reduced. At day 48 (13.5–14.0 mm) there were melanophores over the cephalic region that increased in size and number with development. Between day 25 and day 40 (10.0–13.0 mm) all other fin rays were present (D1=VI; P=21–22 (18–23)) By day 50 (14.5–15.0 mm) there was a row of melanophores extending over the vertebral column. Dorsal and pectoral fin pigmentation started at day 52 after hatching (15.5–16.0 mm).

Larvae started to settle at day 25 after hatching at a size of 10.0–10.5 mm. The change to a benthic mode of life was gradual. Initially, they only touched the substratum returning immediately to the water column. Gradually, they began to stay longer at the bottom until definitely standing there. At metamorphosis the fishes became heavily pigmented. The general pattern of pigmentation was maintained but the anterior part of the body became strongly pigmented. There were two horizontal stripes of melanophores in the first dorsal fin and a horizontal stripe in the second dorsal fin and in the anal fin (less marked in this one). There was also an increase of pigmentation on the flanks and at the border of the scales in the posterior region of the body. Metamorphosis was a gradual process. In most fishes a marked change of body form to that of a juvenile became apparent at day 36 after hatching, when they were about 14.0 mm (at 16.0–16.5 ºC). At this time they began to show typical behaviours of a benthic fish, like jumping and hiding in the substrate. However, the acquisition of juvenile pigmentation only appeared at day 51 at a size of 17.0 mm TL. This agrees with observations made in the field, were the smallest fish collected in tidepools were about 17.0 mm (mean=16.8 mm; SD=0.17; range: 13.0–19.0 mm; N=64), with some fish still lacking the juvenile general body shape and pigmentation (C.F. personal observations). At day 89 after hatching fishes measured 2.5–3.0 cm.

Table 3. Ontogenetic events of embryonic development of *Gobius paganellus* in order of first appearance: (1) embryo recognizable; (2) cephalic and caudal dilatation; (3) brain; (4) myomeres; (5) eye lens; (6) notochord; (7) gut differentiation; (8) heart beatings; (9) pigmented eyes; (10) anus visible but closed; (11) embryo movements; (12) auditory vesicles; (13) otoliths; (14) median fin fold; (15) anus opened; (16) pectoral fin buds; (17) gas bladder; (18) mouth visible but closed; (19) hatching glands; (20) opercula differentiation; (21) liver differentiation; (22) mouth opened; (23) opercula opened; (24) hatching.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
15.0–16.0	d1	d1	d1	D2	d2	d3	d3	d3	d3	d3	d3	d3	d3	d3	d4	d4	d4	d5	d5	d6	d7	d8	d8	d11
18.5–19.5	d1	d1	d2	D3	d2	d2	d2	d3	d5	d2	d2	d3	d3	d2	d3	d4	d4	d4	d4	d4	d7	d5	d7	d9

Table 4. Ontogenetic events of larval development of *Gobius paganellus* in order of first appearance: (1) exogenous feeding; (2) filled gas bladder; (3) hypurals; (4) teeth; (5) caudal fin bud; (6) caudal fin rays; (7) notochord starts to flex; (8) anal fin rays; (9) notochord flexion completed; (10) ventral fin bud; (11) 2nd dorsal fin rays; (12) median fin fold reabsorption; (13) first dorsal bud; (14) ossified vertebrae; (15) larvae started to settle; (16) first dorsal fin rays; (17) ventral fin rays; (18) scales visible; (19) juvenile typical pigmentation. Size ranges are also included

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
16.0–17.0	d1	d1	d3	d6	d7	d7	d10	d12	d12	d12	d12	d14	d18	d23	d25	d26	d27	d66	d90
	4.0–5.9 mm				6.0–7.9 mm			8.0–9.9 mm					10.0–11.9 mm					18.6	25.0
17.0–21.5	d1	d1	d3	–	d5	d7	d7	d7	d9	d16	d9	d16	d16	d16	d24	d19	d19	d58	d66
	4.0–5.9 mm				6.0–7.9 mm							8.0–9.9 mm			10.0–11.9 mm			19.4	23.0
18.5–21.5	d0	d1	d4	–	–	d4	d5	d6	d7	d8	d7	d8	d17	d8	d18	d22	d22	–	d41
	4.0–5.9 mm							6.0–7.9 mm							10.0–11.9 mm				20.0

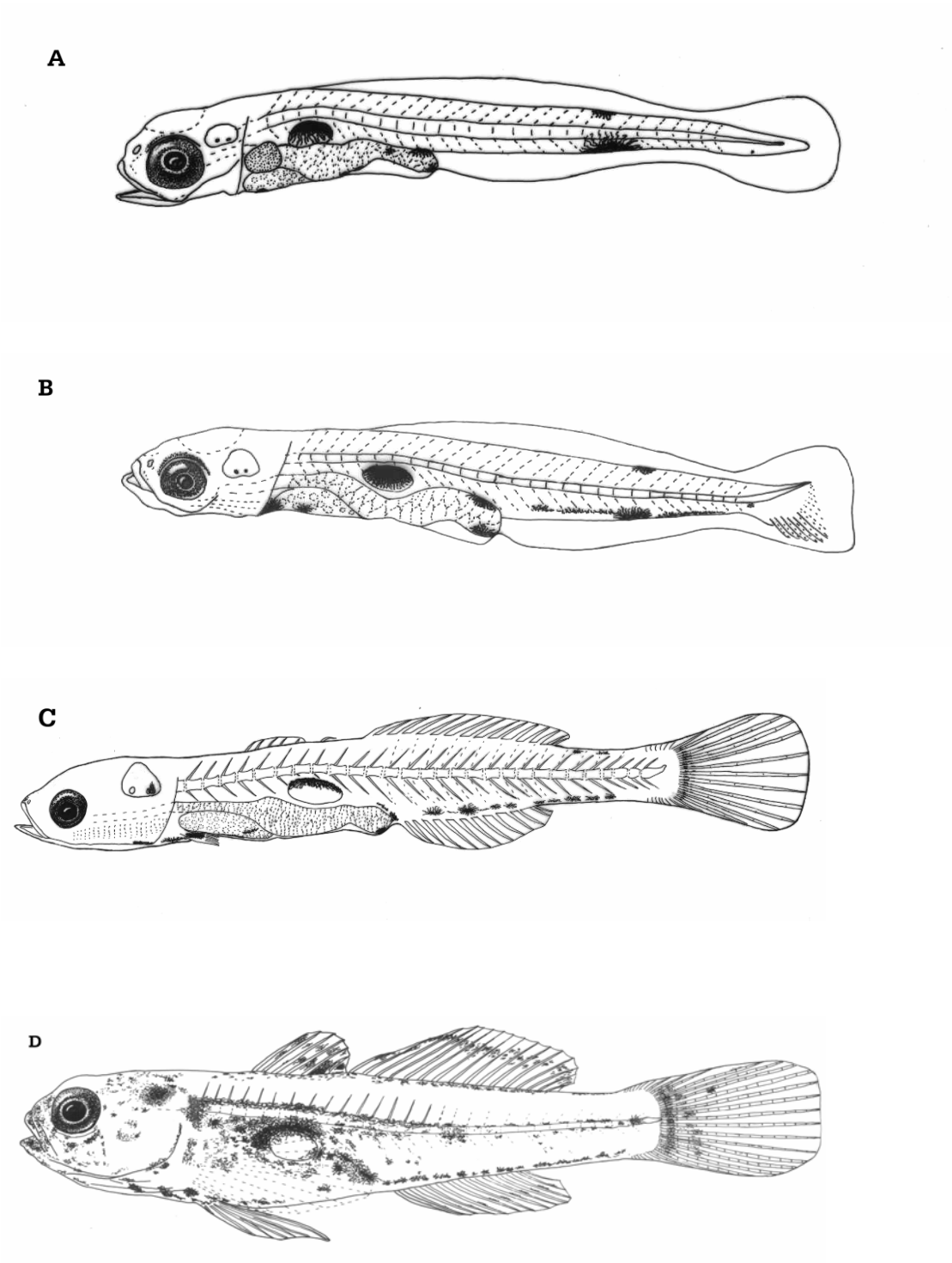

Figure 2. Larvae collected at different developmental stages: (A) newly hatched larva (day 0, 3.9 mm); (B) caudal fin rays (day 9, 6.2 mm); (C) all fin rays formed (day 28, 11.4 mm); (D) juvenile (day 94, 22.2 mm).

DISCUSSION

Egg structure agreed with the descriptions available (Lebour, 1919a; Sparta, 1934; Padoa, 1956; Miller, 1961; Russel, 1976) but egg size was smaller than data presented by several authors: 2.0–3.0 mm height and 0.74–1.0 mm wide (see Sparta, 1934; Miller, 1961; Russel, 1976). However, they are similar to those presented by Holt & Byrne (1898) who described eggs laid in an aquarium (1.84–1.9 mm), and with data from eggs measured in the field, in Portugal (Faria & Almada, 1995). This data should be analysed with care because we observed not only some variability in egg size from the same batch, but also a reduction in egg size with the number of broods of the same female which could be related to metabolic exhaustion of the female, an observation also made by Potts & Wootton (1984). As temperature changed between the second and third batch analysed, it could be argued that it was the drop in temperature and not female exhaustion that caused the decrease in egg size. Although the available data are insufficient to assess these two hypotheses, the finding that between the first and second batch produced at similar temperatures there was already a significant decrease in egg size, provides a preliminary indication that female exhaustion may be a more important factor than temperature in the reduction of egg size. Further work is however needed to resolve this issue.

The basic sequence of embryonic developmental events was maintained at different temperatures. However there was some delay in the timing of appearance of some structures at a lower temperature, a long known phenomenon in fish development (Blaxter, 1969). In another common gobiid species, *Gobius cobitis*, where the embryonic development was studied at two temperature ranges: 12.0–16.0°C (Gil *et al*., 1997) and 15.0–18.0°C (our unpublished data) this was also observed. However, in our

study the delay in the timing of appearance of some structures at a lower temperature was more evident. This could be related to the minimum temperature of the first batch (16.0–17.0°C) that was much lower than the subsequent batches (17.0–21.0°C).

In the present study larvae hatched at 3.90 mm, a size that is slightly smaller than the published values for this species, 4.00–4.80 mm (Hefford, 1910; Lebour, 1919; Spartà, 1934; Padoa, 1956). However, no information on temperature is provided for the previous studies, thus the extent to which this discrepancy is due to temperature differences, population characteristics or is simply a consequence of a large variability present in this species remains an open issue.

The basic sequence of larval development was similar at different temperatures, being faster at higher temperatures, with some differences: when the minimum temperature was higher the differentiation of the second dorsal fin rays and the ossified vertebrae was faster. The larval sequence obtained for this species agreed largely with the one presented by Gil *et al*. (1997) for *G. cobitis*. The main larval structures were all present between day 19 and day 27, and the larvae attained a similar size at the completion of development regardless of temperature. From this time to metamorphosis, the main emphasis seemed to be in growth and in the acquisition of juvenile characters (pigmentation patterns and body form), these changes being faster at higher temperatures (larvae took 49 days to grow 8.9 mm at 16.0°C–17.0°C and only 19 days at 18.5°C–21.5°C, see Table 4).

At the current stage of knowledge about the development of north-eastern Atlantic gobiids, we believe that it is worthwhile to attempt a summary of

characteristics that may help in the identification of eggs and larvae of the most common gobiids of this area. The eggs of gobies are demersal and laid in a single layer (Russel, 1976). For most species they are pear shaped (see Russel, 1976), but those of the common species of the genus *Gobius*, *Gobius niger* (Ballard, 1969; Iglesias, 1979), *Gobius paganellus*, *Gobius cobitis* (Gil *et al*., 1997) and *Gobius cruentatus* (Gil *et al*., 2002), are elongated and fusiform.

Within the genus *Gobius*, besides differences in egg size (height/width: 3.44–3.74/1.11–1.26 mm for *G. cobitis* (Gil *et al*., 1997); 1.90–2.10/0.50–0.60 mm for *G. cruentatus* (Gil *et al*., 2002); 1.60–2.20/0.70–0.90 for *G. paganellus* (our results); 1.50 mm height for *G. niger* (Iglesias, 1979), the eggs of *G. cobitis* and *G. cruentatus* show the apex less rounded than *G. niger* and *G. paganellus*. Between these two pairs of species, egg size is sufficiently different to allow species identification. Also, the habitat of *G. niger* (sand) is very different from that of *G. paganellus* (rock) (Miller, 1986).

The newly hatched larvae of Gobiidae show a typical shape, slender and elongate, with a prominent gas bladder. The basic pattern of pigmentation consists of a ventral post-anal row of melanophores, often with a larger one at about half the distance between the anus and the urostyle, and strong pigmentation in the dorsal part of the gas bladder (Petersen, 1919; Lebour, 1919a,b; Gil *et al*., 1997, 2002; see Russel, 1976 for a review). Most larvae hatch with functional mouth and anus that allow exogenous feeding, and pigmented eyes and developed pectoral fins that allow active swimming immediately after hatching (Gil *et al*., 1997, 2002; see Russel, 1976 for a review).

In spite of these similarities, there are some differences that can distinguish early larvae of *Gobius* from other gobiid genera present in our study area, and between the

four *Gobius* species discussed here, based mainly on the pigmentation patterns and body shape.

Newly hatched larvae of *Lebetus* can be distinguished from other Gobiidae by a heavily pigmented body (except on the caudal region) with a well marked median lateral row of melanophores (see Petersen, 1919; Demir & Russel, 1971; Russell, 1976; Ré, 1980/1981). *Crystallogobius* with less than 6.0 mm can be distinguished by a strong pigmentation on the head (in front of the eyes, on the lower jaw and in the otic vesicle) and by five large melanophores between the head and tail (see Lebour, 1919a; Petersen, 1919; Russel, 1976). Newly hatched larvae of *Gobiusculus flavescens* and *Pomatoschistus* spp. described in the literature, present a pigment in the angle of the lower jaw which is never present in the described *Gobius* larvae (see Petersen, 1919; Lebour, 1919a, b, 1920; Padoa, 1956; Russel, 1976).

Distinction between *Gobius* and other genera in the subsequent stages of development is possible by counting fin rays and vertebrae and by analysing pigmentation patterns (see Petersen, 1919; Lebour, 1919a, b, 1920; Russel, 1976).

Within the genus *Gobius*, newly hatched larvae can be distinguished mainly by their pigmentation patterns and size at hatching: both *G. cobitis* and *G. cruentatus* have a melanophore ventro-posterior to the otic vesicle that is not present in *G. niger* or *G. paganellus* larvae (however, in these species otolith pigmentation appears later in development).

Larvae of *G. cobitis* hatch with a size of 5.08-5.5 mm, and already present several dorsal pre-anal melanophores (which are maintained as larvae develop – see Gil *et al*., 1997), while newly hatched *G. cruentatus* larvae, hatch with a size of 3.3 mm

(Gil *et al*., 2002), and have no dorsal pre-anal melanophores (larval development of this species is not known).

According to our results for *G. paganellus* and the descriptions available in the literature for *G. niger* newly hatched larva (see Petersen, 1919; Lebour, 1919a; Ballard, 1969; Iglesias, 1979), it is not possible to distinguish these two species based on pigmentation patterns. *G. niger* larvae are smaller at hatching (2.5–3.0 mm) when compared with *G. paganellus* (3.9mm) and present a faster development: settlement occurs when larvae measure about 9.0 mm (vs 10.0–10.5 mm in *G. paganellus*) and at this time almost all adult characters are present, while in *G. paganellus* metamorphosis only occurs at about 17.0 mm.

For the other species of the genus *Gobius* present in our study area, *Gobius bucchichi*, *Gobius gasteveni*, *Gobius roulei* and *Gobius xantocephalus* there are no information on larval development (Miller, 1986). Although information on egg and larval development is not available for some *Gobius* species present in our study area, the four species discussed here are the most common *Gobius* both in the plankton (R.B. personal observations) and as adults (Henriques *et al*., 1999). Therefore, the comparison presented may help to perform a preliminary screening of *Gobius* larvae when inspecting icthyoplankton samples for this biogeographic region. It should however be taken in consideration that laboratory reared larvae may present some important differences to those collected in the field (Leis, 1987).

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V-D. LARVAL DEVELOPMENT OF *GOBIUS XANTHOCEPHALUS* AND GENETIC VALIDATION OF LARVAL IDENTIFICATION

Monteiro, J.; R. Borges; Robalo, J.; Almada, V.C.; Henriques, S.; E.J. Gonçalves (*to be submitted*).

ABSTRACT

The ontogenetic development of *Gobius xanthocephalus* larvae is described for the first time. Larvae were collected through bottom trawls using a plankton net attached to an underwater scooter and a light trap, over rocky reefs at the Arrábida Marine Park (Portugal). Ontogeny of the main structures, changes in pigmentation patterns and allometric relationships are described. Identifications were validated through larval hatching under controlled conditions and DNA analysis. The developmental sequence obtained agreed with those described for other gobiidae species. The pigmentation pattern was distinct from that of other Gobiidae occurring in the area. Otolith microstructure analysis showed a linear age-length relationship, with an estimated larval growth rate of 0.28 mm day^{-1}.

INTRODUCTION

*Gobius xanthocephalus (*Heymer and Zander 1992) is found along the coast, from North-West Spain to Madeira and the Canary islands, and in the North-West Mediterranean (Agbayani 2005). It is one of the most common gobies found along the portuguese continental rocky shores (Henriques *et al*. 1999).

This species has long been misidentified as *Gobius auratus* (Risso 1810) and *Gobius luteus* (Koiombatovic 1981). However, Almeida and Arruda (1998) confirmed that the fishes previously described as *G. auratus* in Portuguese waters were actually *G. xanthocephalus* (Almeida and Arruda 1998; Henriques *et al*. 1999). The morphology and ecology of the adults is relatively well studied (Heymer and Zander, 1992, 1994; Almeida and Arruda 1998). In Portuguese waters *G. xanthocephalus* breeding "peak" occurs in May, and juveniles recruit from May to October (Almada *et al*. 2000). Like other Gobiidae species, *G. xanthocephalus* spawns demersal eggs, which are laid in a single layered patch under stones and shells and guarded by the male (Miller 1986). However, to date there are no descriptions of the embryonic or larval stages for this species.

There are six *Gobius* species that breed at the Arrábida Marine Park, including *G. xanthocephalus* (Henriques *et al*. 1999; Almada *et al*. 2000); complete descriptions of larval development are only available for three of these species (*Gobius cobitis* Pallas, 1811 (Spartà 1950; Gil *et al*. 1997), *Gobius niger* Linnaeus, 1758 (Lebour 1919; Petersen 1919; Ballard, 1969; Iglesias 1979) and *Gobius paganellus* Linnaeus, 1758

(e.g. Hefford 1910; Lebour 1919; Spartà 1934; Borges *et al*. 2003). Given the difficulty in distinguishing larvae of related species, more detailed descriptions are needed. The correct identification of eggs and larval fishes is the base for ecological and taxonomic studies of the pelagic stage of fishes (Leis and Rennis, 1983; Powles and Markle 1984; Leis and McCormick, 2002).

In this study the development of *G. xanthocephalus* larvae is described for the first time, based on larvae from plankton collections. Newly hatched larvae under controlled conditions and DNA analysis were used to confirm larvae identity. The age-length relationship and the larval growth rate were determined from otolith microstructure analysis.

MATERIALS AND METHODS

Laboratory reared larvae

Three pairs of adult fishes were obtained at the Arrábida Marine Park, 30 km South of Lisbon (9º00'15'' – 9º03'48''W and 38º26' – 38º27'N), and maintained in two 250 L tanks at a public institute of marine research (IPIMAR) since 15 June 2005. The bottom of the tanks was covered with a layer of sand, and some large stones and vessels were provided as shelters and spawning sites. Tanks were illuminated with a 30 W fluorescent light from 6:00 to 21:00 at a mean temperature of 17.4º C (SD= 0.47, N=139). Fish were fed daily with shrimp and mussel. The newly hatched larvae were obtained from three batches, spawned on the 29th July, 19th September and 10th October 2005. Embryonic development lasted 9 days at a mean temperature of 17.8ºC

(SD=0.46, N=9) for the first batch, at 17.4º C (SD=0.35, N=9) for the second, and 17.3ºC (SD=0.38, N=9) for the last batch. After hatching larvae were collected by aspiration and anaesthetized with MS-222. Larvae were observed and notochord length (NL) was measured under an Olympus stereomicroscope. Photographs were made using a digital camera attached to the stereomicroscope. After this, larvae were preserved in 4% saline formalin buffered with sodium borate.

Developmental series

Sampling methods

Larvae used for the development description were collected at the Arrábida Marine Park. Between 26 June and 27 July 2001, 32 samples were collected near the adults habitats using a plankton net attached to an underwater scooter, during 5 minute trawls. Larvae were immediately preserved in 4% saline formalin buffered with sodium borate for at least one month.

A light trap was used on the 22nd of July 2003 to collect four ichthyoplankton samples (1h each) at the same location. This method was used in an attempt to complete information for the developmental series since the most developed larvae could not be caught using the scooter method.

Larval identification

After sorting, larvae were identified as *G. xanthocephalus* through the "series" method as recommended by Neira *et al.* (1998), by comparison with the descriptions available for other *Gobius* species and by meristic counting in the more developed

larvae. Certainty in the identification of the smallest larvae could be confirmed by comparison to the laboratory reared larvae and through DNA analysis.

Body length (BL, defined as notochord length (NL) in pre-flexion and flexion larvae, and as standard length (SL) in post-flexion larvae (according to Leis and Carson-Ewart 2000) was measured to the nearest 0.1 mm. This measure was used instead of Total Length because caudal fin membrane was frequently damaged. Larvae were grouped in 0.5mm interval BL classes.

Ontogenetic Development

The "dynamic approach" method (Ahlstrom and Ball, 1954) was applied to describe the main ontogenetic events. These included notochord flexion, fin and gill filaments development, vertebral ossification, teeth presence and pigmentation pattern.

Each characteristic was considered to be "present" at the BL at which it appeared in all larvae, and from that BL on.

Morphometrics

For the morphometric analysis several other measurements besides BL were taken to the nearest 0.1 mm. These measurements included: total length (TL), pre-anal length (PAL) and head length (HL) according to Leis and Carson-Ewart (2000), head depth as described in Olivar (1986) and body depth at anus (BDA) following Neira *et al.* (1998). Allometric relationships between these measurements and BL were calculated using the allometric law described in Krickeberg *et al.* (1971). The obtained allometric relationship between BL and TL was used to estimate the TL classes used to compare the *G. xanthocephalus* larval development sequence described here to the

results obtained in other studies. Eye Diameter (ED) was measured (Leis and Carson-Ewart, 2000) to the nearest 0.0001 and allometrically related to Head Length.

Based on the relationships between these measurements, body form, size of head and eye were classified according to Leis and Carson-Ewart (2000) and size of gut following Neira *et al.* (1998).

Validation of the identification: DNA analysis

In order to confirm the correct identification of the larvae used in the developmental series, the DNA of 4 adults, 4 juveniles and 3 larvae, all identified as *G. xanthocephalus*, was analysed. Larvae were caught using a light trap and were fixed in 70% ethanol.

Total genomic DNA was extracted from specimens preserved in ethanol by an SDS/proteinase-k based protocol (adapted from Sambrook *et al*., 1989). For all specimens two mitochondrial genes were sequenced: 12S rDNA and D-loop (mitochondrial control region). The choice of these genes was based on their common use in phylogeny and their different mutation rates (e.g. Kocher and Stepien, 1997).

The 12S rDNA is a slowly evolving gene (by mitochondrial standards) that usually shows little intraspecific variation, but differs sufficiently between closely related species to discriminate them reliably (e.g. Henriques *et al*. 2002; Almada *et al*. 2005). D-loop, on the other hand has a very high mutation rate and is often used to study intraspecific variability (e.g. Fauvelot *et al*., 2003; Astolfi *et al*., 2005). Thus, if larvae and juveniles share a given haplotype with the adults, which are unambiguously identified as *G. xanthocephalus*, we have a high level of confidence that the different forms belong to the same species. Part of the mitochondrial 12S gene (390 bp) was

amplified, using the primers 12SFor 5'-AAC TGG GAT TAG ATA CCC CAC-3' and 12SRev 5'-GGG AGA GTG ACG GGC GGT GTG-3' (Almada *et al.* 2005). PCR conditions followed those in Almada *et al*. (2005). A fragment of 388 bp of the D-loop gene was amplified, using the primers L-PRO1 5'- ACTCT CACCC CTAGC TCCCA AAG -3' and H-DL1 5'-CCTGA AGTAG GAACC AGATG CCAG-3' (Ostellari *et al*., 1996). PCR conditions followed those in Stefanni (2000). Sequencing reactions were performed by Macrogen Inc. in a MJ Research PTC-225 Peltier Thermal Cycler using a ABI PRISM BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq DNA polymerase (FS enzyme) (Applied Biosystems), following the protocols supplied by the manufacturer and the same primers used for PCR. Sequences were aligned in Clustal X (Thompson *et al.*, 1997) followed by visual inspection.

Otolith microstructure

In order to analyse the relationship between age and size, otolith from 15 larvae, from 5.0 to 11.0 mm larval BL classes (5.5 – 13.1 mm TL) were removed and treated following Secor *et al*. (1992): Sagittae otoliths were extracted and then fixed with Crystal Bond (Aremco Products®, USA). Polishment was performed with 3.0 and 0.3 µm lapping film along the sagittal axis. For each otolith, daily increments were counted three times under an Olympus microscope with 1000 x magnification, using transmitted light. Although increment deposition pattern was not validated for this species, a daily deposition as proven for other gobiids (Iglesias *et al*., 1997; Hernamen *et al*., 2000; Shafer, 2000) was assumed. The first otolith increment was considered to form at hatching as in other gobiids (e.g. Sponaugle and Cowen, 1994). Age (in days) was considered to correspond to the number of increments in the otolith.

RESULTS

From the plankton collections, 376 larvae with BL between 3.0 and 11.0 mm BL (3.2 – 13.1 mm TL) were identified as *G. xanthocephalus* and 124 of these were selected for the descriptions. The samples caught with the light trap were mainly composed by juveniles. The only larva caught by this method had 15.00 mm BL (18.5 mm TL), and was used to complete the ontogenetic description.

Laboratory reared larvae

The newly hatched larva (Figure 1) measured 2.83 mm BL (SD= 0.23, range= 2.70 - 3.10 mm, N= 3). The mouth and anus were opened, with formed lips and differentiated jaws. The yolk was almost fully absorbed. Eyes were fully pigmented, the liver developed and the opercula were opened, with four branchial arches. The gas bladder was filled. Pectoral fins were differentiated and fin fold was complete. Sagittae and lapilli otoliths were visible.

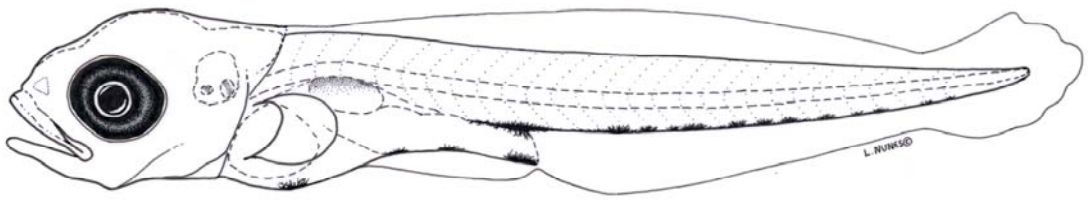

Figure 1 Newly hatched larva under controlled conditions (BL= 3.0 mm).

The dorsal membrane of the gas bladder was totally pigmented. Ventrally, there were some melanophores under the liver, gut and anus, one above the anus and a post-anal row of similar, slightly ramified melanophores regularly spaced, from the anus to the caudal peduncle (Figure 1).

Developmental series

Ontogenetic development

Figure 2 shows larvae from the plankton collection in different developmental stages while in Figure 3 the sequence of the main ontogenetic events is represented.

The developmental stage of the smallest larvae caught in the plankton (Figure 2-a) was similar to the newly hatched larvae at the laboratory (Figure 1). The only difference was that the former was bigger and had no yolk. The pigmentation pattern (Figure 2-a) was also similar to the observed in the larvae hatched under controlled conditions (Figure 1). The same melanophore pattern was maintained until around 11.0 mm BL (13.1 mm TL), with a slight decrease in the intensity and number of ventral pre-anal melanophores.

Caudal fin rays were the first to start differentiation, immediately after the beginning of the notochord flexion, between 4.0 and 4.9 mm BL (4.3 - 5.4 mm TL) (Figure 3). By this time, pigmentation appeared on the base and/or rays of its ventral portion and then spread dorsally during larval development.

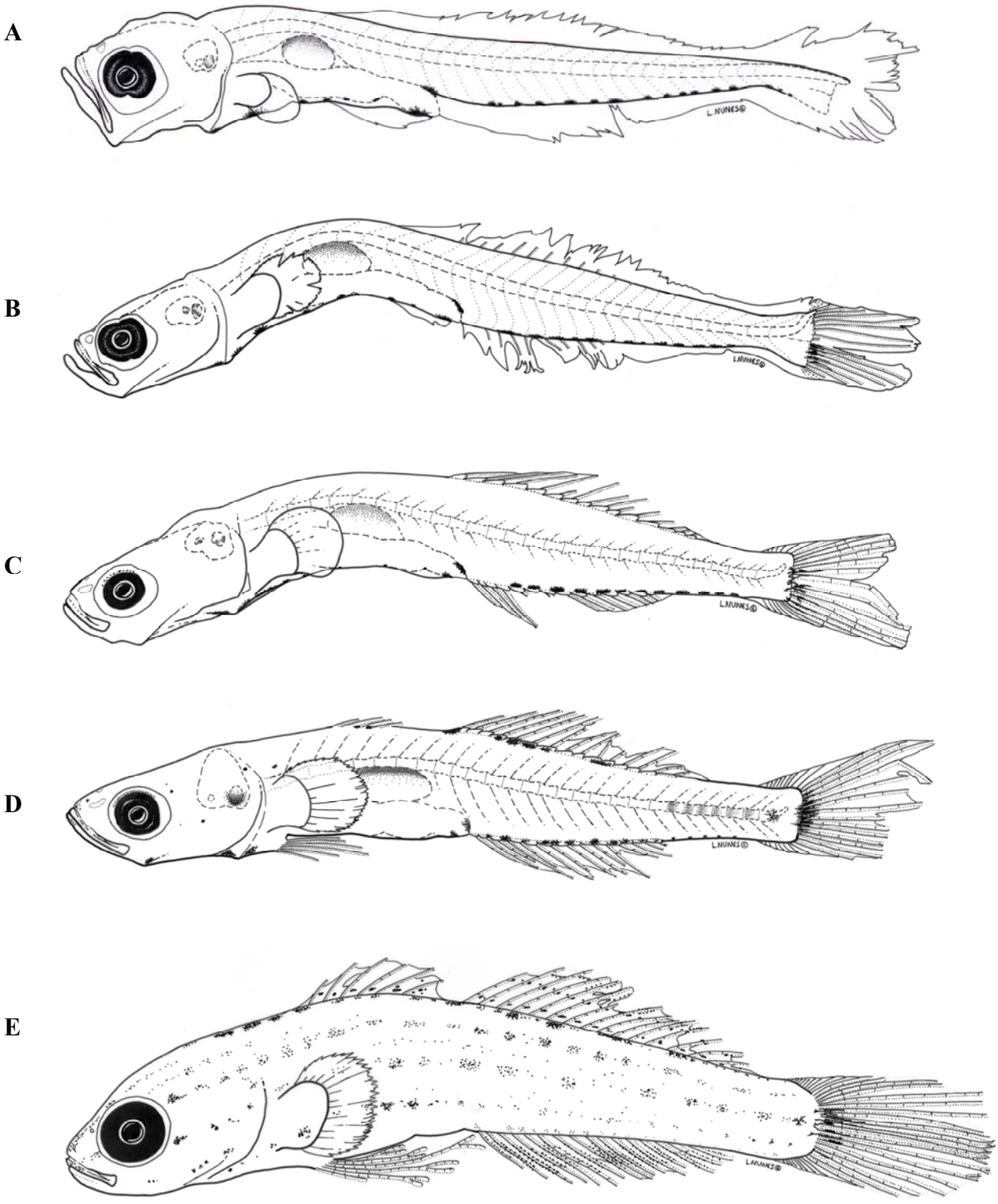

Figure 2 Early life stages of *G. xanthocephalus* collected in the plankton A- Pre flexion larva (3.95 mm BL); B- post-flexion stage larva (5.6 mm BL); C- larva having otolith pigmentation, ossified vertebrae and caudal fin pigmentation (8 mm BL); D- larva with vertebral pigmentation (11.1 mm BL); E- larva with dermal pigmentation (15.0 mm BL).

At 5.5 mm BL (6.1 mm TL), anal and second dorsal fin rays were starting to develop (Figure 2-b; Figure 3); 1-2 melanophores appeared immediately anteriorly to the angle of the throat and another 1-2 posterior to it (Figure 2-b). A double ventral row of melanophores was visible at the anterior portion of anal fin insertion; through development, this double line was spread to the whole length of the anal fin insertion. Around 6.5mm BL (7.4 mm TL), all the 13-15 principal caudal rays were present, vertebrae ossification was completed (Figure 3) and all larvae had conspicuous pigmentation in the otolith capsule. At 8.5 mm BL (9.9 mm TL), when all anal and second dorsal fin rays were present in all larvae (A= 14-15; D2= 15-16), caudal fin pigmentation was evident as a clear spot at the central portion of the fin base and rays (Figure 2-c). Around 11.0 mm BL (13.1 mm TL), all fins and major internal structures were present (Figure 2-d; Figure 3), a melanophore was visible in the angle of the lower jaw, and the pigmentation on dorsal fins was initiated.

The first dorsal fin was the last to form completely (Figure 3), presenting six rays at 15.0 mm BL (18.5 mm TL) (Figure 2-e). At this size, the caudal fin exhibited 17 segmented rays, 6 of which were branched. Ventral fins reached the anus, with 5 segmented and branched rays, and a marginal one, non-segmented and unbranched. The pigmentation pattern had completely changed: the body was pigmented with dermal melanophores from head to caudal peduncle, forming “stripes” along the body axis and anal and dorsal fins were pigmented at the base and between rays (Figure 2-e).

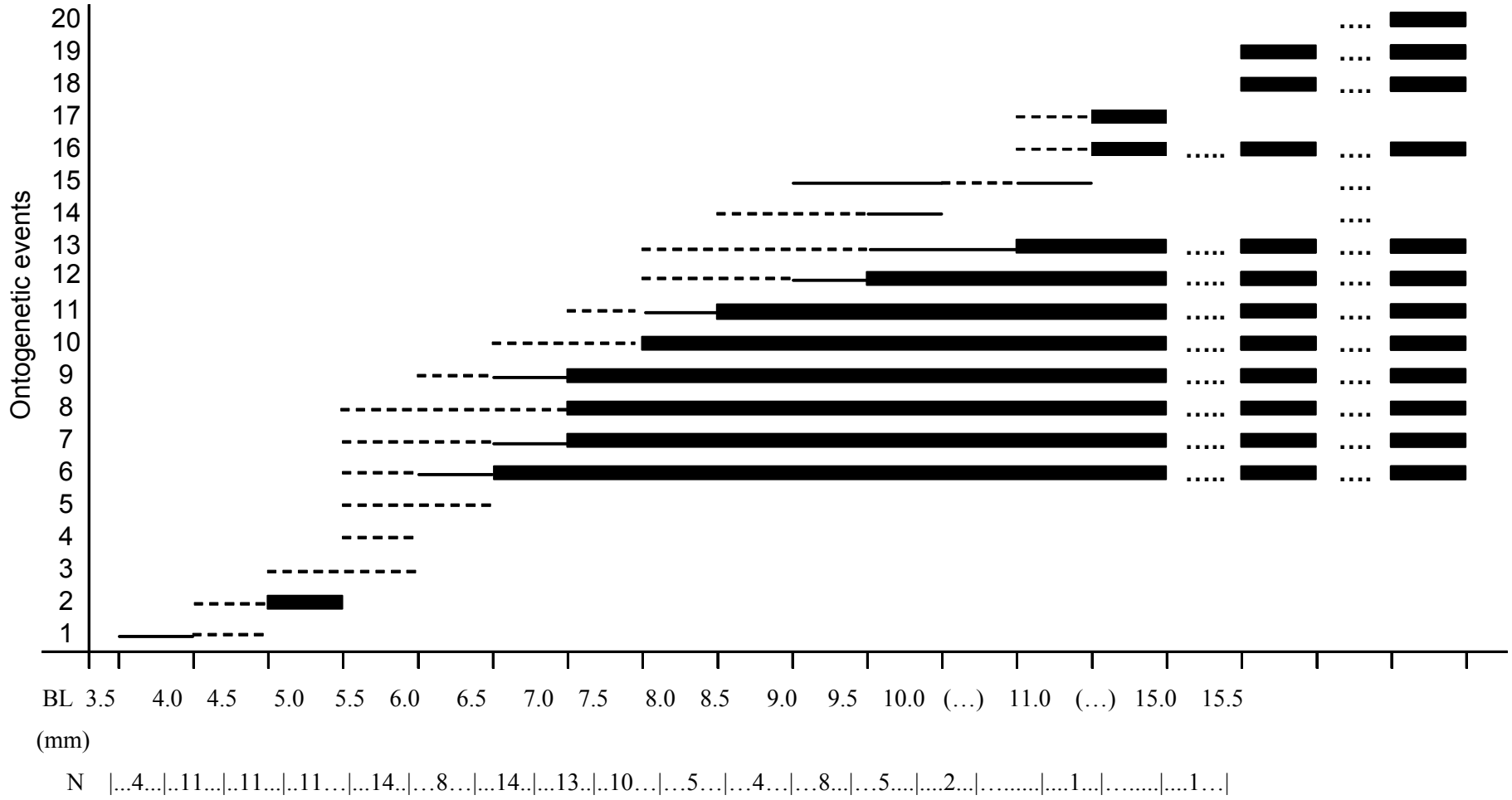

Figure 3. Principal ontogenetic events of *G. xanthocephalus* larval development: (1) hypurals start to develop; (2) beginning of notochord flexion; (3) caudal fin bud; (4) anal fin bud; (5) second dorsal fin bud; (6) hypurals formed; (7) notochord flexion completed; (8) ossified vertebrae; (9) all principal caudal fin rays present; (10) teeth present; (11) all anal fin rays present; (12) all second dorsal fin rays present; (13) fin fold reabsorbed; (14) pectoral fin rays bud; (15) ventral fin bud; (16) branchial filaments in the 4th branchial arches; (17) first dorsal fin bud; (18) pectoral fins formed; (19) ventral fins formed (20) first dorsal fin formed. (**---**) event occurring in less than 75% larvae of that BL class. (—) Event occurring in 75%-99% larvae. (▬) event occurring in 100% larvae.

Morphometrics

The more pronounced positive allometry was found to occur between BDA and BL (k = 1.27± 0.07, r = 0.96, N = 125) (Figure 4). Thus, body growth was faster in depth than in length. Positive allometry was also found between BL and other measurements: TL (k = 1.10± 0.02, r = 0.99, N = 125), PAL (k = 1.12± 0.03, r = 0.99, N = 125), HL (k = 1.15± 0.04, r = 0.98, N = 125) and HD (k = 1.09± 0.05, r = 0.97, N = 125). One single negative allometry was found, between ED and HL (k = 0.66±0.05, r = 0.93, N = 125) (Figure 5).

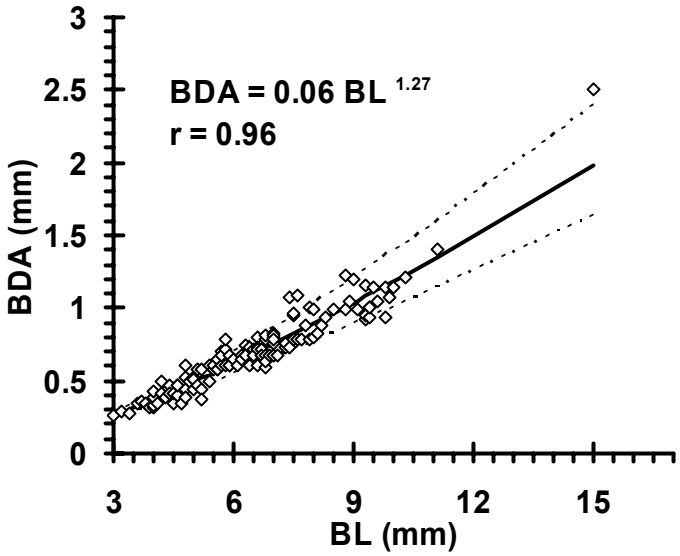

Figure 4. Allometric relation between Body Depth at Anus (BDA) and Body Length (BL). (◊) observed "y"; (—)expected "y"; (----) 95% confidence limits of expected "y".

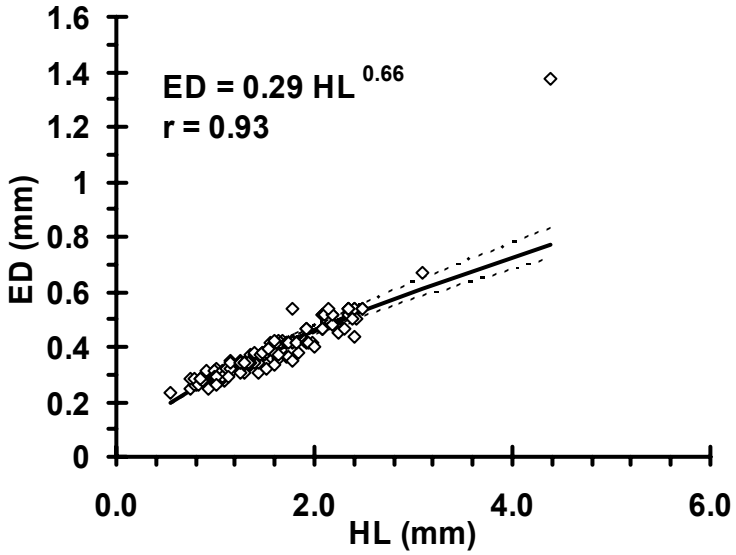

Figure 5. Allometric relationship between Eye Diameter (ED) and Head Depth (HD). (◊) observed "y"; (—)expected "y"; (----) 95%confidence limits of expected "y".

Table I presents the evolution of body shape and head, gut and eye sizes during development. There was a visible change of the body shape with growth: in most larvae (65%) smaller than 5.5mm BL (6.1 mm TL) the body could be considered "very long", but all larvae from that size on had "long" bodies. There was also a change in the eye diameter in relation to the head length. Because eye Diameter varied little as the Head Length increased, the eyes became smaller in relation to the head length and remained "small" in larvae from 6.5mm BL (7.4 mm TL) on (Table 1).

Table I. Evolution of the body shape and head, gut and eye size between 3.0 and 15.0mm BL: variation range, mean, standard deviation (SD) and larvae number (N).

	Range (min.-max.)	**Mean ± SD**	**N**
Body shape (% BD/BL)	Very long→ Long 7.2 - 23.5	Long 10.7 ± 1.9	125
Head size (% HL/ BL)	Moderate 20.0 - 29.2	Moderate 23.6 ± 1.8	125
Gut size (% PAL/ BL)	Moderate/ Long 46.8 - 59.5	Long 54.2 ± 2.9	125
Eye size (% ED/ HL)	Big → Small 49.7 - 18.3	Moderate 26.3 ± 5.2	125

Confirmation of the identification: DNA analysis

All sequences have been deposited in GenBank (accession number DQ382237 to DQ382251 for 12S gene, and DQ382252 to DQ382266 for D-loop gene). In the 12S rDNA only two haplotypes were found, differing by one mutation. One was shared by 9 specimens (3 adults, 4 juveniles and 2 larvae). The other one was shared by an adult and a larva. Concerning the D-loop gene 4 haplotypes were present. The most common was found in 7 individuals (2 adults, 4 juveniles and 1 larva). Another haplotype was shared by one adult and one larva, while the two remaining ones where found in individual fish. Least frequent haplotypes differed from the most common one by one or two mutations only.

Otolith microstructure

There was a linear relationship between the number of increments and body length (Figure 6).

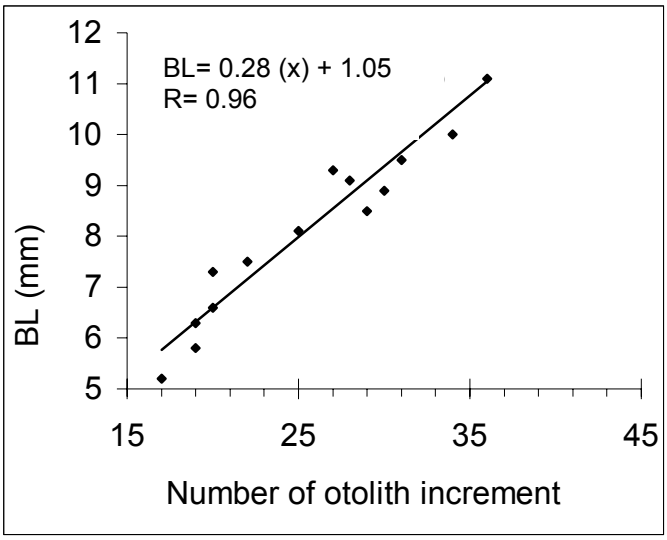

Figure 6. Relation between body length (BL) and the number of increments in the larvae otoliths. "x"=total number of rings in each otolith.

Assuming a daily pattern of the increment deposition, the 5.0 - 11.0 mm larval BL classes (5.5 – 13.1 mm TL) range studied corresponded nearly to the age range 14-36 day, with a growth rate of approximately 0.28 mm/day.

DISCUSSION

The newly hatched larvae showed the typical features characteristic of Gobiidae larvae (see Russell, 1976; Ruple, 1984; Neira and Miskiewicz, 1998; Leis and Rennis, 2000): they presented a typical body shape, slender and elongated, with a prominent gas bladder. The same general pattern of development at hatching was observed in other species of the same genus, present in the studied area: *G. cobitis* (Spartà, 1950; Gil *et al.*, 1997); *G. cruentatus* (Gil *et al.*, 2002), *G. niger* (Lebour, 1919; Petersen, 1919; Padoa, 1956; Russell, 1976), *G. paganellus* (Hefford, 1910; Lebour, 1919; Spartà, 1934;

Borges *et al.*, 2003). This advanced developmental level at hatching is typical of marine fishes with male parental care (Thresher, 1984; Sponaugle *et al*. 2002; Hickford and Schiel 2003) that spawn demersal eggs.

Although differences between reared larvae and larvae collected in the plankton may occur (Leis, 2000), in this study the smallest larvae captured in the plankton showed a developmental level and pigmentation pattern similar to that of the laboratory reared ones.

The basic larval development sequence also agreed with the known descriptions for other species of the same genus: *G. cobitis* (Spartà, 1950; Gil *et al.*, 1997); *G. paganellus* (Hefford, 1910; Lebour, 1919; Spartà, 1934; Borges *et al.*, 2003), *G. niger* Linnaeus, 1758 (Lebour, 1919; Petersen, 1919; Padoa, 1956; Russell, 1976).

However, the size at which some ontogenetic events occurred differed between species: while all caudal, anal and 2nd dorsal fin rays were present in *G. xanthocephalus* at 9.9 TL, in *G. niger* (Petersen 1919, Padoa 1956, Russell 1976) and *G. paganellus* (Lebour 1919, Borges *et al*. 2003) these structures were present with 7.0-8.0 TL and in *G. cobitis*, only at 11.0 mm (Gil *et al*. 1997). Size at settlement can also vary: 9.0 mm in *G. niger* (Petersen 1919; Russel 1976); 10.0-13.0 in *G. paganellus* (Lebour 1919, Borges *et al*. 2003) and 13-14 in *G. cobitis* (Gil *et al*. 1997). In *G. xanthocephalus*, the exact size at which larvae start to settle could not be detected in this study. The absence of larvae bigger than 13.1 mm TL (11.0 mm BL) in plankton net samples may be due to the beginning of the settlement process or to an increased ability to avoid the net (Cowen, 2002). The only *G. xanthocephalus* larva caught with the light trap had approximately 18.5.mm TL (15.0 mm BL). Its pigmentation pattern had completely

changed and post-anal scales were present, both considered as juvenile characteristics (Kendal *et al.,* 1984). These features, and the fact that light traps usually capture mostly late-stage larvae (Doherty 1987; Choat *et al.*, 1993), suggest that this larva could be already settled. Therefore, *G. xanthocephalus* settlement should start between 13.1 and 18.5 mm TL. Future work should be directed to understand when settlement occurs. This could be accomplished by using otoliths through the analysis of settlement marks (see e.g. Raventós and MacPherson, 2001), using recently settled juveniles.

Borges *et al.* (2003) summarised the characteristics that may help in the identification of larvae of the most common gobiids of the north-eastern Atlantic. In the present study, we focus on the features that may help in the distinction of *G. xanthocephalus* larvae from other Gobiidae larvae, in particular from the *Gobius* species present in north-eastern Atlantic, and whose larval stage descriptions are available: *G. niger* (Petersen, 1919; Padoa, 1956; Russell, 1976)*, G. cobitis* (Gil *et al.,* 1997), *G. paganellus* (Hefford, 1910; Lebour, 1919; Spartà, 1934; Borges *et al.*, 2003) and *G. cruentatus* (Gil *et al.*, 2002), the latter described only at hatching.

G. xanthocephalus newly hatched larvae are clearly distinguishable from other Gobiidae genera by their pigmentation pattern and myomer counting (see Lebour, 1919; Petersen, 1919; Padoa, 1956; Russell, 1976): they typically present no pigmentation at the angle of the lower jaw and have 28 myomeres. These two features are especially important in the distinction of newly hatched *Gobius* larvae from *Pomatoschistus* spp. e *Gobiusculus flavescens* (see Lebour, 1919; Petersen, 1919).

Within the genus *Gobius*, *G. xanthocephalus* differs at hatching from the other described species by the lack of dorsal pigmentation and the presence of a ventral post-anal row of similar melanophores regularly spaced, from anus to caudal peduncle. They also differ from *G. cobitis* (Spartà, 1950; Gil *et al.*, 1997) and *G. cruentatus* (Gil *et al.*,

2002) by the absence of a conspicuous melanophore, ventral and posteriorlly to the otic vesicle (the "median head cromatophore" described by Petersen, 1919)

At intermediate sizes (5.5 - 10.5 mm TL approximately; 5.0 - 9.5 mm BL), *G. xanthocephalus* larvae continued to be easily identified by the same pigmentation pattern shown at hatching. However, new melanophores appeared: around 5.5 mm TL (5.0 mm BL), a ventral post-anal double row of melanophores appeared at the anal fin insertion. *G. paganellus* also showed this feature with 10.0-10.5 mm TL (Borges *et al.*, 2003). Caudal fin pigmentation became visible sooner than in the other *Gobius* species described (between 4.3-5.4 mm TL (4.0 – 4.9 mm BL) vs 9.0-11.0 mm TL in *G. niger, G. cobitis*, and *G. paganellus*), and formed a spot in the central portion of the fin base and/or rays, around 9.3 mm TL (8.0 mm BL). Around 7.4 mm TL (6.5 mm BL) the otolith capsule became pigmented in both *G. xanthocephalus*, *G. paganellus* (Borges *et al.*, 2003) and *G. niger* (Lebour, 1919; Petersen, 1919). However, it was much more conspicuous in *G. xanthocephalus* than in the other two species.

In subsequent developmental stages, dermal pigmentation pattern develop. However, by this time, the distinction between *Gobius* and other Gobiidae genera, and between *Gobius* species, can be accomplished by comparing the meristic counts of fin rays and vertebrae (see Miller, 1986; Heymer and Zander, 1992). Still, there may be some difficulty in separating *G. xanthocephalus* (A= 14 - 15; D2= 15 - 16 according to Heymer and Zander, 1992) from *G. bucchichi* Steindachner, 1870 and especially from *G. gasteveni* Miller, 1974. Larval stages of these two species are unknown, and the meristic count is very similar to that of *G. xanthocephalus* (see Miller, 1986). However, *G. bucchichi* never presents 16 rays in the second dorsal fin (Miller, 1986). Distinction between *G. xanthocephalus* and *G. gasteveni* was based on a global evaluation of morphological and pigment features integrated in a developmental series. Also, *G.*

xanthocephalus is much more abundant than *G. bucchichi* and *G. gasteveni* at the Arrábida Marine Park.

The interspecific comparisons made in this study should be taken into account with some caution, since they were based on larvae reared under different temperature conditions. Because the decrease of the developmental time with higher temperatures is known for many fish species (Blaxter, 1969), the descriptions of the same species under different temperature conditions may indicate different sizes for the same ontogenetic events focused here. The ontogenetic index proposed by Fuiman (1994) is a good tool to be used in interespecific comparisons allowing comparisons on the basis of larval size or age at a certain ontogenetic event (Fuiman, 1994; Fuiman *et al.*, 1998). The ontogenetic index (OL) expresses "the state of ontogeny of a larva at any point in a developmental sequence", according to the formula (OL=logL/Logjuv.100), where L= standard length and Ljuv= SL at the beginning of the juvenile stage. If we consider that settlement occurs with c.a. 13 mm TL, the ontogenetic development of some structures can be compared between species with OL (Table II).

Table II- Ontogenetic Index for some ontogenetic events of *Gobius* species.

	G. xanthocephalus	*G. niger*	*G. paganellus*	*G. cobitis*
fin rays	89.38	82.50	73.17	81.48
caudal pigmentation	61.56	94.28	98.94	88.47
otolith pigmentation	78.03	81.95	86.00	
flexion begins	62.75		83.61	
complete flexion	56.92		87.80	55.56

Although the most complete descriptions for other *Gobius* species are based on laboratory reared larvae, which often differ from field-caught larvae in pigment or other

morphological characteristics (Leis, 2000), in *G. xanthocephalus* structures seem to develop faster than in *G. pagennelus* and *G. cobitis* (Table II).

Future work should be done on the standardization of the methodology used in the larval descriptions, creating objective categories of development, in order to allow the application of the ontogenetic index. This should help in phylogenetic and taxonomic studies among others (e.g. Fuiman 1984).

The mutations found in the DNA analysis were consistent with the variability present within species, and the adults analysed were unambiguously identified as *G. xanthocephalus*. Consequently, concerning the correct identification of the described larvae as *G. xanthocehalus*, little doubt remains. Therefore, the characteristics described above, used to distinguish *G. xanthocephalus* from other species, seem to be valid and adequate. The use of genetic markers is proving to be a powerful tool to ascribe larvae collected in the plankton to the correct species. For the North-Eastern Atlantic and the Mediterranean there is a considerable number of fish families for which genetic markers are available that can be used to identify individuals, including embryos and larvae, with high levels of certainty (e.g. blennids, Almada *et al.* 2005; tripterygids, Carreras-Carbonell *et al.*, 2005; labrini, Hanel *et al*. 2002, Henriques *et al*, 2002; sparids, Hanel and Sturmbauer, 2000). We propose that a comprehensive program based on unambiguously well identified adults may help to identify many larval forms that have not yet been ascribed to the species level.

In what concerns the otolith analysis performed, the linear relationship between age and BL found is common in larval fish (Ré, 1984). This seems to indicate that the relationship between the ontogenetic development and body growth found actually

reflects the larval development through time. However future validation of daily deposition is still needed (Thorrold and Hare, 2002).

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VI. DISCUSSION

The difficulty in sampling ichthyoplankton in nearshore environments with traditional methods led to a poor knowledge of the composition and dynamics of nearshore larval assemblages (Smith *et al*. 1987; Kingsford and Choat 1989; Kobayashi 1989). The lack of complete descriptions of larval stages and their ontogenetic development is particularly notorious as far as reef species are concerned. In most temperate nearshore studies of fish larval assemblages, reef species as the gobies, labrids or blennids are seldom identified to the species level; most information is given to the family level (e.g. Kingsford and Choat 1989; Brogan 1994; Palomera and Olivar 1996; Tilney *et al*. 1996; Sabatés *et al*. 2003). This can lead to serious problems when investigating ecological patterns that can vary from species to species. Nonetheless, many of the described patterns have been identified based on "family level" identifications; therefore, generalizations from existing models should be made with caution when applied to these nearshore assemblages. Detailed descriptions like those in Chapter **V** are needed to facilitate distinction between larvae of related species (Balon 1984; Powles and Markle 1984; Leis 1991a, 2000). With these detailed studies, it is possible to identify distinctive features which can help in the accurate identification of species. There are at least nine species of the family Gobiidae living at the Arrábida Marine Park (Henriques *et al*. 1999). From these, the complete sequence of larval development has been described for *Gobius cobitis* (Gil *et al*. 1997), *Gobius paganellus* (**V-C**), *Gobius xanthocephalus* (**V-D**) and the newly hatched larvae of *Gobius cruentatus* (**V-B**); older studies exist for *Gobius niger*, *Pomatoschistus pictus*, *Pomatoschistus minutus*, *Pomatoschistus microps*, and *Gobiusculus flavescens* (see descriptions of Lebour 1919, 1920; Petersen 1919; Padoa 1956; review by Russell 1976).

From the nine blennid species living at the Arrábida Marine Park (Henriques *et al*. 1999), complete descriptions of larval development exist for *Lipophrys pholis* (**V-A;** Fives 1970), *Lipophrys trigloides* (Faria *et al.* 2005), *Parablennius pilicornis* (Olivar 1986), *Parablennius gattorugine* (Fives 1970), *Parablennius sanguinolentus* (Santos 1989) *Blennius ocellaris* and *Coryphoblennius galerita* (Fives 1980) (see also Russell 1976 for older descriptions).

For Gobiesocidae larvae, the descriptions available are incomplete (see Russell 1976). Larval stages of some labrids are known (Fives 1976; review by Russell 1976): *Symphodus melops* is the species of the genus *Symphodus* with more complete descriptions of larval stages; for the other *Symphodus* species the descriptions of Quignard (1967a,b, 1968) for newly hatched larvae allow the distinction of some "types" of *Symphodus* species, but the precise separation between all the seven *Symphodus* species living at the Arrábida Marine Park is still difficult. *Trypterygion delaisi* larvae have not been described, but this is the only species of the family present at the Arrábida Marine Park and the identification to the family level was possible through myomer counting and general body shape.

Rearing larvae from eggs spawned by identifiable adults is a good way to allow valid descriptions of larval stages. However, this methodology can be time consuming and not always successful. On the other hand, it must be considered that reared larvae may be different from field captured larvae (Leis 2000). Coupling descriptions from plankton collections with further genetic validation can allow complete descriptions of larvae growing in the natural environment (**V-D;** Taylor *et al*. 2004). Distinctive morphological characteristics and pigmentation patterns can however be used for the separation of several related species. This was the case of *G. xanthocephalus* (**V-D**). There were no previous descriptions of larval stages for this species. In the smaller

larvae, the distinctive pigmentation pattern coupled with myomer counts allowed identification of a distinctive species of *Gobius*; the pigmentation pattern remained relatively stable with development and in the more developed larvae fin ray counting was possible, confirming the identification of these individuals as *G. xanthocephalus*. However, although less probable, there were still some other possibilities. These were excluded taking into account the results of the genetic analysis, which validated the identification. This method could be used in the future to solve multiple identification problems that are still to be solved. The Gobiidae and Labridae larvae, for example, present many unsolved difficulties, mainly in the identification of pre-flexion stage larvae. There are many "types" of larvae, based on larval pigmentation alone and myomer counting. These "types" are often more numerous than the possible number of species. This leads to the hypothesis that variability in pigmentation patterns within the same species occurs, even in those pigments that are usually described as species distinctive (e.g. Petersen 1919; Russell 1976; Ruple 1984). This is also a consequence of the little replication that rearing experiments necessarily offer (when space and human resources are limited) given that the reduced number of eggs and high mortality rates limit the number of larvae to be used in the descriptions when the objective is that larvae survive successfully until settlement.

Although the above discussed descriptions are useful for larval identifications, caution must be taken in inter-specific comparisons. Larval development is flexible and varies with size, age or the species considered (Ditty *et al.* 2003). Size is often used in comparisons. However, at the same ontogenetic state size can be variable, depending on genetic and environmental factors (Ditty *et al.* 2003). Therefore, size is not a reliable indicator of the developmental stage as far as inter-specific comparisons are concerned. Fuiman (1994) proposed the ontogenetic index which expresses the state of ontogeny of

a larva at any point in a developmental sequence as a relative measure between the standard length at that point and the standard length at the beginning of the juvenile phase. This allows a correction of differences in size at a given comparable ontogenetic state (Fuiman 1994; Fuiman *et al.* 1998; Ditty *et al.* 2003). In order to use the ontogenetic index in comparisons, the same ontogenetic events must be considered for the different species. Therefore, future descriptions should define comparable categories of development for each ontogenetic event, including body pigmentation pattern (see Ditty *et al*. (2003) for definition of character states in blennies).

Despite the difficulties in reef fish larval identification, some work has been conducted in nearshore reef environments, directed at investigating the composition of larval assemblages and identifying patterns of variation at small temporal and spatial scales (e.g. Leis 1986, 1991b, 1993; Leis and Goldman 1987; Kobayashi 1989; Gray 1996, 1998; Harris *et al*. 1999; Cowen 2002; Sponaugle *et al*. 2003). In some of these studies, the composition of nearshore assemblages has proven to be different from that of oceanic assemblages (e.g. Leis and Miller, 1976; Leis 1982; Smith *et al*. 1987; Sponaugle *et al*. 2003, **III**). Understanding the temporal patterns of variation of those nearshore assemblages can provide useful information about the spawning events of adults and on the patterns of larval supply that potentially limit the recruitment of local populations (Cowen 2002).

At the Arrábida Marine Park nothing was known about the structure of larval assemblages and their temporal and spatial patterns. Moreover, nearshore environments are poorly investigated in what regards larval fish assemblages. Studies presented in Chapters **II, III, IV** pretended to contribute to fill this gap.

1. Composition

The Arrábida Marine Park is located in the southern limit of distribution of several species and in the northern limit of others. This contributes to a high diversity of coastal fish species living there as adults (Almada *et al*. 1999; Henriques *et al*. 1999). This diversity was well reflected in the larval assemblages studied. These were composed mainly by larvae of nearshore species, which is in agreement with the results of other nearshore studies that found shore fish larvae to be an important component of the assemblages (e.g. Kingsford and Choat 1989; Brogan 1994; Palomera and Olivar 1996; Tilney *et al*. 1996; Sabatés *et al*. 2003; Veléz *et al*. 2005). The rarity or absence of larvae considered to be "oceanic", even along transects (**III**), probably reflects a weak influence of oceanic waters in these nearshore assemblages both in the upwelling season and in the remaining periods. Other studies have detected the presence of these larvae as indicative of offshore influence (e.g. Sabatés *et al.* 2003).

Gobiesocids are very abundant at the Arrábida Marine Park. However in studies **II, III**, and **IV** few larvae of this family were found. Although *Lepadogaster lepadogaster* larvae have been referred to occur in offshore waters of the North Sea (e.g. Fives and O'Brien 1976; Riley *et al*. 1986; Tully and ÓCéidigh 1989; Lee *et al*. 2005), the studies along the Portuguese coast did not found larvae of these species (except for the presence of *Diplecogaster bimaculatus* obtained by Afonso 1995). The absence of gobiesocids from both the surface (**II, III**) and bottom (**IV**) samples seems to indicate dispersal of these larvae. However this may not be the case. Gobiesocid larvae have been found in several studies conducted in other nearshore rocky environments (Marliave 1986; Kingsford and Choat 1989; Brogan 1994, Tilney *et al.* 1996; Sabatés *et al.* 2003). The methods used in the present study may not be adequate to sample larvae

of these species. In fact, high numbers of gobiesocid larvae were collected in preliminary light trap-experiments (unpublished data).

2. Temporal patterns

Inter-annual differences in larval density, diversity and in assemblage structure, were evident, within the same season (**II**). Wind patterns and sea surface temperatures (SST) off the Portuguese coast are influenced by the North Atlantic Oscillation (NAO) (Borges *et al*. 2003). In southwest Europe and in the Mediterranean, years of positive NAO index (NAOI) are associated with dry and cold winters with prominent NW winds and lowered SST; upwelling can also occur, further cooling nearshore waters (Hurrel *et al.* 2003) . Positive winter NAOI can change the wind patterns off Portugal (Borges *et al.* 2003), influencing the frequency and intensity of winter upwelling events and lowering SST values off Portugal (Ribeiro *et al*. 2005). It can be speculated that the overall decrease in larval supply in the spring-summer and in the autumn 2000 could be related to the high NAOI values of 1999-2000. In fact, in the years 1999 and particularly 2000, winter NAO was very positive (Ribeiro *et al*. 2005; Santos *et al*. 2005). Recruitment of planktivorous populations, including sardine can be strongly affected by this variation (Borges *et al*. 2003). However, Ribeiro *et al*. (2005) found that the February 2000 winter upwelling had a positive effect in the survival of sardine larvae off the northern coast. Larvae were maintained in good condition due to an increase in phytoplankton, caused by the presence of a less saline riverine plume (Chícharo *et al*. 2003). Given the different locations of these studies, it is not known the extent to which the winter NAOI affected the nearshore assemblages at the Arrábida Marine Park. The NAOI can have a more direct influence on temperature fluctuation and this can also have a local impact on adult populations. Being a transitional zone,

inter-annual variations in SST seem to affect directly the occurrence of adults (E.J. Gonçalves, personal communication). Temperature greatly influences the reproductive processes of fishes (Thresher 1984) and has strong impact on larval growth and on other early life history features (Houde and Zastrow 1993), even in tropical waters (Meekan *et al*. 2003).

The NAO Index influences directly river inflow (Alveirinho *et al.* 2004) and this, in turn, can have strong impact over the coastal area (Wolanski *et al*. 2004). The Sado river has an almost null flux during the spring and summer months but during the winter, average values of 60 m^3s^{-1} are usual (Martins *et al*. 2001), and flow can reach 1800 m^3s^{-1} (Martins *et al*. 2002). Although the exact patterns of circulation of the Sado outflow and its interaction with the nearshore adjacent waters of the Arrábida Marine Park are poorly understood, winter flow can potentially fertilize nearshore waters and influence the reproductive patterns of adults and the dynamics of planktonic assemblages. In 2000 the winter flow was minimal. The extent to which this fact had influence on the composition and dynamics of the larval assemblages in that year is not known.

The succession dynamics of the larval assemblages showed a clear seasonal pattern. Most nearshore reef species breed in the spring and summer period (Henriques *et al*. 1999) and this was the period with the highest larval diversity (**II, III**). The observed temporal patterns observed and the fact that most larvae were in the pre-flexion stage is indicative that larval assemblages' dynamics were closely following the spawning patterns of adults.

Some authors have outlined the fact that factors acting at assemblages at a small scale can confound the results obtained at large-scales (Gray 1996). For instance, Gray (1996) sampled at two locations on two consecutive days and at different times at two

depths. He found variation between the number of taxa and density between days and times within each day. This raises the question whether the composition of the assemblages described in Chapter II could have been different if different days had been sampled. In order to eliminate small scale spatial variation a high number of samples was used, however the temporal sampling could have been biased by the specific patterns of the sampled days. To test the influence of daily variations in the composition of larval assemblages, 24 hour sampling needs to be done in different days and results compared in an hourly basis. If sampled regularly, the planktonic stages caught with surface sampling can allow monitoring fluctuations of the adults spawning patterns of activities and their seasonal variation. This should involve small scale sampling (Gray 1996). In the same sense, inter-annual differences in the larval composition may be used to monitor population changes in face of environmental variation (Neira and Sporcic 2002). Assemblage composition can change rapidly from year to year, depending on sea temperature. Those species that have their southern limit of distribution at the Arrábida Marine Park can become rarer while the abundance of the "southern" species may increase (E.J. Gonçalves, personal communication). If larval composition is directly linked to the composition of the adult assemblage, intensive sampling directed to monitor the presence of "indicator" species may be used to monitor the changing patterns of the adult populations.

3. Spatial patterns

As traditionally assumed in the hypotheses developed to explain recruitment fluctuations in fisheries (e.g. Hjort 1914; Lasker 1981; Cushing 1990; Parish *et al.* 1981; Iles and Sinclair 1982; Bakun 1996), the need to understand the patterns that affect the larval phase assumes major importance in reef fishes, given their influence

over larval supply and replenishment of adult populations. It is well established that variation in larval supply can have a strong influence over the recruitment patterns of reef fishes (Victor 1986 b; Robertson *et al*. 1988; Milicich *et al.* 1992; Meekan *et al*. 1993; Swearer *et al*. 1999; Valles *et al*. 2001; Breitburg 1991; Sponaugle and Cowen 1996). When a pluralistic view about the factors affecting population dynamics is adopted, both post-settlement processes and larval supply need to be considered when studying the mechanisms that influence recruitment patterns (Jones 1991; Shima 2001; Jones and McCormick 2002). In this sense, larval distribution and the existence of possible retention mechanisms can have a strong impact over larval supply and recruitment variability (Jenkins *et al*. 1998).

The observation of large schools of reef fish larvae at the very-nearshore over rocky reefs motivated the investigation of possible larval retention patterns at the Arrábida Marine Park. Assuming passive dispersal as the only process occurring near the reefs, and considering that the same oceanographic factors would act over the larvae occurring at a given time, the same pattern of variation would be expected for the different species collected. However, the results found seem to indicate a different scenario.

Considering the horizontal distribution of the assemblages during the spring-summer period, we have found that:

i) Overall, larval density and diversity was higher at the extreme nearshore than at two miles from shore. Patterns of variation could be found at a very small spatial scale, with a clear decrease from the extreme nearshore to the first mile, and with further decrease with increasing distance from shore. The extreme low numbers of larvae occurring in more offshore waters led to the first two miles being identified as an obvious distinct group;

ii) Some species had a clear limited distribution at the extreme nearshore; other species were limited to the first miles, while others showed broader distributional ranges;

iii) Larvae of reef fish species dominated both distances from shore.

Mullus surmuletus was the only species with larvae having a predominant offshore distribution. These observations agree with published results in which larvae (Russell 1976) or juveniles (Deudero 2002) of this species have been found in offshore waters.

The offshore assemblage's composition was most probably underestimated taking into account the sampling method used. Comparison with other studies along the Portuguese shore revealed higher abundances of larval fishes, given that in those studies the whole water column was sampled (Afonso and Lopes 1994; Afonso 1995; Lopes and Afonso 1995).

The spawning mode of fishes is among the principal life history trait that can influence their dispersal potential (Leis 1991a, 2002; Cowen and Sponaugle 1997; Sponaugle and Cowen 1997; Leis and McCormick 2002; Sponaugle *et al.* 2002). Several of the reef species present at the Arrábida Marine Park have larvae that hatch from benthic eggs. It is known that these larvae hatch with a more advanced developmental stage and a bigger size (when compared to larvae hatching from pelagic eggs); these larvae can have enhanced abilities to avoid dispersal (Thresher 1984; Sponaugle and Cowen 1997; Leis and McCormick 2002; Sponaugle *et al*. 2002; Hickford and Schiel 2003). On the other hand, the pelagic egg is a dispersive stage, in opposition to the demersal eggs of reef fishes. Therefore, considering the hypothesis that larvae from demersal eggs would be retained and that eggs and larvae from pelagic eggs would disperse, a decrease in the abundance of larvae from benthic eggs and an

increase of larvae from offshore taxa hatching from pelagic eggs would be expected with increasing distance from shore (Leis and Miller 1976; Kingsford and Choat 1989; Brogan 1994).

Considering the results presented in Chapter **III**, one would expect species which showed a broader range of distribution to have hatched from pelagic eggs. A higher proportion of newly hatched larvae from demersal eggs would also be expected nearshore than offshore, while the reverse pattern should occur to larvae hatching from pelagic eggs. This has been described by Leis and Miller (1976), which considered the absence of pelagic eggs and larvae from nearshore waters as a result of offshore displacement by oceanographic factors. However the results found in chapter **III**, indicated that from those taxa found in more offshore waters, both larvae hatching from pelagic eggs (e.g. *Mullus surmuletus*) and from demersal eggs (e.g. *G. niger* type or *P. pilicornis*) could be found. Therefore, the patterns obtained were different from the ones expected and indicate that other factors beside the spawning mode of the adults are acting on the dispersal of larval stages. This is in agreement with the results obtained in other coral reef (e.g. Leis 1982, 1993) and temperate nearshore (e.g. Hickford and Schiel 2003; Sabatés *et al*. 2003) studies, in which the patterns obtained were taxon specific, and do not dependent only on the spawning mode of the species.

The abundance of larvae from pelagic eggs decreased along the inshore-offshore transects, with larvae being present at the very-nearshore and more abundant in the first miles. However, offshore dispersal during the egg stage should be expected. Incubation periods of pelagic eggs depend on the temperature and range from days to weeks, depending on the species (Russell 1976). Like planktonic larvae, pelagic eggs are dispersive stages. Larvae from many species hatching from pelagic eggs are small and

undeveloped being "little more than a yolk sac with a tail" (Leis *et al.* 2006). One possible explanation to the inshore distribution of these larvae is that physical mechanisms could allow the passive retention of part of the eggs and early larvae at the nearshore environment. If such passive mechanisms are acting, they could influence the retention of eggs and larvae in the early developmental stages, nearshore, at least in surface (**II, III, IV**).

In the surface samples, most larvae were in the pre-flexion stage. Three hypotheses were raised to explain the absence of more developed larvae from the samples (**I**):1) net avoidance; 2) offshore dispersal; 3) depth distribution.

Bigger larvae may be able to avoid nets (Leis 1991 b; Choat *et al*. 1993). It is possible that part of the larvae could have that ability, even before flexion of the urostyle is completed. However, preliminary data show that most larvae caught with night trawling were also in the pre-flexion stage (unpublished data), indicating that visual avoidance is improbable. There remains, however, the possibility of avoiding the noise caused by the boat, given that larvae can actively react to sounds (e.g.. Tolimieri *et al*. 2000, 2004; Leis *et al*. 2002; Leis *et al*. 2003; Simpson *et al*. 2004; Leis and Lockett 2005; Simpson *et al*. 2005). Nevertheless, it is improbable that almost all the taxa share the same hearing capabilities from very early stages in development (see Myrberg and Fuiman 2002). On the other hand, the net was towed sub-superficially at some distance from the boat, and unless all the larvae would have the same reaction of "escaping towards the bottom", more developed larvae would have been caught by the net, at least for some species.

The second possibility, offshore dispersal of bigger larvae, must be investigated with other sampling methods rather than surface sampling. However, at least at the

surface, no bigger larvae were found offshore (**III**). From the taxa living at the Arrábida Marine Park, few species were caught in other studies that have sampled with oblique towing along shelf waters. For instance, some species known to be abundant nearshore like tripterygiids and some gobiesocids were never found offshore (see Ré 1984; Ré *et al.* 1990; John and Ré 1993; Afonso and Lopes 1994; Afonso 1995; Lopes and Afonso 1995).

The results of Chapter **IV** showed the existence of clear vertical structure in the assemblages at the extreme nearshore with ontogenetic patterns of variation for some species. This is not surprising given that small scale vertical patterns have been described to occur even at small spatial scales in other systems (Leis 1986, 1991a,b; Marliave 1986; Breitburg 1989, 1991; Breitburg *et al.* 1995; Sponaugle and Cowen 1996, Sponaugle *et al*. 2003; Vélez *et al.* 2005, **IV**).

Although the towing method used was different at the surface (boat) and at the bottom (scooter), both nets had the same diameter and mesh size and were pulled at the same speeds. The differences obtained in the extreme nearshore sampling seem therefore to reflect real differences in the observed patterns between the surface and bottom rather than a hypothetical avoidance of the net by the larvae only at the surface.

The depth distribution of larvae in shelf waters near the Arrábida Marine Park remains to be tested. In offshore waters, larvae probably spread vertically in the water column. In fact, vertical distribution of larvae in oceanic or shelf waters is well documented (e.g. Kendall and Naplin 1981; Southward and Barret 1983; Conway *et al*. 1997; Olivar and Sabatés 1997; Gray 1998; Somarakis *et al*. 2002; Sabatés 2004). In future studies, it would be interesting to try to specifically sample more competent larvae using for instance light traps, at different depths and at several distances from

shore. Light traps are more selective devices but they are also more efficient than towed nets in capturing bigger larvae (Doherty 1987; Choat *et al*. 1993). Another possible way of attracting more developed larvae is through the use of artificial substrata, a good technique to quantify late stage larval supply (Steele *et al*. 2002).

Combining the results obtained in chapters **II-IV**, some patterns can be identified for some taxa, and these will be discussed for the most relevant families:

Gobiidae: there were few larvae from this family at the surface; these surface larvae were little developed, and hence difficult to identify to the species level. On the contrary, Gobiidae larvae were the most abundant larvae at the bottom (**IV**), with the assemblages being dominated by a few number of species: *P. pictus*, *G. xanthocephalus* and *G. niger,* all demersal spawners. *P. pictus* inhabits gravel and sand in inshore waters at a depth range from 1-55m (Miller 1986). Adults of *G. xanthocephalus* also occur inshore and little is known about its reproduction. No larvae of these two species were found in offshore samples (**III**).

Although not so abundant, *G. flavescens* and *G. niger* also presented a vertical pattern of distribution. Adults of *G. flavescens* inhabit exclusively nearshore shallow waters, while those of *G. niger* can be found in estuaries or inshore over sand or mud with a depth range from 1 to 75 m (Miller 1986). For these species, the fact that larvae were present within all the size classes at the bottom suggests that they may soon be able of controlling their vertical positioning early in development which could facilitate nearshore retention (Brogan 1994; Leis 1994; Leis *et al*.1998; Planes *et al*. 1998 b; Leis *et al*. 2003). However, *G. niger* type larvae also showed some dispersal ability: it was one of the species with a more "broader" distribution along the shelf (**III**). These two

results together seem to indicate that although some retention could occur, the degree of self-recruitment for *G. niger* could be lower than, for instance that of *P.pictus* or *G. xanthocephalus*. Other possible explanations are that *G. niger* type could correspond to other *Gobius* species present at Arrábida (however, the offshore occurrence of this taxa points to a dispersive pattern, at least to some extent). The fact that gobies were the main group represented at the extreme nearshore is in agreement with several other studies in which Gobiidae species showed a clear "inshore" distribution (e.g. Leis 1986; Smith *et al*. 1987; Kingsford and Choat 1989; Kobayashi 1989; Gray 1993; Brogan 1994; Gray and Miskiewicz 2000; Kingsford 2001; Sabatés *et al*. 2003; Sponaugle *et al*. 2003).

Labridae: within this family, *C. julis* larvae were abundant in the spring-summer period (**I**); larvae of this species had some spatial dispersal at the surface (**III**) and were rarely present at the bottom (**IV**). This species lives near reefs and spawns pelagic eggs. Other studies have also indicated dispersal of *C. julis* larvae (e.g. Sabatés *et al*. 2003).

Symphodus melops larvae were also found at the bottom within all size classes. These larvae hatch from demersal eggs that are laid in nests made of seaweeds among rocks or in crevices (Quignard *et al.* 1986). Like *G. niger*, larvae of *S. melops* type were also found offshore and are referred in other studies (**III**, Russell 1973; Fives and O'Brien 1976; Riley *et al.*1986; Tully and O'Ceidigh 1989; Afonso 1995; Acevedo *et al.* 2002; Koutrakis *et al*. 2004; Lee *et al.* 2005).

S. roissali larvae were also found, both at surface (**II,III,IV**) and at the bottom (**IV**). Although only 14 individuals were found at the bottom, they were bigger than those at surface, indicating the same pattern found for *S. melops*.

The other *Symphodus* species are less abundant than *S. melops* at the Arrábida Marine Park occurring also in lower densities in samples. Several species of *Symphodus*, have their northern limit at the Arrábida Marine Park (Henriques *et al.* 1999). It is the case of *S. mediterraneus*, *S. ocelatus*, *S. roissali* and *S. rostratus* (Henriques *et al*. 1999). These species spawn during the spring-summer season. During this period, when upwelling occurs, the coastal circulation pattern is dominated by currents over the shelf, directed towards the south, and extending to about 300m depth; there is a current with opposite direction at more than 300m deep, where water with Mediterranean influence exists (Fiúza 1984). Considering an "offshore dispersive scenario", two situations could provide adequate transport towards the shore at this site: 1) the larvae of these shore species migrate to depths greater than 300m; or 2) they can swim against currents during their short PLD until they are returned to the reefs. These two hypotheses are improbable. This raises the question of how will the larvae of these warm water species spawned at the Arrábida Marine Park or elsewhere, be able of reaching the coastal environment of the Arrábida coast. Other scenarios must then be considered when trying to explain the persistence of populations of these warm water species at Arrábida: either the larvae are transported by alongshore currents, from populations located southerly to the region, or there is a high degree of self-recruitment.

Moreover, during the winter, the direction of the alongshore current on the Portuguese shore is reverted (Fiúza 1984) and the northward current can transport juveniles and adults from other populations to the Arrábida Marine Park. Swearer *et al*. (2002) defended that populations located at the edges of a species' range, under unidirectional flow regimes, will be transitory unless they have some "capacity for self-recruitment".

Sparidae: Sparidae larvae were abundant at inshore surface waters (**II, III, IV**) and could also be found more offshore (**III**). *Boops boops* is one of the most common of the eighteen species of this family known to occur at the Arrábida Marine Park (Henriques *et al.* 1999). Although larvae of this species hatch from pelagic eggs, they were also present at the bottom in several size classes (**IV**).

Tripterygiidae: *T. delaisi* was one of the species with the highest larval abundances found (**II, III, IV**). This is, in fact, one of the commonest species inhabiting the shallow rocky environment (Henriques *et al*. 1999). Larvae could be found in the extreme nearshore both at the surface and bottom, but no vertical pattern of distribution was found (**IV**). In fact, larvae at the surface were slightly bigger than at the bottom and no larvae bigger than 9 mm were found. This could indicate a high dispersal pattern for this species. For instance, Hickford and Schiel (2003) found tripterygiid larvae in offshore waters, at a more exposed coast. However, at offshore waters close to Arrábida, no larvae of *T. delaisi* could be found (**III;** Ré 1984; John and Ré 1993; Afonso and Lopes 1994; Afonso 1995; Lopes and Afonso 1995). On the other hand, tripterygiid larvae are often referred to be retained at temperate rocky nearshore environments (Kingsford and Choat 1989; Brogan 1994; Sabatés *et al*. 2003) and have been associated with surface waters (Hickford and Schiel 2003). Our data show that *T. delaisi* larvae are more abundant at intertidal shallower waters, than over other habitats (unpublished results), which could indicate that larvae of this species could migrate to specific coastal habitats early in the ontogeny.

Blenniidae: *P. pilicornis* was the most abundant species at the surface within the spring-summer period (**II, III, IV**). This species is a very common species at the

Arrábida Marine Park, which spawns demersal eggs, from March to August (Gonçalves 1997; Henriques *et al*. 1999; Almada *et al.* 2000). Larvae of this species hatch with 3.1 mm (C. Faria, personal communication) with a developmental level similar to the one of the other species already discussed. Although abundant when newly hatched at the surface, they were rare in the bottom collections (**IV**) and also occurred offshore (**III**). This seems to indicate a "dispersive" pattern for this species. Olivar (1990) found *P. pilicornis* larvae over shelf waters of the Benguela region, being more abundant in the upper layers of the water column and suggested that this distribution could facilitate offshore transport associated to the coastal upwelling. The other blennid larvae, *C. galerita* and *P. gattorugine* were less abundant, and never occurred at the bottom.

The ability of staying near the bottom has been reported for some species. Larval schooling near reefs has been observed in gobies (Breitburg 1989, 1991; Breitburg *et al.* 1995) and in other families (see Leis 1986; Steffe 1990), prior to settlement. This behaviour has been proposed to reduce offshore dispersal (Leis 1986; Leis and McCormick 2002) and can occur when larvae select microhabitats with reduced flow, as the benthic boundary layer (Breitburg *et al.*1995).

With exception of *B. boops*, all the species discussed as being possibly "retained" nearshore at the vicinity of the rocky bottom hatch from demersal eggs. For these species, size at hatching is variable, but within a very small range: *P.pictus*: 2.7-3.0mm (Petersen 1919; Lebour 1920); *G. xanthocephalus*: 2.8 mm (**V-D**); *G. flavescens*: 2.2-2.6 mm (Lebour 1919; Petersen 1919); *G. niger*: 2.5 mm (Iglesias 1979); *S. melops*: 2.5-3.0 mm (Quignard 1967b; Fives 1976). For *T. delaisi*, the smallest larva caught had 3.72 mm. This should be about the size at hatching for this species, given that larvae of this size still had a small yolk.

B. boops, being a sparid, hatches with a big yolk sac and without pigmented eyes at an undeveloped stage (Ranzi 1956). All the other referred species have, when newly hatched, the mouth and anus open, little or no yolk and the eyes fully pigmented (see the above cited references), as often happens in larvae hatching from demersal eggs (Thresher 1984; Hickford and Schiel 2003). The ontogenetic stage of development of the sensorial structures in these species is not known, but probably these larvae must be able of interacting, early in development, with the physical oceanographic features acting at the nearshore environment of the Arrábida Marine Park.

4. Planktonic larval duration

Considering the traditionally accepted relationship between dispersal potential and planktonic larval duration (PLD) (e.g. Sponaugle and Cowen 1997; Planes 1998 a, 2002; Leis 2002; Mora and Sale 2002; Sponaugle *et al*. 2002; Sale 2004), it is worthwhile to analyze the information available on the known PLD for these species (**Table 1**). This information is obtained both from the analysis of settlement marks in otoliths (Raventós and Macpherson 2001), from descriptions based in rearing experiments (**V-A, B, C;** Gil *et al.* 1997; Faria *et al*.2005) or from plankton collections (**V-D**). It can be easily seen that variability in PLD can be high within the same family or even genus. From the above discussed species, *G. xanthocephalus* has a long PLD which, in theory, could facilitate dispersal. As larvae of this species were present nearshore within all size-classes, this seems to indicate the existence of behavioural capabilities by the larvae that, although growing for an extended period, apparently can be maintained in the nearshore environment. For the other gobid species above discussed there is no information on the PLD, although other *Gobius* species can have lower PLD than *G. xanthocephalus* (Table 1).

T. delaisi and the labrids of the genus *Symphodus* have shorter PLD's. During this period *Symphodus melops* larvae grow from 2.5-3.0 mm (Quignard 1967 b) to about 10 mm (Fives 1976) or less (Raventós and Macpherson 2001, 2005). This is indicative of a fast development suggesting that these larvae may soon develop sensorial structures that allow them to actively behave.

B. boops is within the sparids with known PLD, the species with the shorter time spent in the plankton and the largest size at settlement. Like for *S. melops*, larval growth as well as probably the development of behavioural capabilities must be fast. Temperate sparids as small as 5-7 mm can show directional swimming and swimming speeds that allow them to actively affect their dispersal soon in development (Leis *et al.* 2006).

Blennid larvae have longer PLD's, therefore having a higher potential for dispersal (Roberts 1997, Sale 2004). Larvae of *L. pholis* (**V-A;** Fives 1970), *L. trigloides* (Faria *et al.* 2005); *P.pilicornis* (Olivar 1986), *P. gattorugine* (Fives 1970) and *C. galerita* (Fives, 1980), have well developed pectoral fins. On the other hand, blennid larvae can have strong swimming abilities. In coral reef blennies, swimming speed can reach 26.4 BL s^{-1} (Leis and Carson Ewart 1997). These characteristics could favour survival in the pelagic environment and facilitate transport back to the reefs in the end of the pelagic stage. However, results from other temperate studies indicated both retention (e.g. Tilney *et al.* 1996) and dispersal (Brogan 1994; Sabatés *et al.* 2003) of blennid larvae.

Gobiesocids on the contrary, have small PLD's (Table 1). Larvae of these species soon develop the adhesive disk (personal observations), which is an adaptation to the benthic mode of life. We hypothesise that these larvae are able of remaining at the epibethic layer very early in development, at least during the day. In fact, temperate gobiesocids are known to occur at very shallow waters, at mid depths or near the bottom

(Kingsford and Choat 1989). If this is the case, the absence of these larvae both from the surface and scooter samples could be explained.

Pelagic larval duration can vary within the same species (Sponaugle and Cowen 1997) and this variation can be a reflex of the ability to delay metamorphosis (Victor 1986 a). Growth rates and size at settlement can also be flexible and vary among locations (Sponaugle and Cowen 1997). By dividing the size at settlement by the PLD for each species, different values were obtained (Table 1). Growth rates cannot however be estimated with precision and only a rough comparison is possible by this method. Taking this in account, these values allow nevertheless a relative comparison of larval growth.

It seems that, for some species, larvae will have to grow much faster than for others, until they reach the respective settlement sizes. In fact, different larval growth rates can occur in the pelagic environment, even within the same species (Sponaugle and Cowen 1997; Searcy and Sponaugle 2000; Sponaugle and Pinkard 2004). Moreover, temperature has a major influence in larval growth and thus geographic differences in PLD can be explained by this factor (Houde and Zastrow 1993). For most species listed in Table 1 data were obtained from Raventós and MacPherson (2001) at the NW Mediterranean and mean PLD may be different for the same species at the Arrábida Marine Park. On the other hand, differences between species at the NW Mediterranean can also reflect variation in water temperature in different spawning seasons. The other measurements of PLD were obtained from laboratory reared larvae and can be different from those occurring under natural conditions (Leis 2000).

Table 1- Relationship between Planktonic larval duration and size at settlement for species present at the Arrábida Marine Park

Family	Egg	Species	PLD	Size at settlement (mm)	Size /PLD	Reference
Blenniidae	D	*C. galerita*	26-27 days			1
	D	*L. pholis*	29	13/14	0.45-0.48	(**V-A**)
	D	*L. trigloides*	52	20	0.38	1
			39-42	16-17	0.41	2
	D	*P- gattorugine*	52-66			3
	D	*P. pilicornis*	66-69			3
Gobiesocidae	D	*Apletodon dentatus*	15	7	0.47	1
	D	*L. candolei*	13			1
Gobiidae	D	*G. cobitis*	22	11	0.5	4
	D	*G. pagenellus*	25	10.25	0.41	(**V-C**)
	D	*G. xanthocephalus*	≥36	13.1-18.5	**0.28mm d^{-1}**	(**V-D**)
Labridae	P	*C. julis*	28.9	16	0.55	1
	P	*C. rupestris*	21.5	11	0.51	1
	D	*S. mediterraneus*	13.4			1
	D	*S. melops*	15			1
	D	*S. roissali*	12.8	5-7	0.47	1,5
	D	*S. rostratus*	13-14			1
Scorpaenidae	D	*S. porcus*	29			1
Serranidae	D	*S. cabrilla*	26			1
Sparidae	P	*B. boops*	16.7	12	**0.72**	1
	P	*D. annularis*	18	9	0.5	1
	P	*D. cervinus*	17	9	0.53	1
	P	*O. melanura*	15.8	10	0.63	1
	P	*P. pagrus*	38			1
	P	*S. cantharus*	38			1
	P	*S. salpa*	31.2			1
Tripterygiidae	D	*T. delaisi*	17-18			1

1- Raventós and Macpherson 2001; 2) Faria *et al*. 2005; 3) C. Faria personal communication; 4) Gil *et al*.1997; 5) Raventós and Macpherson 2005

Photoperiod is another factor that can influence the length of the larval period and growth rates of reef fishes (see Arvedlund *et al*. 2000). Larvae reared under 24-hour light: 0- darkness showed slower growth rates than larvae reared under 16L: 8D photoperiod regime (Arvedlund *et al*. 2000). The studies of larval development described in Chapter **V** were conducted in rearing conditions under a 24 hour light regime **(V).** Different results could thus have been obtained if the larvae would have been maintained under alternate darkness and light regimes. Despite these cautions, the comparison is indicative of some possible differences in growth rates among species living at the same locations, in temperate reef environments. We can thus assume that at

the Arrábida Marine Park the growth rates among species must also be variable, but the exact differences between species should be further investigated.

Starvation has been considered by many authors as the main factor influencing larval growth and recruitment (Hjort 1914; Lasker 1981; Cushing 1990). The growth during the larval phase of reef fishes and their size at hatching can influence post-settlement survival, as happens in temperate pelagic species (Bergenius *et al*. 2002; Vigliola and Meekan 2002). Besides temperature, other factors can influence food availability of reef fishes. These include the levels of solar radiation, wind and rainfall and the importance of such factors may vary seasonally and ontogenetically during the larval phase (see Bergenius *et al*. 2005). All these effects remain to be investigated at the Arrábida Marine Park. In the future, bottom trawling (**IV**) or light traps could be used to catch bigger larvae in order to investigate the growth and mortality patterns of these species. Otolith microstructure analysis could be used to investigate the growth patterns. Since the discovery of daily ring deposition in otoliths, age estimation from otolith microstructure has been extensively used (Brothers 1981, 1984; Campana and Neilson 1985). In temperate species, daily increments have been used to determine size-at-age, while in reef fish ecology, attention has been centred on the analysis of settlement marks for estimation of PLD (e.g. Victor 1991; Raventós and Macpherson 2001; Thorrold and Hare 2002). Studies on larval growth of reef fishes through otolith analysis have been conducted recently, giving relevant results to the understanding of recruitment patterns (e.g. Searcy and Sponaugle 2000; Meekan *et al.* 2003; Sponaugle and Pinkard 2004; Raventós and Macpherson 2005).

5. Summary of the patterns observed

In summary, the present study gives some evidence of possible active retention patterns for some species: *P. pictus, G. xanthocephalus, G. flavescens, G. niger, S. melops, B. boops* and possibly *T. delaisi* and other *Symphodus* species. Some other species showed more "dispersive patterns". It can be speculated that the long PLD found in blennies could give these species "time" for their pelagic phase to be completed offshore; their probable good swimming capacities would then facilitate successful shoreward transport at the end of the pelagic phase. For those larvae hatching from benthic eggs for which the PLD is short (e.g. *S. melops*, *T. delaisi* and clingfishes), dispersal could have serious risks and therefore retention near the adults habitat is advantageous. This could have led to the evolution of behavioural mechanisms which favoured retention. The specific behaviours and environmental cues associated are not known, but different patterns seem to exist: *S. melops* larvae seem to be able of being retained near the bottom, while *T. delaisi* showed no vertical pattern of distribution, and seems to be more abundant at the intertidal (unpublished data). Interestingly, larvae of *G. xanthocephalus*, although having slow growth and long PLD, must have behavioural skills that allow them to stay nearshore during all the larval stage. Larvae hatching from pelagic eggs and having long PLD are more prone for dispersal as they do not have skills to avoid it. However, for those with shorter PLD, a situation in which larvae are passively retained in the beginning of the pelagic phase, growth is fast and sensorial structures and swimming capabilities develop early in ontogeny, could facilitate retention. This could be the case of the sparid *B. boops* at our study site since at least part of the larvae occur near reefs at different developmental stages.

6. Temperate reef and coral reef fish larvae compared

Leis and McCormick (2002) identified several differences between temperate fish larvae of pelagic species and coral reef fish larvae. As these authors referred, most published work is about pelagic species rather than rocky reef species, and this "may confound temperate/coral reef comparisons". In fact, rocky reef fish larvae share several early life history features with coral reef fish larvae that distinguish them from temperate larvae hatching from pelagic eggs. Both coral and rocky reef fish larvae suffer habitat change from the pelagic to the benthic environment, with precise habitat requirements. In particular, habitats available to settle are less extensive and more discrete than in soft bottoms. On the other hand, adults of both coral and rocky reef species are more sedentary than adults of pelagic and soft bottom species. This makes the pelagic larval stage the "dispersive stage" of reef fishes both at coral reefs and temperate rocky reefs. Leis and McCormick (2002) identified larvae of coral reef fishes at any particular size to be more developed than pelagic temperate fish larvae. Temperate reef fish larvae hatching from demersal eggs are also more developed at hatching than larvae from pelagic eggs (**V**). Leis and McCormick (2002) also stated that coral reef fish larvae have better swimming abilities than pelagic temperate larvae. These authors hypothesised that reduction in coastal upwelling in the tropics would reduce opportunity to passive retention of the larvae and would increase the selective pressure to active larval retention through better swimming capabilities. However, rocky reef fish larvae can also be strong swimmers (Dudley *et al.* 2000; Leis *et al.* 2006). These capabilities, interacting with possible physical mechanisms (like upwelling, or others), could maximize the retention near reefs. Leis and McCormick (2002) also outlined differences in the embryonic period, which is much faster in coral reef species. Many rocky reef species have longer incubation periods that are usually linked to the

occurrence of male parental care. Almada *et al*. (1999) concluded that the relative importance of male parental care in temperate reefs is higher than in coral reefs, with fishes of bigger sizes still showing male parental care. Barlow's (1981) model to explain the advantage of sending propagules away from reefs considered that in coral reefs parental care is restricted to small fishes and that bigger fishes would take advantage in the dispersal of eggs in a patchy unpredictable environment subject to storms. If that is the case in coral reefs, where retention seems to occur, in temperate environments disturbance can be more predictable (e.g. Ebeling and Hixon 1991 *in* Almada *et al*. 1999) reducing the selective pressure to a dispersive stage and making larval retention near reefs also probable.

In coral reefs, predation on fish larvae is known to be intense (Leis and McCormick 2002). Plankton feeding is common and has strong impact on the trophy ecology of these systems (Hobson 1991). Planktivore species may be abundant in reefs, where they can compose 45% of the existing species (Hobson 1974 *in* Hobson 1991). In temperate systems, however, the trophic structure can be different. Almada *et al.* (1999) suggested that, given the seasonal changes in plankton abundance, preying zoobenthos could be the most efficient feeding strategy for reef fish of higher latitudes. They analysed the reef species occurring in the biogeographic area where the Arrábida Marine Park is included (Europe, Macaronesian islands, Mediterranean and Baltic). From the 316 species listed, only 12% were included in the planktivores group, while most species (74. 3%) were benthivores. In this context, if predation is the main evolutive forcing to a dispersive stage of reef fishes as proposed by Johannes (1978), at least in temperate waters and for coastal species this is less strong and evolutionary advantages could be minimal, when compared to the advantages in staying near the reefs.

Evidence of larval retention in coral reefs has grown in recent years (see Swearer *et al.* 1999, Jones *et al*. 1999; Taylor and Hellberg 2003; Paris and Cowen 2004; Jones *et al*. 2005). Given the similarities in ecology and some life history traits between larvae of coral and rocky reefs, larval retention is also highly probable near temperate rocky reefs. These environments having less predation on larval stages and having more stable and predictable conditions could have even more favourable conditions to the evolution of behaviours that could favour larval retention.

The advantages of growing at a more productive environment and near the settlement habitats (Leis 1991a; Swearer *et al*. 2002) must overcome the disadvantageous of dispersal, with the risk of permanent loss if the right shoreward transport fails.

7. Evidences contributing to the "Emerging View"

The paradigmatic view that reef fish populations are "open" is changing given the growing evidence that self-recruitment can be much more common than what was supposed. The "Open Population Paradigm" was based on the assumption that reef fish larvae are passive and are moved by currents that operate far from the reefs (which Leis (2002) called "far-field" currents) and that genetic panmixia is the evidence that populations are open at large scales (Planes *et al*. 1998 a; Bonhomme and Planes 2000; Leis 2002;, Planes 2002;). However the results of recent research show that these assumptions can be refused, and that, depending on the scale considered, populations may be more closed than expected (Planes *et al.* 1998 a, b; Jones *et al.* 1999, 2005; Swearer *et al*. 1999; Taylor and Hellberg 2003; Paris and Cowen 2004). In opposition of the traditional view, Leis (2002) considered this new "Emerging View" of dispersal as consisting of four major elements. Each of Leis' assumptions will be analysed, when

possible, in the context of the results obtained in this study, and the characteristics found at the Arrábida Marine Park.

The first assumption of Leis (2002) "Emerging view" is that ***reef fish larvae are not passive and can influence their distributional patterns***. This is based on growing recent evidence showing that larvae from reef fishes can be strong swimmers and can have complex behavioural repertoires (see below).

At the Arrábida Marine Park, the fact that some species were present at the very nearshore within all size classes and sometimes with clear vertical patterns of distribution seems to be a consequence of behavioural interactions with local physical factors. Given that other species with similar life history patterns seem to disperse, the differences observed between species should rely on different behavioural patterns. In particular, the swimming performance of larvae, the underlying sensory abilities of the different species and the ontogeny of such capabilities must be fundamental to explain the differences observed.

However, nothing is known about the behavioural capacities of these larvae and further studies are necessary. Temperate larval fishes are usually considered to be weak swimmers (Dudley *et al*. 2000; Leis and McCormick 2002). Blaxter (1986) reviewed swimming speeds of larvae of temperate pelagic species and found a maximum speed of about 4 to 5 BL s^{-1}. However, recent studies showed that some reef fish larvae can have much stronger swimming capacities, in particular on coral reefs. Leis and Carson-Ewart (1997) measured in situ swimming speeds of several coral reef species. They found average speeds of 20.5 cm s^{-1}, corresponding to13.8 BL s^{-1}, about three times higher than those reported by Blaxter. Leis and Carson-Ewart (2003), found that six out of seven species showing similar swimming speeds to those reported by Leis and Carson-

Ewart (1997), were able of swimming faster than local currents. Additionally, information on sustained swimming and distances swam by larvae has been given by experiments in swimming chambers (Fisher *et al*. 2000; Fisher and Bellwood 2002, 2003; Fisher 2005). Leis and Stobutzki (1999) compared both capabilities and concluded that these studies gave complementary information and that both have increased evidence in favour of the strong swimming capabilities of coral reef larvae. Experiments of Fisher *et al*. (2000), Fisher and Bellwood (2002, 2003) and Fisher (2005) investigated the ontogeny of the sustained swimming capabilities in larvae at different developmental stages, and concluded that different species had different patterns, but that sustained swimming and critical swimming capabilities increased early in development. Fisher (2005) concluded that the potential for reef fish larvae to significantly influence their dispersal relatively to ocean currents may be present at least from half their pelagic phase.

Leis and Carson-Ewart (1997) attributed the different results obtained between their study and those of Blaxter (1986), to the differences in development between the two groups of larvae, given that coral reef larvae fins develop sooner, at a smaller size than those of temperate larvae. Another possible explanation has to do with taxonomic differences. Most temperate larvae investigated were mainly from clupeiforms, pleuronectiforms or gadiforms, while in coral reefs most fishes belonged to the Perciform order. In rocky reef environments as those found at the Arrábida Marine Park, Perciformes also dominate the rocky coastal assemblages.

Swimming can be more efficient in environments with higher temperatures as coral reef environments (Leis and Carson Ewart 1997). However, Dudley *et al*. 2000 investigated the swimming performance of perciform larvae from temperate reef species and obtained swimming speeds from 4.5 to 13.5 BL s^{-1} for long periods of time. Direct

comparisons of the distances swum within the same family showed that larvae of a temperate reef species could travel longer distances than coral reef larvae of the same family. Dudley *et al.* (2000) suggested that these differences could be due to the largest size of the temperate larvae, or to a higher metabolic rates of coral reef larvae. Leis *et al*. (2006) followed in situ larvae of temperate fishes spawning pelagic eggs, as small as 5.0 mm SL. They showed a linear increase in larval swimming capabilities with development, and also that even these small larvae from pelagic eggs are able of controlling their position in the water column, through directional swimming.

Houde and Zastrow (1993) found that larvae from cold pelagic environments require two to four times less daily energy ingestion than larvae from coral reefs in relation to their respective growth rates. It would be interesting to investigate the energetic component in the growth of temperate reef larvae, and to incorporate energetics information in the swimming performance of fish larvae from both reef environments.

Condition and growth will influence the development of sensory and swimming abilities of larvae (Mora and Sale 2002). Thus, it is also important to understand which factors affect larval condition at the Arrábida Marine Park. In particular, possible differences in feeding patterns between larvae that stay near the bottom and at the surface (for example, through the analysis of their gut contents) and their impact over larval condition (evaluated through biochemical indices, see Ueberschär *et al*. 1992; Bergeron 1997), should be investigated.

Other examples of larval behaviour include the ability of larvae to actively choose between different habitats (e.g. Marliave 1977; Breitburg 1989, 1991; Breitburg *et al.* 1995; Doherty *et al*. 1996; Risk 1997; Watt-Pringle and Strydom 2003). The

results of the above cited studies clearly indicate that larvae are not passive, presenting complex and flexible behavioural patterns that can strongly affect their dispersal.

The second assumption proposed by Leis (2002) is that ***"far-field" currents are little relevant to dispersal of the early (and possibly passive) stages of the larval period and that small scale circulation have stronger impact on this phase.***

The large scale currents traditionally viewed as the transport mechanisms from source to sink populations operate many times at a large distance from shore. Oceanographic conditions change greatly with proximity from shore where several particular characteristics can affect the retention or the transport of larvae at a smaller scale (Pineda 2000; Cowen 2002; Leis 2002; Largier 2003). Some of these factors, acting over scales of meters to kilometres, can have a "buffering" effect, by increasing the retention time of water masses nearshore (Leis 2002).

At the Arrábida Marine Park several physical factors occurring nearshore could contribute to the retention of larval stages. As it was already discussed, the abundance of larvae from pelagic eggs at very nearshore waters could be indicative that such mechanisms may be operating. Although at the present little is know on the exact factors that could affect retention, one can speculate about their possible influence.

Tidal currents are present at an alongshore direction in our study area (E.J. Gonçalves, personal communication). These can interact with the irregular shallow bottom topography (Leis 1991a; Pineda 2000; Sponaugle *et al*. 2002) creating micro-circulation patterns around the rocky habitats with different flow layers, to which larvae could react (see Breitburg *et al.* 1995). Also the "coastal boundary layer" (*sensu* Largier 2003) can slow water movement. In particular, at the "benthic boundary layer" flow may be reduced to nearly zero (Breitburg *et al*. 1995). If this is the case at the Arrábida

Marine Park, staying near the bottom could be a good energy saving mechanism of avoiding dispersal.

Several studies have proved the influence of upwelling events over larval distribution (Olivar 1990; Pitts 1999; Reiss and McConaugha 1999; Bjorkstedt *et al*. 2002). The summer upwelling can occur at very shallow waters very close to the Arrábida Marine Park (Fiúza 1984). The patterns of larval dispersal associated to these upwelling events are not known, but they probably influence offshore displacement of larvae that are positioned at the outer border of the upwelling front. However, upwelling areas are enriched in nutrients and are known to retain larval fishes, affecting their distribution (Bjorkstedt *et al*. 2002). The relationship between the intensity of the summer upwelling in fertilization of nearshore waters is not known, but it can be speculated that inter-annual differences in the patterns of larval distribution, growth and mortality must be influenced by this factor. Wind forcing can strongly influence flow patterns (Voss and Hinrichsen 2003) and cause disturbance of phytoplankton and zooplankton communities, affecting both the transport and the survival of planktivorous species (Nakata *et al*. 2000; Wilson and Meekan 2001; Voss and Hinrichsen 2003). However, the interaction with the shallow bottoms of the Arrábida Marine Park could modulate wind forcing, reducing water displacement (Largier 2003). The fact that the shore at the Arrábida Marine Park faces south and the prevailing wind direction is from North, can originate upwelling relaxation events at the area. Evidence of warmer waters in the region than in the surrounding coastal area has been registered leading to a retention of phytoplankton nearshore (Moita *et al*. 2003). This effect has been demonstrated to potentially concentrate planktonic organisms nearshore (Graham *et al*. 1992 *in* Cowen 2002; Largier 2003; Marín *et al.* 2003; Roughan 2005a,b).

The influence of the lower Sado estuary over the Arrábida Marine Park is also not studied. However, alongshore tidal currents can facilitate larval transport. Therefore, although alongshore spatial homogeneity was found at the Arrábida Marine Park (I**I**), the possible larval exchange between these two environments cannot be excluded. The presence of larval stages of *Engraulis encrasicolus* at the Arrábida Marine Park, as already discussed, can be indicative of such possible larval flux. The influence of the vertical eddies that form at the estuary mouth (Martins *et al*. 2001, 2002) over larval retention should also be investigated in the future.

For winter species, saline fronts from the estuarine flow of the Sado river interacting with coastal waters could promote larval retention or transport. Riverine plumes or their associated fronts can be important mechanisms of dispersal/ retention of larval fishes (Grimes and Kingsford 1996; Thorrold and McKinnon 1995; Reiss and McConaugha 1999).

Internal waves are known to occur at Setúbal bay (Small and Dovey 1999). They are formed through the interaction between tidal flow and shelf edges (Cowen 2002) and are propagated towards the shore, being a possible way of transporting organisms (Lamb 1997). Internal bores can generate through the breaking of internal waves (Leichter 1998; Lennert-Cody and Franks 1999) and can also encompass the cross-shelf transport of planktonic organisms (Pineda 1991, 1994, 1999; Leichter 1998).

The extent to which some or all these factors can affect larval distribution patterns at the Arrábida Marine Park is not studied. However, the existence of such predictable oceanographic features together with the relative isolation between local populations from the nearest rocky bottom habitats, make the overall conditions affecting the Arrábida Marine Park potentially favourable to retention.

A third assumption of the "Emerging View" is that ***at ecological relevant scales populations can be more "closed" than "opened"***

When considering biogeographical spatial scales, all the populations are "closed". On the other hand, at a meter scale, all populations are "open" (Leis 2002); therefore, the extent to which dispersal or retention can occur is scale-dependent. Patterns of dispersal are also species specific and variable depending on the location considered (Leis 1991a; Cowen *et al*. 2000; Cowen 2002; Leis 2002; Mora and Sale 2002; Sponaugle *et al*. 2002; Kritzer and Sale 2004). At small ecological scales, populations can present some degree of self – recruitment. In fact, evidence is growing for coral reef species, that self- recruitment can be high in certain populations. Evidence of larval retention is based on tagging studies (e.g. Jones *et al*. 1999, 2005; Swearer *et al*. 1999), modelling studies that consider larval biophysical interactions (Paris and Cowen, 2004) or studies that show the genetic isolation between populations (Planes *et al*. 1998 a, b; Taylor and Hellberg 2003; see also reviews by Cowen, 2002; Leis and McCormick, 2002; Sponaugle *et al*. 2002; Swearer *et al*. 2002).

The last assumption of Leis' "Emerging view" is that ***"long-distance dispersal may not be relevant to ecological and management questions"***

Genetic similarities between populations may be indicative of connectivity between them and these results led researchers to consider populations as "open" (see reviews of genetic studies by Planes 1998 a, 2002 and Bonhomme and Planes 2000). However, a reduced number of migrants between populations per generation may be sufficient to maintain genetic connectivity between populations (Planes 2002, Swearer *et al*. 2002). Considering relevant ecological scales, the degree of self-recruitment may have strong impact on local population dynamics (Cowen 2002, Leis 2002, 2003). In

this sense, and considering the spatial scale investigated in the present study, some species living at the Arrábida Marine Park may present some degree of retention nearshore, being potentially more "self-seeded" than previously considered. If this is the case, retention can have impact on local recruitment and, indirectly, over the adult populations living at the Arrábida Marine Park (considering that at least to some extent larval supply influences the patterns of recruitment). If self-recruitment occurs, this must be considered at relevant scales in management options (that must act at the population level).

Different species living at the same location, can present different degrees of dispersal. Considering the Arrábida Marine Park and the local scale investigated, the observed distributional patterns (II, III, IV) must depend on the interaction between specific larval behaviours and the local oceanographic conditions. In Figure 1 the biological and physical factors that can potentially affect larval retention patterns at the Arrábida Marine Park are shown. The degree of self-recruitment must be different from species to species, depending on several biological factors including adult behaviour, temporal and spatial patterns of spawning, fecundity, mode of spawning or egg size (Leis 1991a, 2002; Sponaugle and Cowen 1997; Trippel *et al*. 1997). Size at hatching and larval growth patterns can also influence larval condition and mortality (Searcy and Sponaugle 2000; Bergenius *et al.* 2002, 2005; Raventós and Macpherson 2005). Pelagic larval duration, morphology and sensory abilities determine the patterns of larval behaviour and the ontogeny of such behaviours must explain much of the differences found between species (Leis 1991a, 2002, 2003; Cowen 2002; Leis and McCormick 2002; Sponaugle *et al*. 2002).

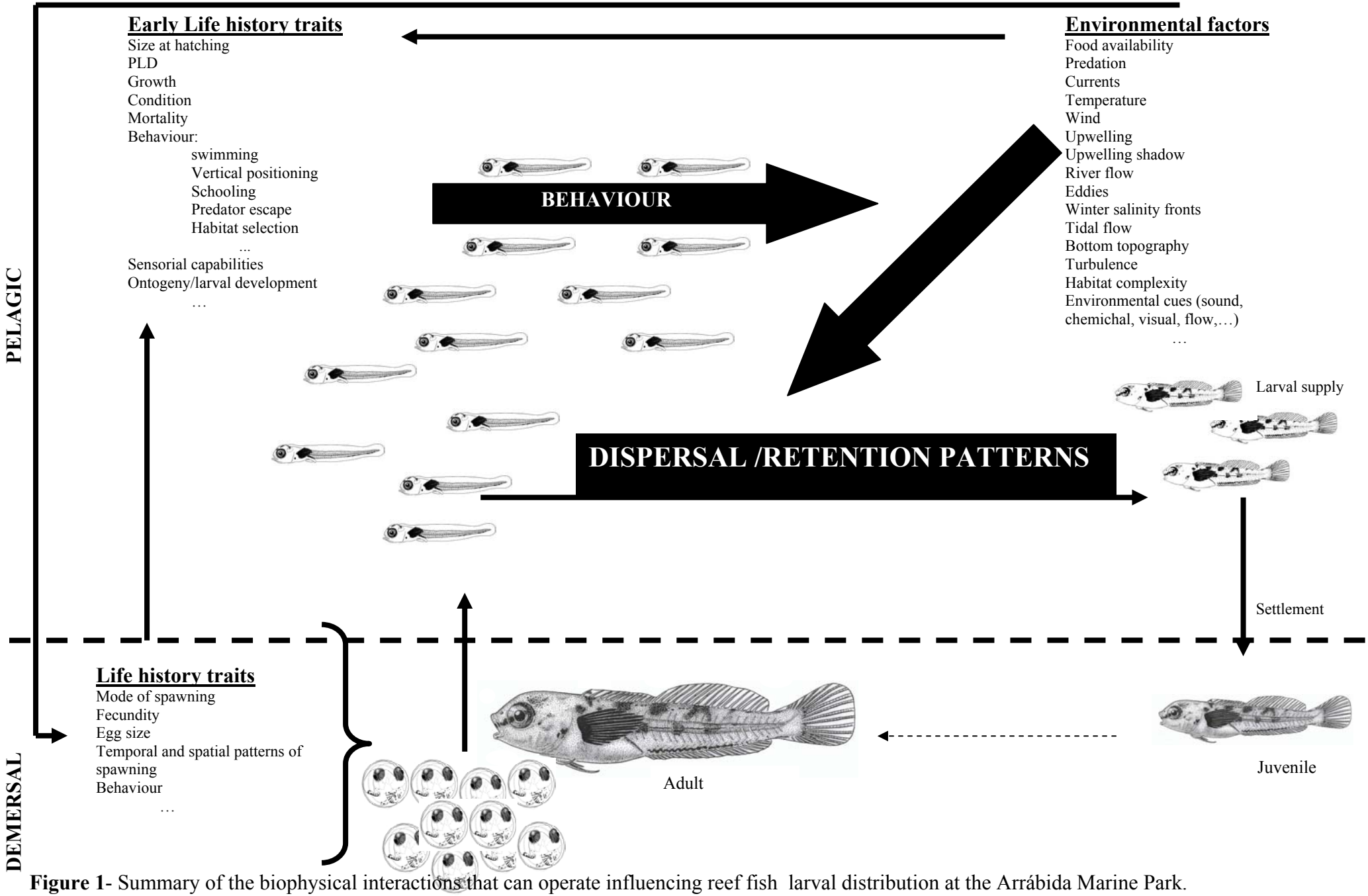

Figure 1- Summary of the biophysical interactions that can operate influencing reef fish larval distribution at the Arrábida Marine Park.

Even when small scales are considered, if the patterns of retention are influenced by biophysical interactions, variability is expected. The ontogeny of larval sensory systems and behaviour is flexible (Myrberg and Fuiman 2002); as already discussed, larval PLD, growth and condition also vary depending on the species, adult's biology and on the environmental conditions. In turn, the physical oceanographic conditions are variable in time and space. Therefore, the same factors acting in different locations can promote different patterns of larval distribution. So it is expected temporal and spatial variability of the distributional patterns of a certain species. This variability makes the patterns species and locally- specific and some careful should be taken with comparisons and generalizations from models.

8. Final Remarks: Relevance of this study at the Arrábida Marine Park

The Arrábida Marine Park is an area of major ecological importance given its high biodiversity (Almada *et al.* 1999; Henriques *et al.* 1999; Gonçalves et al., 2003). Several of the species that live and reproduce locally have strong commercial value and have been subject to a high level of fishing pressure. On the other hand it is a location with great pressure from tourism and leisure activities, mainly during the spring and summer period (when larval diversity is higher). Given these conflicting interests, protective measures and the management of the protected area must be based on reasonable scientific findings. The present study gives some contribute to understand the basic patterns of larval production. From our results, the Arrábida Marine Park is not just a spawning location for several nearshore species but also for species that can live along the shelf (e.g. sardine). It also seems to function as a nursery area for some of

those species. Local protection will improve larval supply, given that more (and probably bigger) adults will be spawning and hence producing more larvae.

Understanding the temporal and spatial patterns of larval supply, larval retention mechanisms and their relation with the recruitment patterns is of major importance to determine the number and size of the protected areas (Cowen *et al*. 2000; Planes 2000; Stobutzki 2001; Mora and Sale 2002; Shanks *et al.* 2003; Miller and Shanks 2004). For most of the species that are usually locally fished at the Arrábida Marine Park (e.g. sparids, sardine, mullets) there was no evidence of retention patterns, at least for the early stages. If this is true, protective measurements at the Arrábida Marine Park can enhance the export of larvae to other fished populations, as assumed by the metapopulation models (Planes *et al.* 2000; Armsworth 2002; James *et al*. 2002; Kritzer and Sale 2004). For some other commercial and non-commercial species, self-recruitment can occur, and those populations can be more "closed" than expected. Understanding the extent to which the local populations are self seeded, will be necessary to determine the management options. In more closed populations, local measures can contribute to enhance local recruitment (Planes *et al.* 2000;Leis 2003; Jones *et al.* 2005).

Whether considering that for some species local populations are open and others more closed, given the small geographic dimension of the Arrábida Marine Park, protection measures are needed in all its extent. The degree of self-recruitment within the scale of the Arrábida Marine Park is not known and should be investigated in future studies.

9. Future directions

This was the first study dealing with the composition, temporal variation and distribution of larval assemblages at the Arrábida Marine Park. The results identified highly diverse larval assemblages. Their temporal variability seemed to reflect the adults spawning patterns, making the study of these larval assemblages a good way of monitoring the adult populations.

Different spatial patterns of horizontal and vertical distribution were identified for different species. These patterns could be indicative of larval retention for some species and of more dispersive patterns for others. The different patterns found were not explained only considering the spawning mode or PLD of the different species, indicating that other factors must be acting. From the present study many aspects remain to be clarified, and this first approach could be the baseline for future research:

Larval behaviour probably has a strong influence over the distinct distributional patterns obtained for the different species. Therefore, future investigations should include the study of the swimming and sensory capabilities of reared larvae or of larvae captured in the plankton (using for example light traps). This should be accompanied by the study of larval sensorial structures and of the environmental cues underlying behavioural patterns. Investigation should be directed to the study of the ontogenetic development of those behaviours and sensorial structures.

Understanding how the larvae of the different species develop and interact with the environment will be determinant to explain the apparent different patterns of dispersal among species. In the future a strategy of focusing studies on target species should be adopted. Species that seem to be retained as *P. pictus* or *G. xanthocephalus* could be used. Comparisons with other subtidal species (e.g. *P. pilicornis*) with similar life history cycles but with possible opposite dispersal patterns would be interesting.

Gobiesocidae larvae should also be investigated in order to understand their developmental and distributional patterns. In particular, inter-specific comparisons on the ontogeny of behaviours and sensorial structures should be calibrated considering Fuiman's ontogenetic index (Fuiman 1994; Ditty *et al.* 2003).

Biophysical research should also be encouraged in order to understand the mechanisms underlying dispersal and/or retention patterns. Future studies should incorporate the patterns of microcirculation at the nearshore environment and investigate the other physical mechanisms discussed.

The use of oblique tows, light traps or artificial substrata devices at several distances from shore should be considered, in order to provide more realistic information about the offshore larval assemblages, and to catch more developed larvae.

The tidal influence on larval distribution should also be investigated, in order to understand if the apparent higher abundances of larval fishes at low and ebbing tides (**IV**) reflect a higher concentration of larvae in a smaller water column, or if they reflect active behavioural patterns in response to the tidal flows.

Analysis of the otolith microstructure of late stage larvae of those species with apparent different patterns of dispersal, could be a good way to investigate early life history traits that can influence larval distribution (such as PLD, growth, size at hatching). Otolith elemental analysis could also provide a better understanding of the environment where fish grow during their pelagic phase.

It must be considered that the results of the present study only reflect the diurnal patterns of composition, abundance and distribution of larval fishes at the Arrábida Marine Park. Future studies should investigate diel cycles of occurrence of larval fishes both at the surface and at the bottom.

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