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Marta Andreia Duarte Dias

Dissertação de Mestrado

Mestrado em Ecologia Marinha

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Henrique Cabral e pela Doutora Catarina Vinagre

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1. Resumo

As poças de maré, em zonas rochosas, são um importante refúgio e áreas de viveiro para as larvas e juvenis de peixes de várias espécies com interesse comercial. Os objectivos deste trabalho foram: 1) determinar a diversidade de espécies de larvas e juvenis de peixes marinhos que usam as poças de maré, em zonas rochosas, localizadas a oeste da costa portuguesa, 2) a sazonalidade do uso destes habitats por estas espécies, 3) a condição desses indivíduos através da determinação do índice *K* de Fulton, 4) a estrutura de tamanhos ao longo do ano, 5) a composição da dieta das larvas e juvenis dos peixes marinhos transientes mais abundantes, *Diplodus sargus* e *Atherina* spp., e o seu papel trófico nestes habitats. Para este estudo, foram seleccionadas quatro poças de maré em zona rochosa. Estas poças localizam-se em duas praias rochosas expostas (praia A e praia B) na costa ocidental Portuguesa. Cada poça foi caracterizada em termos de profundidade e área. As amostragens foram realizadas mensalmente, durante dois anos, de Janeiro de 2011 a Dezembro de 2012, na primeira baixa-mar do mês, com uma altura de cerca de 0,7 m, sempre em período de marés-vivas. A temperatura da água de cada poça de maré foi registada, bem como se procedeu à caracterização do substrato e formas de vida dominantes. As larvas e juvenis dos peixes marinhos transientes foram capturados utilizando camaroeiros de malha fina. Os peixes capturados foram colocados em sacos de plástico devidamente identificados e transportados numa pequena arca congeladora para o laboratório onde foram armazenados à temperatura de -20 °C. Os procedimentos laboratoriais incluíram a medição das larvas e dos juvenis dos peixes capturados com precisão de 1 mm, a sua pesagem com precisão de 0,01 g, excisão dos estômagos das larvas e juvenis,

preservação em álcool a 70% e identificação dos conteúdos estomacais. O estágio de desenvolvimento das larvas pós-flexão das espécies de peixes transientes foi classificado como larva *a, b, c, d* e *e* para *D. sargus* e larva *a, b* e *c*, para *Atherina* spp.. Os procedimentos analíticos incluíram o cálculo de índices alimentares (índice numérico, índice volumétrico e índice de ocorrência) e do índice de condição *K* de Fulton.

Neste estudo foram identificadas 6 espécies de peixes transientes que utilizam as poças de maré das zonas rochosas: o sargo legítimo, *Diplodus sargus*, o peixe-rei, *Atherina* spp., a taínha, *Liza ramada*, o bodião de Bailloni, *Symphodus bailloni*, o sargo-veado, *Diplodus cervinus* e a sardinha Europeia, *Sardina pilchardus*. Uma elevada variabilidade inter-anual, tanto nas densidades, como na altura de colonização das poças, foi observada. Enquanto que em 2011 as poças foram colonizadas em Março, em 2012 apenas foram colonizadas em Maio. O número de meses de colonização também foi inferior em 2012. A colonização das poças, tanto por *D. sargus*, como por *Atherina* spp. começou na Primavera e terminou no Outono. As densidades mais elevadas foram registadas para *D. sargus* em todas as poças, seguidas das de *Atherina* spp. com a excepção da poça 2 da praia B onde *Atherina* spp. estiveram ausentes. O número de espécies que colonizou as poças de maré variou entre dois (*D. sargus* e *Atherina* spp.) na poça 1 da praia A e cinco, tanto na poça 2 da praia A (*D. sargus*, *Atherina* spp., *L. ramada*, *S. bailloni*, *D. cervinus*), como na poça 1 da praia B (*D. sargus*, *Atherina* spp., *L. ramada*, *D. cervinus* and *S. pilchardus*). Os picos de densidade de *D. sargus* foram mais pronunciados na primavera enquanto que os de *Atherina* spp. foram mais pronunciados no Verão. Os picos de densidade foram ainda mais elevados em 2011 do que em 2012, quer para *D. sargus*, quer para *Atherina* spp. em todas as poças e meses. As espécies transientes raras (*L. ramada*, *S. bailloni*, *D. cervinus* e *S. pilchardus*) estiveram presentes principalmente na Primavera-Verão, com uma grande variabilidade entre poças, e num maior número de meses em 2012 do que em 2011. As larvas pós-flexão de *D. sargus* colonizaram as poças em Março e Abril de 2011 e em Maio de 2012, no primeiro estágio (larva *a*), os juvenis apareceram em Junho, em ambos os anos. *Atherina* spp., nas poças 1 e 2 da praia A, apareceram pela primeira vez no segundo estágio de desenvolvimento (larva *b*), enquanto que na poça 1 da praia B todos os estágios de desenvolvimento estiveram presentes, os juvenis começaram a aparecer em Junho de 2011 e Agosto de 2012. A condição de *D. sargus* aumentou ao longo do ano, em ambos os anos, com excepção da poça 2 da praia B. Para *Atherina* spp. a condição manteve-se na poça 2 da praia A e decresceu na poça 1 da praia B. O tamanho médio de *D. sargus* e *Atherina* spp. mostrou frequentemente um aumento contínuo da Primavera para o Outono, em ambos os anos.

Relativamente à dieta de *D. sargus*, esta apresenta uma grande variabilidade de grupos de presas: algas, cnidários, platielmintes, entoproctas, anelídeos, crustáceos, insectos, acarídeos, moluscos e teleósteos. Por outro lado, *Atherina* spp. consumiram uma menor diversidade de presas que *D. sargus*, as suas presas incluem apenas crustáceos, insectos, acarídeos e moluscos. Sendo, tanto *D. sargus* como *Atherina* spp., consideradas espécies generalistas.

A dieta das larvas *a* e *b* de *D. sargus* é principalmente constituída por copépodes harpacticoides, larvas de insecto da família Chironomidae e ostracodes; nas larvas *c* e nos juvenis os ostracodes foram substituídos, em termos de importância na dieta, por anfípodes e nas larvas *d* e *e* por insectos adultos da família Chironomidae.

A dieta das larvas *a*, *b* e *c* e dos juvenis de *Atherina* spp. é principalmente constituída por copépodes harpacticoides, ostracodes e gastrópodes. Foram detectadas diferenças estatisticamente significativas entre as larvas e adultos de *D. sargus*. À medida que as larvas de *D. sargus* se desenvolvem, a proporção de copépodes harpacticoides na sua dieta diminui, sendo mínima nos juvenis. Além disso, os juvenis de *D. sargus* alimentam-se de algas, sendo *D. sargus* considerado uma espécie omnívora. Por outro lado, *Atherina* spp. não se alimentam de algas em nenhuma fase do seu desenvolvimento. Anfípodes e isópodes só começam a aparecer na dieta de *D. sargus* e *Atherina* spp. no segundo e terceiro estágio do desenvolvimento larvar, respectivamente. Este resultado pode estar relacionado com a dimensão destas presas relativamente ao tamanho das larvas destas espécies nos primeiros estágios de desenvolvimento. Ao longo do desenvolvimento ontogénico, tanto *D. sargus* como *Atherina* spp., consomem presas de maiores dimensões, o que pode estar relacionado com um aumento das necessidades energéticas. Os juvenis de *D. sargus* apresentam uma maior diversidade de itens alimentares que os estágios de desenvolvimento anteriores, incluindo, para além das algas, o isópode *Cyathura* sp., o quíton *Lepidochitona cinerea* (Linnaeus, 1767) e poliquetas. *D. sargus* e *Atherina* spp. são espécies oportunistas, como algumas espécies de peixes residentes nas poças de maré, tirando proveito da elevada disponibilidade de presas do ambiente em que vivem. Neste trabalho foi possível concluir que as espécies de peixes transientes *D. sargus* e *Atherina* spp. provavelmente utilizam as poças de maré de costas rochosas como zona de viveiro, dado as elevadas densidades das larvas e juvenis destes indivíduos nos meses mais favoráveis em termos de temperatura e de disponibilidade de alimento. Estudos futuros permitirão avaliar a importância da contribuição dos peixes que

ocorrem nestes habitats durante a fase de pós-larva e juvenil para os mananciais de adultos das respectivas espécies.

2. Summary

Transient marine fishes were sampled monthly in four tidal pools of a rocky reef, for two years, in the west Portuguese coast. Species diversity was higher than that found in previous studies in other parts of the world. The transient fish population was comprised of six species: *Diplodus sargus*, *Atherina* spp., *Liza ramada*, *Symphodus bailloni*, *Diplodus cervinus* and *Sardina pilchardus.* Fish abundance varied seasonally, between years and among pools, with peak numbers in spring and summer. *D. sargus* and *Atherina* spp. were present in most pools, from spring to autumn, with their size often increasing along this period, in both years. Rare species were present mostly in spring-summer. In most pools, the overall condition of *D. sargus* increased throughout the year, in both years; whereas in *Atherina* spp. it was maintained or decreased. The diet composition of larvae and juveniles of *D. sargus* and *Atherina* spp., was investigated. *D. sargus* consumed Algae, Cnidaria, Platyhelminthes, Entoprocta, Annelida, Crustacea, Insecta, Acari, Mollusca and Teleostei. *Atherina* spp. only consumed Crustacea, Insecta, Acari and Mollusca. The diet of *D. sargus* was composed mainly of harpacticoid copepods, chironomid larvae, ostracods, chironomid adults and amphipods; whereas the diet of *Atherina* spp. was composed mainly of Harpacticoid copepods, ostracods and gastropods. Along the ontogenetic development, the proportions and diversity of food items in the diet of *D. sargus* varied. The importance of harpacticoid copepods decreased with increasing size, while the importance of

amphipods and isopods increased. The trophic role of larvae and juveniles of *D. sargus* and *Atherina* spp. occurring in tidal pools of rocky reefs is that of opportunistic consumers. The species richness, the high densities of larvae and juveniles, their continuous growth and the food items consumed, strongly emphasize the importance of these environments for larvae and juveniles of *D. sargus* and *Atherina* spp..

3. General introduction

Rocky intertidal communities are very valuable as a model system for the study of the mechanisms that generate community patterns and structure, since intertidal organisms are easily manipulated and the access to this habitat is easily granted (Dayton, 1971; Paine, 1974; Menge, 1976, 1995; Lubchenco, 1978).

Plants and animals inhabiting the shores are closely related to fully marine species. For them, the environmental gradient formed between low water and the limit of the influence of the sea in the splash zone represents stress conditions (Raffaelli and Hawkins, 1996). Organisms experiment air temperatures and relative humidity more variable than those in the sea; also precipitation and evaporation can affect the salinity of rock pools and water on the rock surface (Raffaelli and Hawkins, 1996). Since aquatic organisms require some degree of moisture, the most important stress in temperate and tropical areas is desiccation due to the combination of heat and low relative humidity. In polar and boreal regions, extreme cold is a major problem at high shore levels (Raffaelli and Hawkins, 1996). At increasingly higher shore levels, marine organisms spend increasingly longer periods of time emmersed, being exposed to harsh terrestrial conditions of extreme temperatures and water loss (Bertness and

Leonard, 1997). The shore represents a unidirectional vertical stress gradient for most marine animals, referred commonly as the intertidal gradient. Along this gradient, species are ordered according to their responses to physical processes (e.g. heat, desiccation) and variation in their responses to biological processes (e.g. competition and predation) (Lewis, 1964; Connell, 1972; Carefoot, 1977; Paine and Levin, 1981; Menge and Sutherland 1987; Denny, 1988; Little and Kitching, 1996). This ordering is reflected in the zonation patterns observed on rocky shores. On these habitats, the biological features like habitat structure and complexity, biological productivity, species diversity and the intensity of biological interactions increase towards the lower shore, being environments rich in a variety of species. (Underwood, 1996, 2000; Zander et al., 1999). A universal classification scheme for classifying the zones of all rocky shores was suggested by Stephenson and Stephenson (1972) and it has been used nearly everywhere (Southward, 1958; Lipkin and Safriel, 1971)**.** In this scheme, shore is divided into three major zones:

- a high-shore area, the supralittoral fringe, characterized by encrusting lichens, Cyanobacteria (bluegreen bacteria) and small snails, the periwinkles;
- a broad midshore zone, midlittoral zone, dominated by suspension-feeding barnacles, mussels or sometimes oysters;
- a narrower low-shore zone, the infralittoral fringe, dominated by red algae including pink, calcareous, encrusting forms, often with large kelps (brown seaweeds), or in some places in the Southern Hemisphere, large filter-feeding tunicates (sea squirts).

In the Portuguese coast, the supralittoral fringe is dominated by lichen *Verrucaria maura* Wahlenberg, 1803 and the gastropod *Melaraphe neritoids* (Linnaeus, 1758), with the exceptions of some shores like Avencas and Meco where *V. maura* may be absent (Boaventura et al., 2002). The grey upper zone is composed of cyanobacteria and *M. neritoides*, however, it can be dominated by green algae like *Enteromorpha* sp. if near to a freshwater runoff (Boaventura et al., 2002). The midlittoral zone is dominated by sessile filter feeders such as barnacles and mussels. The barnacle *Chthamalus montagui* Southward, 1976 dominates on the upper mid-shore zone where lichen *Lichina pygmaea* (O.F.Müller) C. Agardh, 1820 is also present (Boaventura et al., 2002). Mussels occur in the lower mid-shore zone of more exposed shores. *Xantho incisus* Leach, 1814, *Carcinus maenas* (Linnaeus, 1758), *Necora puber* (Linnaeus, 1767), *Pirimela denticulata* (Montagu, 1808) and *Pilumnus hirtellus* (Linnaeus, 1761) can be observed in the lower zone of the central Portuguese coast; *Pachygrapsus marmoratus* (Fabricius, 1787) and *Eriphia verrucosa* (Forskål, 1775) exploit all the intertidal range (Flores and Paula, 2001). Algae like *Corallina* spp., *Caulacanthus ustulatus* (Mertens ex Turner) Kützing, 1843, *Mastocarpus stellatus* (Stackhouse) Guiry, 1984 and *Lithophyllum lichenoides* Philippi, 1837 can occur together with mussels. Throughout the midlittoral zone, gastropod species like *Littorina saxatilis* (Olivi, 1792)*, Phorcus lineatus* (da Costa, 1778)*, Gibbula umbilicalis* (da Costa, 1778) and *Nucella lapillus* (Linnaeus, 1758) are common. Amongst grazing limpets, *Patella vulgata* Linnaeus, 1758*, Patella rustica* Linnaeus, 1758*, Patella depressa* Pennant, 1777 and *Patella ulyssiponensis* Gmelin, 1791 inhabit the midlittoral zone along the Portuguese coast (Boaventura et al., 2002).

The infralittoral fringe is occupied by an agglomeration of macroalgae. Northern shores present large Brown algae such as *Laminaria* spp., *Saccorhiza polyschides* (Lightfoot) Batters, 1902 and *Himanthalia elongata* (Linnaeus) S. F. Gray, 1821. In the infralittoral fringe of the Centre and South shores dominate *Corallina* spp., *Plocamium cartilagineum* (Linnaeus) P. S. Dixon, 1967*, C. ustulatus, Gelidium* spp. and *Laurencia pinnatifida* (Gmelin) Lamouroux. On more exposed shores, *Corallina* spp. dominate over the other algal species. The abundant animal species in the infralittoral fringe are *P. ulyssiponensis, Paracentrotus lividus* (Lamarck, 1816)*, Sabellaria alveolata* (Linnaeus, 1767) and *Pollicipes pollicipes* (Gmelin, 1790) (Boaventura et al., 2002).

The fishes inhabiting the pools formed by tide cycles on the rocky shore can be classified as either residents or transients, depending on the time spent in this environment (Gibson, 1969, 1982; Thomson and Lehner, 1976; Grossman, 1982; Mahon and Mahon, 1994; Griffiths, 2003a). Fishes that spend all their life cycle in tidepools are defined as resident and are generally small benthic fishes, such as the blennies and gobies; while the transient ones are defined as those that only spend part of their life in this environment, they are primarily infralittoral, but occur in tidepools, particularly as juveniles. (Gibson, 1982; Castellanos-Galindo et al., 2005). Generally, the pattern observed is that higher pools are dominated by resident species and lower pools are occupied by a greater proportion of transient species (Castellanos-Galindo et al., 2005). Rocky tidal pool fish communities have been widely investigated in many parts of the world. In Brasil, the principal resident species are *Bathygobius soporator* (Valenciennes, 1837) (Gobiidae), *Parablennius pilicornis* (Cuvier, 1829) (Blenniidae), *Gobiesox barbatulus* Starks, 1913 (Gobiesocidae) and *Labrisomus nuchipinnis* (Quoy and Gaimard, 1824) (Labrisomidae), while the transient species are *Abudefduf saxatilis*

(Linnaeus, 1758) (Pomacentridae), *Epinephelus marginatus* (Lowe, 1834) (Serranidae) and *Diplodus argenteus* (Valenciennes, 1830) (Sparidae) (Barreiros et al., 2004).

In South Africa, Cryptic Tripterygiidae, Clinidae, Blenniidae, Gobiidae, Acanthuridae and Gobiesocidae represent the resident families, while Pomacentridae, Serranidae, Sparidae, Labridae, Cheilodactylidae and Mugilidae represent the transient families (Beckley, 1985, 2000).

In Colombia, the resident fish include Gobiidae (*Bathygobius ramosus* Ginsburg, 1947), Labrisomidae (*Malacoctenus zonifer* (Jordan and Gilbert, 1882)) and Gobiesocidae (*Gobiesox adustus* Jordan and Gilbert, 1882) and the transient fish include Pomacentridae (*Abudefduf concolor* (Gill, 1862), *Abudefduf troschelii* (Gill, 1862), *Microspathodon bairdii* (Gill, 1862)), Mugilidae (*Chaenomugil proboscideus* (Günther, 1861)), Labridae (*Halichoeres aestuaricola* Bussing, 1972) and Muraenidae (*Echidna nocturna* (Cope, 1872)) (Castellanos-Galindo et al., 2005).

The fish community in Australia is mainly composed of resident fish from the families Clinidae, Blenniidae, Tripterygiidae, Gobiidae, Gobiesocidae and Girellidae, the transient families are Kuhliidae, Apogonidae, Scatophagidae, Chaetodontidae and Pleuronectidae (Griffiths, 2003a, b).

In India, Blenniidae and Gobiidae represent the resident fishes, more abundant in the pools, and Mugilidae (*Mugil cephalus* Linnaeus, 1758) and Terapontidae (*Terapon jarbua* (Forsskål, 1775)) represent the transient fishes (Tsering et al., 2012).

In Japan, Gobiidae, Blenniidae, Pomacentridae, Labridae and Tripterygiidae are the dominant families, Gobiidae and Blenniidae include both resident and transient fish, while Pomacentridae (*Abudefduf sordidus* (Forsskål, 1775), *Abudefduf notatus* (Day, 1870), *Plectroglyphidodon leucozonus* (Bleeker, 1859)), Labridae (*Thalassoma cupido*

(Temminck and Schlegel, 1845), *Thalassoma purpureum* (Forsskål, 1775)), and Tripterygiidae (*Enneapterygius philippinus* (Peters, 1868), *Enneapterygius etheostoma* (Jordan and Snyder, 1902)) only include transient fish. Other families that include transient fishes are the Gobiesocidae (*Pherallodus indicus* (Weber, 1913)), Kyphosidae (*Girella leonina* (Richardson, 1846), *Girella mezina* Jordan and Starks, 1907), Kuhliidae (*Kuhlia mugil* (Forster, 1801)) and Syngnathidae (*Choeroichthys sculptus* (Günther, 1870)) but are less represented families (Murase, 2013).

In California, Stichaeidae *(Anoplarchus purpurescens* Gill, 1861*, Xiphister atropurpureus* (Kittlitz, 1858)) and Gobiesocidae (*Gobiesox maeandricus* (Girard, 1858)) represent the resident fish families. Cottidae comprises both resident and transient fish (*Oligocottus maculosus* Girard, 1856 and *Oligocottus snyderi* Greeley, 1898 as resident species and *Hemilepidotus spinosus* Ayres, 1854 as a transient species). Other families that include transient fish are Paralichthyidae (*Citharichthys stigmaeus* Jordan and Gilbert, 1882), Hexagrammidae (*Hexagrammos decagrammus* (Pallas, 1810), *Hexagrammos lagocephalus* (Pallas, 1810)), Scorpaenidae (*Sebastes melanops* Girard, 1856, *Sebastes mystinus* (Jordan and Gilbert, 1881)) and Liparidae (*Liparis florae* (Jordan and Starks, 1895)) (Grossman, 1982; Moring, 1986).

In New Zealand, Tripterygiidae (*Bellapiscis medius* (Günther, 1861), *Forsterygion lapillum* Hardy, 1989) and Gobiesocidae (*Trachelochismus pinnulatus* (Forster, 1801), *Gastroscyphus hectoris* (Günther, 1876)) represent the resident fishes and Labridae (*Notolabrus celidotus* (Bloch and Schneider, 1801)) represent the transient ones (Willis and Roberts, 1996).

In Azores, Blenniidae (*Parablennius ruber* (Valenciennes, 1836), *Parablennius incognitus* (Bath, 1968), *Parablennius sanguinolentus parvicornis* (Valenciennes, 1836), *Ophioblennius atlanticus* (Valenciennes, 1836), *Coryphoblennius galerita* (Linnaeus, 1758), *Lipophrys trigloides* (Valenciennes, 1836)) constitute the dominant resident fish family in the intertidal pools, the other families of resident fish include Gobiidae (*Gobius paganellus* Linnaeus, 1758), Labridae (*Centrolabrus trutta* (Lowe, 1834)) and Gadidae (*Gaidropsarus guttatus* (Collett, 1890)); the transient fish include Carangidae (*Trachinotus ovatus* (Linnaeus, 1758)), Mugilidae (*Chelon labrosus* (Risso, 1827)), Serranidae (*Epinephelus marginatus* (Lowe, 1834)) and Sparidae (*Diplodus sargus* (Linnaeus, 1758)) (Santos et al., 1994).

In western Portugal, Blenniidae (*C. galerita, Lipophrys pholis* (Linnaeus, 1758)), Gobiidae (*Gobius cobitis* Pallas, 1814*, G. paganellus*) and Gobiesocidae (*Lepadogaster lepadogaster purpurea* (Bonnaterre, 1788)) constitute the resident fish families in the intertidal pools. Sparidae (*D. sargus*) and Atherinidae (*Atherina presbyter* Cuvier, 1829) constitute the transient fish families (Faria and Almada, 1999; Almada and Faria, 2004).

Tide pools are commonly used by various species of transient fish in their early stages of development that later move to subtidal waters as adults (Randall, 1955; Norris, 1963; Major, 1978). It is generally agreed that this happens because tide pools provide refuge from predators through structural complexity (vegetation cover, debris and substratum rugosity) (Bennett and Griffiths, 1984; Davis, 2000), favorable temperatures for growth (Gibson, 1994) and suitable food availability (Grossman, 1982).

Recent definitions of nursery areas include higher survival, faster growth, higher density and higher contribution to the adult habitats (Steves et al., 1999; Beck et al., 2001). Important efforts have been made to quantify the contribution of different marine ecosystems that function as nurseries areas, to adult populations, so that habitats which contribute most can be identified and protected (e.g. Gillanders and Kingsford, 1996; Yamashita et al., 2000; Beck et al., 2001; Gillanders, 2005, Dahlgren et al., 2006).

Alterations to the habitat quality and efficiency of nurseries may be a major cause of adult fish stock decrease (Riley et al., 1981), since the larval and juvenile phases of fishes are crucial for future recruitment to the adult stocks (e.g. Haedrich, 1983; Miller et al., 1985). This way, it is of the utmost importance to indentify and evaluate the importance of all ecosystems and habitats as nusery areas for the most important fish species. However, this is still largely unkown for tide pools of rocky reefs.

The current work is a first step in the investigation of the importance of rocky tide pools as nursery areas for fish and is composed of two scientific articles with the following titles: “Use of rocky intertidal pools as nursery areas by marine fish larvae and juveniles” and “Trophic role of marine fish larvae and juveniles that use rocky intertidal pools”.

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4. Use of rocky intertidal pools as nursery areas by marine fish larvae and juveniles

4.1 Summary

Fishes were sampled monthly, in four tidal pools, for two years, in the west Portuguese coast. Species diversity of transient fish was higher than that found in previous studies, in other parts of the world. The transient fish population comprised six species: the white seabream, *Diplodus sargus*, sand smelt, *Atherina* spp., the thinlip grey mullet, *Liza ramada*, the Baillon's wrasse, *Symphodus bailloni*, the zebra seabream, *Diplodus cervinus* and the European pilchard, *Sardina pilchardus.* Fish abundance varied seasonally, between years and among pools, with peak numbers in spring and summer. The most abundant species, both as larvae and juveniles, was *D. sargus*, in all pools. *D. sargus* and *Atherina* spp. were present in most pools, from spring to autumn. Rare species were present mostly in spring-summer. Lower mean sizes were registered in the beginning of spring of 2011 (March-April) and in the end of spring/ beginning of summer of 2012 (May-June), when pools were colonized. Mean size often showed a continuous increase from spring to autumn in both years. The highest density peaks were due to the high number of post-larvae entering the pools in spring. In most pools, the overall condition (Fulton's *K*) of *D. sargus* increased throughout the year, in both years. The overall condition of *Atherina* spp. was maintained or decreased throughout the year, in both years. The species richness, the high densities of larvae and juveniles and their continuous growth, observed in tidal pools, strongly emphasize the

importance of these environments for larvae and juveniles of several transient marine fishes.

Keywords: Rocky reefs, biodiversity, fish assemblages, habitat use, Fulton’s *K*.

4.2 Introduction

Often, marine juvenile fish enter nursery areas after metamorphosis, having been spawned somewhere else in the sea. They spend months to years in these habitats and then move to their adult ones (Randall, 1955; Norris, 1963; Major, 1978). This pattern of habitat utilization has long been recognized and it is known to occur because sheltered habitats provide advantages over the marine environment regarding protection from predators and an abundant food supply (Clark, 1974; Cushing, 1975; Lenanton, 1982; Boesch and Turner, 1984; Gibson, 1994).

Nursery habitats have also been described as being areas where growth and survival of settled juveniles are enhanced (Gibson, 1994), or areas where post-settled juvenile fish can prevail and grow prior to first spawning (Steves et al., 1999). Recent definitions of nursery areas are based on four factors: higher survival; faster growth; higher density and contribution of nurseries to the adult habitats (Steves et al., 1999; Beck et al., 2001). Important efforts have been made to quantify the contribution of different marine ecosystems that function as nurseries areas, to adult populations, so that habitats which contribute most can be identified and protected (e.g. Gillanders and Kingsford, 1996; Yamashita et al., 2000; Beck et al., 2001; Gillanders, 2005, Dahlgren et al., 2006).

Alterations to the habitat quality and efficiency of nurseries may be a major cause of adult fish stock decrease (Riley et al., 1981), since the larval and juvenile phases of fishes are crucial for future recruitment to the adult stocks (e.g. Haedrich, 1983; Miller et al., 1985).

Various coastal habitats are well known as nurseries for juvenile marine fish (Clark, 1974; Lenanton, 1982). These include estuaries (Wallace and Van Der Elst, 1975; Day et al., 1981; Wallace et al., 1984, Costa and Bruxelas, 1989; Vinagre et al., 2010), coastal lagoons (Franco et al., 2006; Abecasis et al., 2009; Ribeiro et al., 2012), salt marshes (Veiga et al., 2006), coral reefs (Nagelkerken et al., 2000), mangroves (Nagelkerken et al., 2000; Dorenbosch et al., 2004), surf zone reefs (Berry et al*.,* 1982), seagrass beds (Nagelkerken et al., 2000; Dorenbosch et al., 2004; Verweij et al., 2008) and the sandy beach surf zone (Lasiak, 1981, 1983).

Estuarine nurseries are the best documented to date, with numerous studies, from as early as the larval phase of numerous fish species (e.g. Miskiewicz, 1986; Roper, 1986; Boehlert and Mundy, 1988; Whitfield, 1994; Harris and Cyrus, 2000). Rocky tidal pools are probably the least studied nurseries for marine fish, leaving a considerable research gap worldwide.

Several studies have been developed in rocky tidal pools that include transient juvenile fish, concerning their recruitment in tidal pools of South Africa (Beckley, 2000) and New Zealand (Willis and Roberts, 1996); species diversity and seasonal changes, in Brazil (Barreiros et al., 2004), India (Tsering et al., 2012) and California (Moring, 1986); community structure, in Colombia (Castellanos-Galindo et al., 2005), Australia (Griffiths, 2003a), Azores (Santos et al., 1994), Japan (Murase, 2013), Barbados (Mahon and Mahon, 1994) and Brazil (Cunha et al., 2008); concerning spatial and temporal dynamics in Australia (Griffiths, 2003b), Japan (Arakaki and Tokeshi, 2006), spatial and vertical patterns in Hawaii (Cox et al., 2011), re-colonization after experimental elimination, in California (Thompson and Lehner, 1976; Grossman, 1982) and in South Africa (Beckley, 1985b) and feeding guild structure in Colombia (Castellanos-Galindo and Geraldo, 2008). However, the only studies that investigated the nursery function of these habitats were carried out in South Africa (Beckley, 1985a; Bennett, 1987; Gibson and Yoshiyama, 1999).

Among the fish that use rocky tidal pools, the best studied fish are from the families Gobiidae and Blenniidae (Almada et al., 1983, 1990, 1992, 1994; Almada and Santos, 1995; Faria et al., 1996, 1998a, 1998b; Faria and Almada, 2001). However, these are resident fish. Larvae and juveniles of some marine transient fish species are often also observed in these environments (Gibson, 1982; Beckley, 1985a; Santos et al., 1994; Almada and Faria, 2004; Barreiros et al., 2004). In southern Europe, the very high densities of early forms of marine fish in rocky tidal pools are indicative that these habitats may be important nurseries for highly commercial fish species, like the white seabream *Diplodus sargus* (Linnaeus, 1758) (Garcia-Rubies, 1997).

The use of tidal pools during early ontogeny may enhance the growth, condition and survival chances of the individuals that use them (Thompson and Lehner, 1976; Moring, 1986, 1990; Mahon and Mahon, 1994; Macpherson, 1998). Refuge from larger predators is granted in tidal pools during ebb tide, because larger animals remain in the subtidal (Metaxas and Scheibling, 1993; Mahon and Mahon, 1994; Gibson, 1994). In spring and summer, water in tidal pools is warmer than in subtidal environments because they are exposed to the warmer atmospheric temperatures (Prochazka, 1996). This allows faster development of early fish stages, since temperature is a crucial variable for growth (Haedrich, 1983). Tidal pools may also be preferential feeding grounds for marine juvenile fish, since these highly structured environments harbor high biodiversity, abundant macroalgae and small invertebrates that are potential prey for juvenile fish (Beckley, 1985a; Moring, 1986; Amara and Paul, 2003; Cunha et al., 2007).

This study aims to investigate 1) the species diversity of marine fish larvae and juveniles using rocky tidal pools, in a southern European coast, 2) the seasonality of the use of these habitats by these species, 3) the condition of the individuals and 4) the size structure throughout the year.

4.3. Materials and Methods

Four rocky tidal pools were selected for this study. They were located in 2 exposed rocky beaches, distanced 1.4 km, in the central coast of Portugal (Fig. 1). Each tidal pool was characterized in terms of depth and approximate area (Table 1). These pools were studied monthly, during 2 years, from January 2011 to December 2012. They were visited every month in the first diurnal spring tide of the month, when tide height was approximately 0.7 m. Tides at this latitude are semi-diurnal. Water temperature was registered (Fig. 2), as well as the dominant substrata and biota. Whenever larvae and/or juveniles of marine fish were present, they were collected with hand nets, with a stretched mesh of 1 mm, transported to the laboratory in refrigerated bags and preserved at -20 °C.

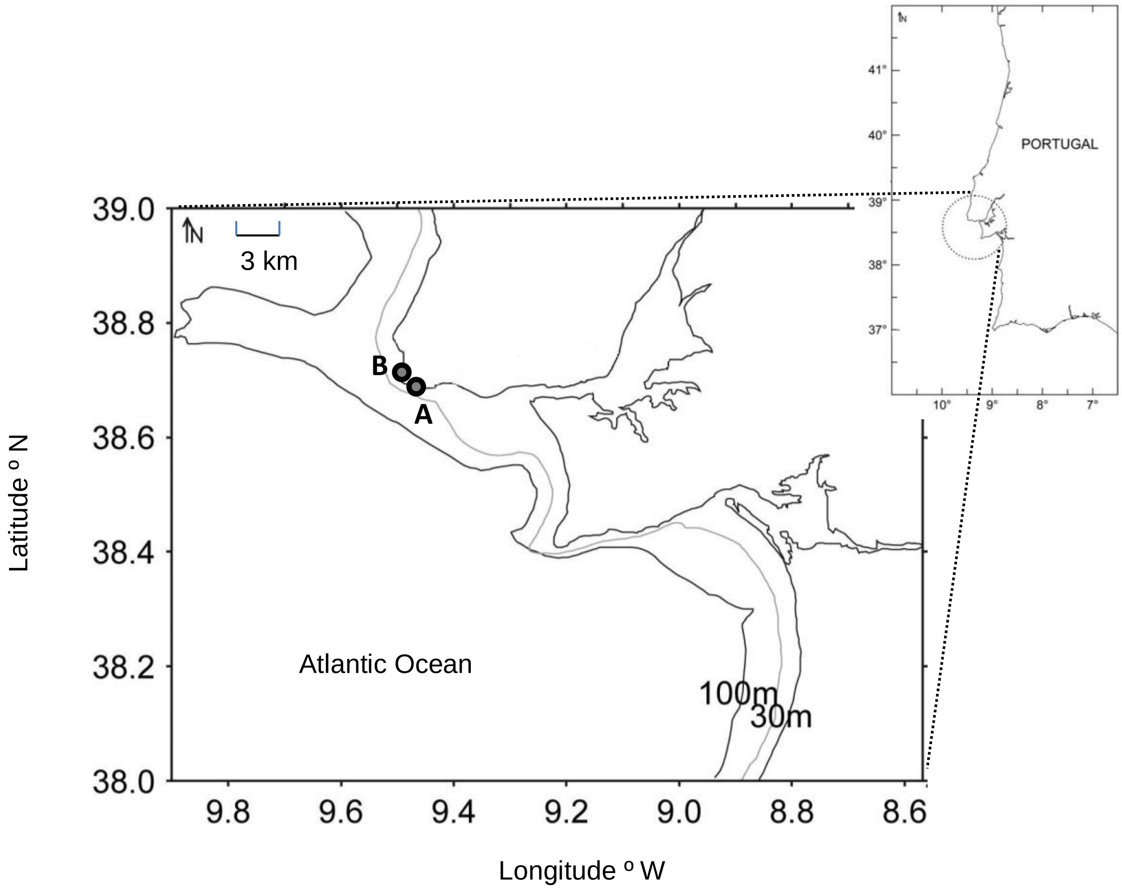


Fig. 1 – Location of the beaches (A and B) studied in the Portuguese coast.

Table 1 – Depth and approximate area of each studied tidal pool and mean densities of the transient fish species present (ind.m^{-2}). Standard deviation values of density are given in brackets

	Depth (cm)	Area (m²)	*D. sargus*	*Atherina* spp.	*L. ramada*	*S. bailloni*	*D. cervinus*	*S. pilchardus*
Beach A Pool 1	54	1.14	18.2 (24.3)	0.9 (0.0)				
Beach A Pool 2	63	1.44	21.1 (25.5)	22.9 (19.2)	2.1 (0.0)	1.4 (1.0)	1.4 (0.0)	
Beach B Pool 1	38	10.50	22.4 (52.3)	7.5 (10.7)	0.2 (0.2)		0.1 (0.0)	0.1 (0.0)
Beach B Pool 2	30	22.00	12.9 (21.0)			0.1 (0.0)	0.1 (0.0)	

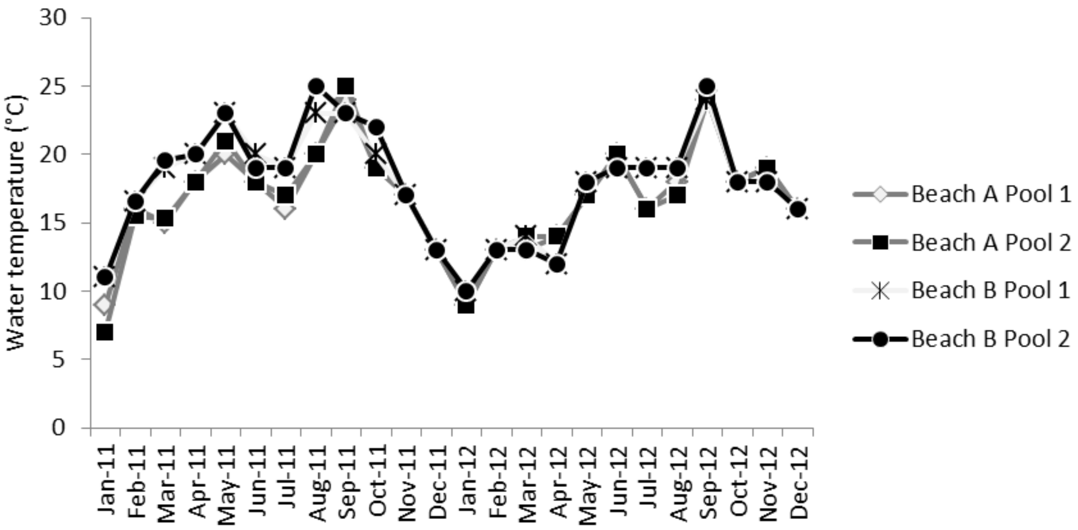


Fig. 2 – Water temperature in the pools during the sampling period.

In the laboratory, fish were identified to the species level, measured to the nearest mm and weighed with a precision of 0.1 g. The ontogenetic stage of the species was identified according to García and Moyano (1990). Post-larval stages were classified as larvae *a, b, c, d* and *e*, for *D. sargus*, and larvae *a, b* and *c*, for *Atherina* spp.. Condition was estimated based on Fulton's *K*, according to the equation:

$K = 100Mt/Lt^3$

where Mt is total wet mass (mg) and Lt is total length (mm) (Ricker, 1975).

4.4. Results

Pools' depth varied between 30 cm and 63 cm and their area ranged between 1.14 m^2 and 22.00 m^2 (Table 1). Temperature varied between 7 °C and 25 °C in 2011 (January and September, respectively) and between 9 °C and 25 °C in 2012 (January and September, respectively) (Fig. 2). The pools' algal communities were dominated by *Ellisolandia elongata* (J. Ellis and Solander) K. R. Hind and G. W. Saunders, 2013, *Phymatolithon calcareum* (Pallas) W. H. Adey and D. L. McKibbin, 1970, *Asparagopsis armata* Harvey, 1855 and *Ulva lactuca* Linnaeus, 1753.

A total of 6 species of non-resident fish were identified in the pools: the white seabream, *D. sargus*, sand smelt, *Atherina* spp., the thinlip grey mullet, *Liza ramada* (Risso, 1827), the Baillon's wrasse, *Symphodus bailloni* (Valenciennes, 1839), the zebra seabream, *Diplodus cervinus* (Lowe, 1838) and the European pilchard, *Sardina pilchardus* (Walbaum, 1792) (Table 1).

A high inter-annual variability in both densities and time of pool colonization was observed. In 2011, the fish colonized the pools earlier, in March, while in 2012 it occurred in May. In 2011 the number of months with marine fish larvae and juveniles was also longer, than in 2012. In 2011, *D. sargus*, colonized the pools in spring (March-April) and left the pools in autumn (September-November); in 2012, it colonized the pools in May-June and left in September-October. In 2011, *Atherina* spp. colonized the pools in March and left the pools in September; in 2012, colonization started in June and fish left in September (Fig. 3).

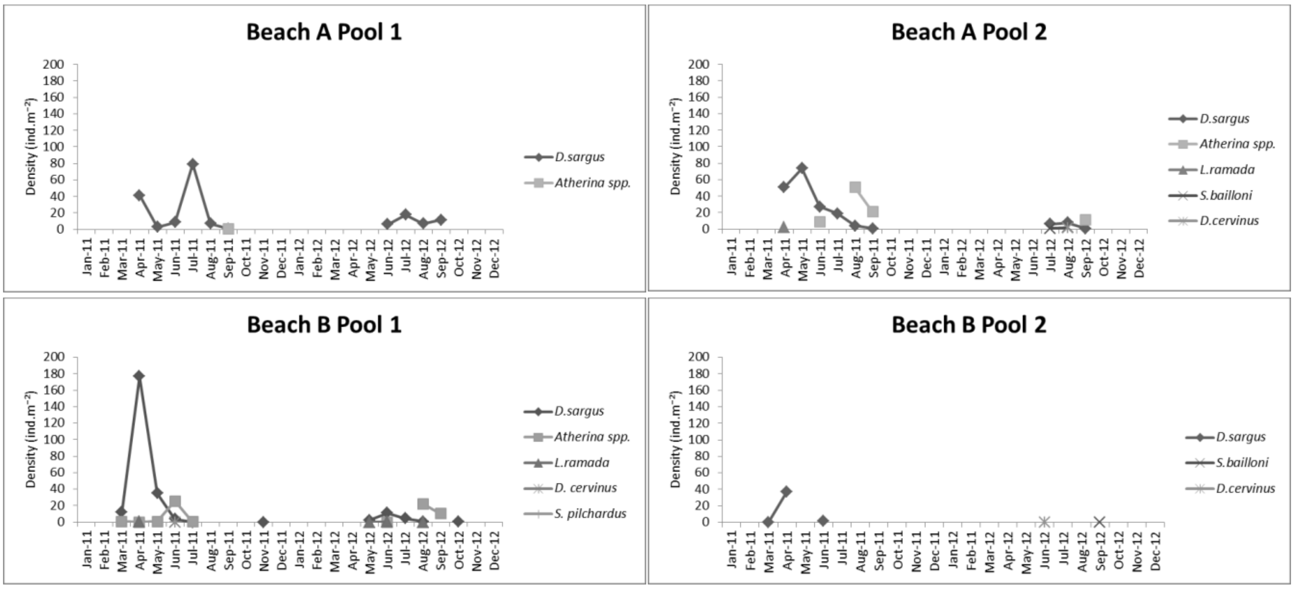


Fig. 3 – Abundance of transient fish species in the tidal pools throughout the sampling period.

The highest densities were registered for *D. sargus* in all pools, followed by *Atherina* spp. with the exception of pool 2 of Beach B, where *Atherina* spp. were absent (Fig. 3). The number of species within the pools was very variable. *L. ramada* was present in pool 2 of Beach A and in pool 1 of Beach B; *S. bailloni* was present in pool 2 of both Beach A and Beach B; *D. cervinus* was present in all pools of Beach B but only was present in pool 2 of Beach A; *S. pilchardus* was only present in pool 1 of Beach B (Table 1).

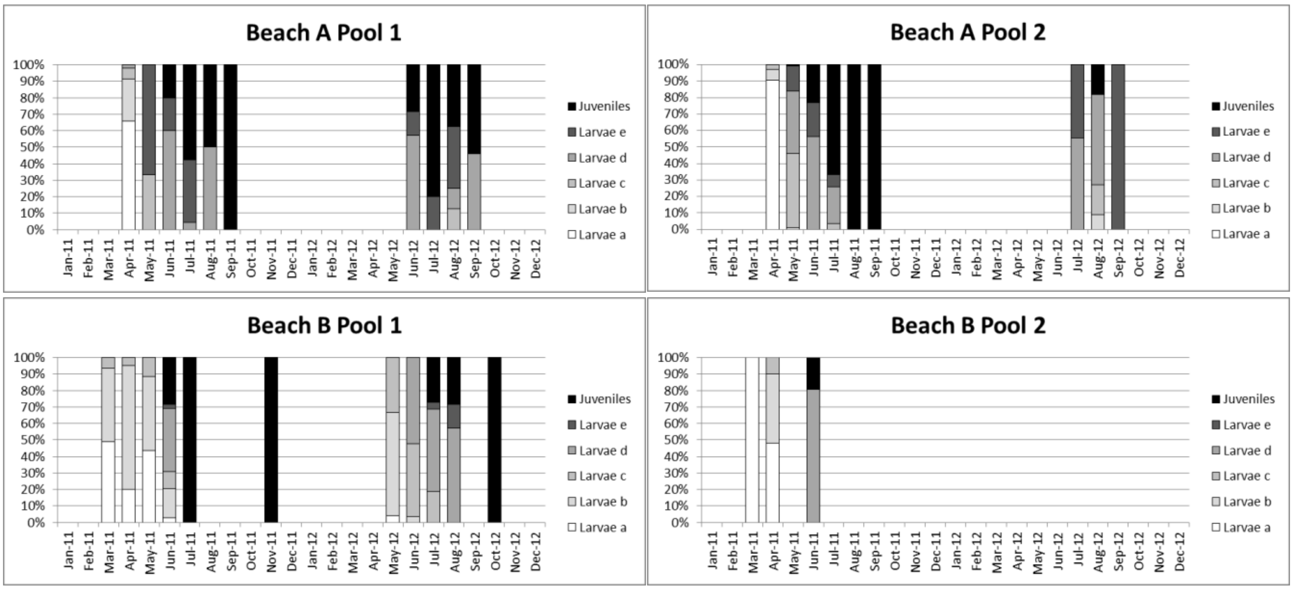
D. sargus was present in all pools in both years with the exception of pool 2 of Beach B, where it was absent in 2012 (Fig. 3). In 2011, density peaks were registered from April to July, with variability among pools, the density peak was more pronounced in April in all pools with the exceptions of pool 2 of Beach A, where the peak was more pronounced in May, and pool 1 of Beach A where the peak was more pronounced in July. In 2012, the density peaks were observed from June to September (Fig. 3).

Atherina spp. were the second most abundant marine transient fish in the pools in both years, with the exceptions of pool 1 of Beach A where they were absent in 2012 and pool 2 of Beach B where they were absent in both years. In 2011, the density peaks occurred in June and August; in 2012, the density peak occurred only in August (Fig. 3).

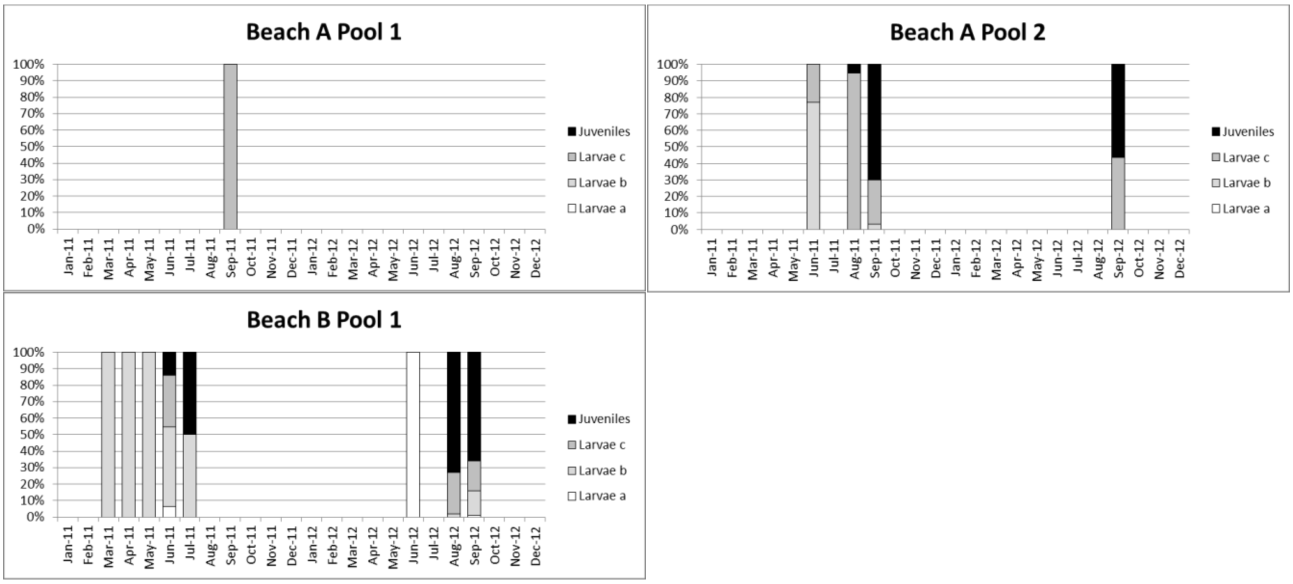
The density peaks were higher in 2011 than in 2012, for both *D. sargus* and *Atherina* spp.*,* in all pools and months (Fig. 3). Rare species were present mostly in spring-summer (Fig. 3) with a great inter-annual variability (e.g. *S. pilchardus* was only present in 2011, on only one occasion, and *S. bailloni* was only present in 2012). Great variability was registered both among pools and months. No rare species were found in pool 1 of Beach A. Rare species appeared in more months in 2012, than in 2011.

Post-larval stages of *D. sargus* colonized the pools in March-April of 2011 and May of 2012, in the first post-flexion larval stage (larvae *a*). As the months passed, the stage of development changed and the larvae reached the ultimate stage of larval development. In June of both years, early juveniles started to appear in the pools, with variability among pools (Fig. 4a).

In pool 2 of Beach A, post-larval stages of *Atherina* spp. started to appear in the second post-flexion larval stage (larvae *b*); in pool 1 of Beach A, post-larval stages of *Atherina* spp. were only present in the third post-flexion larval stage (larvae *c*). In pool 1 of Beach B, all the ontogenetic larval stages of the development were present. The early juveniles started to appear in June of 2011 and in August of 2012, with variability among pools (Fig. 4b).



a)



b)

Fig. 4 – Percentage of larvae and juveniles throughout the sampling period of *D. sargus* (a) and *Atherina* spp. (b).

In all pools, the overall condition of *D. sargus* increased throughout the year, in both years, with the exception of pool 2 of Beach B where the overall condition decreased (Fig. 5). The mean Fulton's *K* varied between 0.6 and 1.8 in 2011 and between 0.6 and 1.3 in 2012 (Fig. 5).

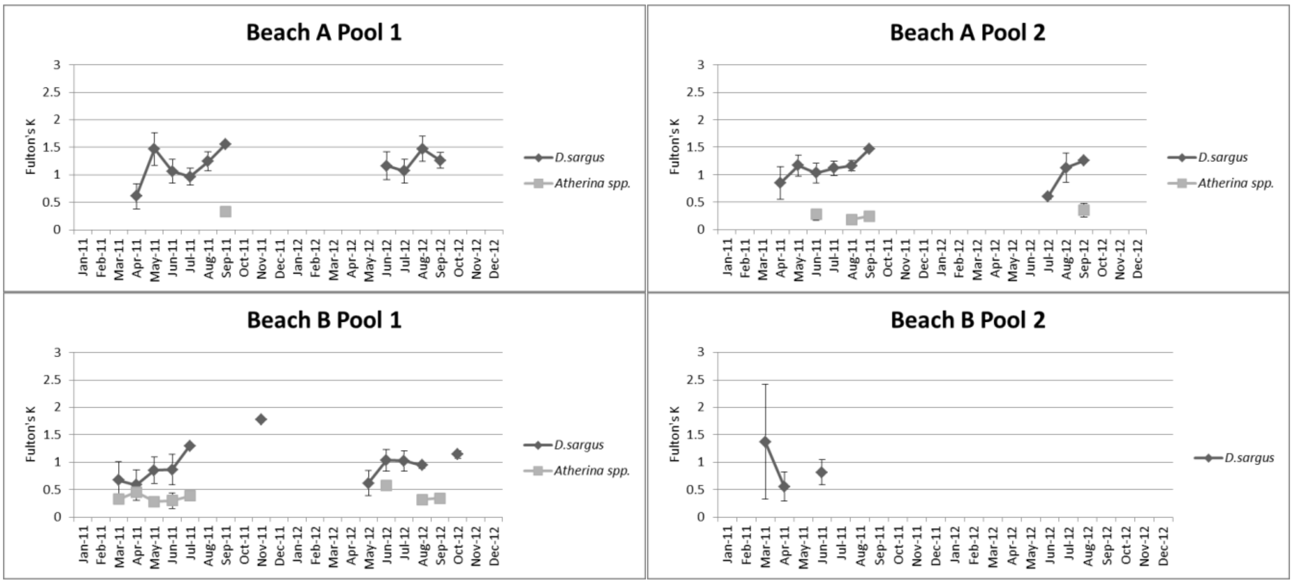


Fig. 5 – Variation of mean Fulton's *K* throughout the sampling period (bars indicate standard deviation).

In pool 2 of Beach A, it was observed the maintenance of the overall condition of *Atherina* spp. in the months that they were present (summer-autumn). In pool 1 of Beach B, a decreasing of the overall condition (spring-autumn) was observed, in both years. The mean Fulton's *K* varied between 0.2 and 0.4 in 2011 and between 0.3 and 0.6 in 2012 (Fig. 5).

Sizes of the most abundant fish, *D. sargus* and *Atherina* spp*.* were different among months for all pools. Lower mean sizes were registered in the beginning of spring of 2011 (March-April) and in the end of spring/beginning of summer of 2012 (May-June) (Fig. 6). Mean size often showed a continuous increase from spring to autumn in both years (Fig. 6).

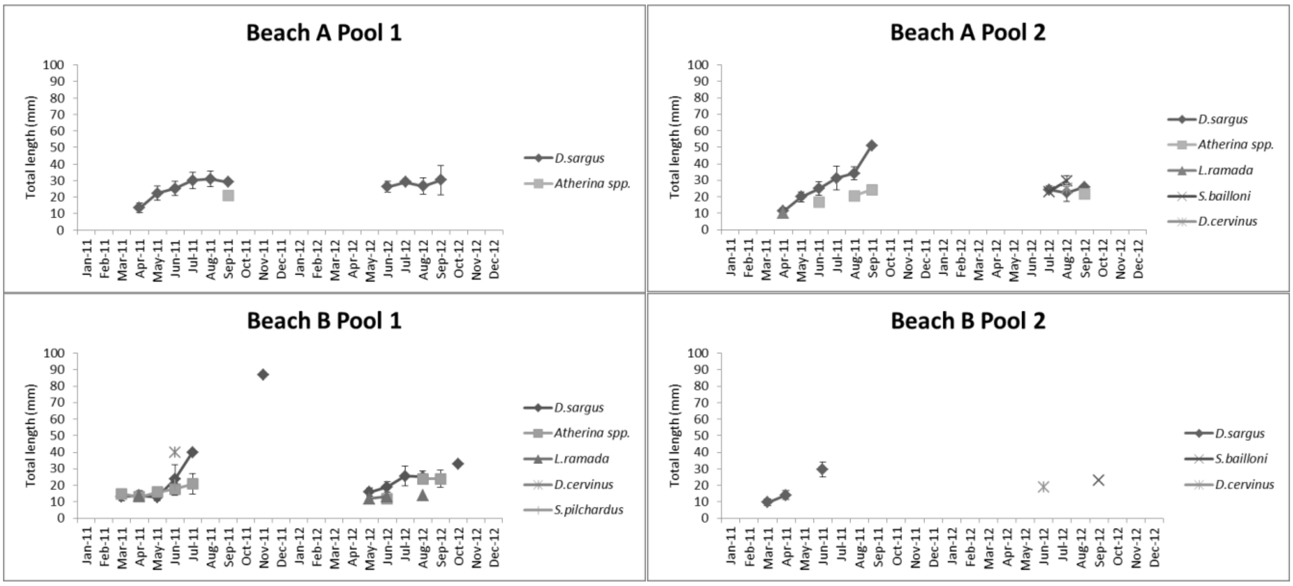


Fig. 6 - Variation of mean length throughout the sampling period (bars indicate standard deviation).

4.5. Discussion

This study showed the importance of tidal pools for late stage larval and early juveniles of marine fish, especially for *D. sargus* and *Atherina* spp., given the high densities and continuous growth observed. The tidal pools studied were used by larvae and juveniles of six species of marine fishes. Previous studies on other rocky tidal pools reported a lower number of non-resident fish species, typically three or four (Santos et al., 1994; Barreiros et al., 2004; Tsering et al., 2012).

The white seabream, *Diplodus sargus* (Linnaeus, 1758), was the most abundant transient species in the present study, both as larvae and juveniles. It was also the species that used these habitats in a more consistent way and within a longer timeframe. It is a species with a coastal rocky reef distribution, although it also occurs in estuaries, ranging from the Mediterranean to the eastern Atlantic (from the Bay of

Biscay to South Africa) (Vigliola et al., 1998) and constitutes an important fishery resource along its coasts (Gordoa and Molí, 1997). After one month of larval life, it settles in very shallow benthic habitats mainly from May to June, where it remains for several months (Gordoa and Molí, 1997). Changes in habitat use with growth have been described for *D. sargus* in Mediterranean rocky reefs, from inhabiting specific settlement habitats within the nursery, to colonizing the whole nursery and finally leaving the nursery to join the adult stock (Harmelin-Vivien et al., 1995; Biagi et al., 1998; Macpherson, 1998; Vigliola and Harmelin-Vivien, 2001).

Sand smelt, *Atherina* spp., includes various species of this genus, most often *A. presbyter* and *A. boyeri*, which are very difficult to distinguish by visual observation. It was the second most abundant marine transient fish found in the present study. These are euryhaline fish which inhabit coastal and estuarine waters, as well as lagoons, salt marshes and shallow brackish water ecosystems. They occur in the Mediterranean and in the Atlantic coast of Europe (Hendersen and Bamber, 1987). It is a relatively important commercial fish in some countries. The reproductive period lasts from the start of March to the end of July (Leonardos and Sinis, 2000), it hatches as a post-larva and forms schools in sheltered, inshore waters (Hendersen and Bamber, 1987).

Early stages of *L. ramada, S. bailloni, D. cervinus* and *S. pilchardus* were also found in the tidal pools, although in lower numbers. These are also typical shallow water transient marine species of west-European coasts (Henriques and Almada, 1998; Nicolas et al., 2010; França et al., 2011; Ribeiro et al., 2012).

In this study, transient fish were observed in the tidal pools from spring to autumn and left in winter. This pattern had been previously observed for various marine fish species that use estuaries as nursery areas (e.g. Elliott and Dewailly, 1995; Cabral and Costa, 1999; Power et al., 2000; Amaral and Cabral, 2004; Vinagre et al., 2005), including *D. sargus* (Ribeiro et al., 2006). This reinforces the results of previous studies from temperate regions, demonstrating that the abundances of species and individuals increase in late spring and summer due to the influx of juveniles of transient species following their breeding seasons (Gibson and Yoshiyama, 1999).

The highest density peaks registered were due to the high number of post-larvae entering the pools in spring. The mean density and species richness of rockpool fish decreased in autumn. This is attributed to a decrease in recruitment of transient species into the rockpool environments. Population densities and diversity of fish species indicate that rocky intertidal pools may have seasonal importance as nursery areas for juveniles of coastal species.

The period of time when these habitats were used by early stages of transient fish presented inter-annual variability for all species studied, which is in accordance with previous studies (Shulman, 1985; Fowler et al., 1992, Doherty and Fowler, 1994). Temporal variability in species abundance and richness between years may be associated with the different precipitation regime among the years analyzed. Intense winter rainfall in 2011 may have enhanced the productivity of coastal waters due to increased terrestrial run-off of organic matter, an effect that has been demonstrated in other coastal areas adjacent to major rivers (Darnaude et al., 2004). The coastal area studied in this work receives organic matter of terrestrial origin carried by the plume of the river Tagus (Vinagre et al., 2011; 2012), the second largest river in the Iberian Peninsula in terms of drainage.

Terrestrial run-off may also increase the amount of chemical cues in coastal waters used by larval fish to direct their movement towards nursery areas (Miller, 1988). The effect of the intensity of the Tagus river plume on juvenile fish recruitment to estuarine nurseries has been shown for other coastal fish, such as the soles, *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858 and the European seabass, *Dicentrarchus labrax* (Linnaeus, 1758) (Vinagre et al., 2007, 2009) and may also be important for the species reported in the present study.

While 2011 had intense rainfall in winter, 2012 was a draught year. The lower numbers of transient fish larvae and juveniles found in the pools in 2012, may be due to lower recruitment, consequence of a decrease in the intensity of chemical cues reaching the spawning areas and to a general lower enrichment in organic matter of this coastal area that possibly lowered the amount of food available for these early life-stages.

The change from larvae to juveniles, as time passed, as well as the gradual increase in mean size registered for the most abundant species, *D. sargus* and *Atherina* spp., indicates that these fish continuously use tidal pools during the period when these habitats are favorable.

K values of *D. sargus* were lower in spring and reached its highest in autumn. The fact that *K* values increased along the months indicates that the fishes enhanced their overall condition while they were using the pools, indicating that this is a favorable habitat for this species' growth. Exception made to *Atherina* spp. that showed maintenance of *K* values throughout the months.

Long-term data time-series would allow the investigation of inter-annual patterns of tidal pool colonization and how they relate to environmental factors, like already known for estuaries (e.g. Vinagre et al., 2007, 2009). Investigations into the differential survival and recruitment to adult stocks of the individuals that use tidal pools *versus* the ones that do not, or that use estuaries as nurseries, should clarify how important tidal pools are for the maintenance of adult stocks of important commercial species, such as *D. sargus*. This has been accomplished for fish that use estuarine nurseries using otolith microchemistry analysis (e.g. Gillanders and Kingsford, 1996; Thorrold et al., 1998; Yamashita et al., 2000; Gillanders, 2005) but remains unknown for transient fish that use rocky reef tidal pools during early life-stages.

Future studies should elucidate if the individuals visiting the pools during ebb tide are always the same. This would reveal if these species have site fidelity to each pool or if the occupation of these habitats is occasional and if individuals that inhabit coastal subtidal waters occasionally use tidal pools during ebb tide. Tagging and release studies would bring new insights into this question.

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5. Trophic role of marine fish larvae and juveniles that use rocky intertidal pools

5.1. Summary

Transient marine fishes were sampled monthly, in four tidal pools of a rocky reef, for two years, in the west Portuguese coast. The diet composition and trophic role of the most abundant marine fish larvae/juveniles in the tidal pools, *Diplodus sargus* and *Atherina* spp., were investigated. The stomach contents of 483 individuals (354 *D. sargus* ranging from 7 mm to 87 mm and 129 *Atherina* spp. ranging from 10 mm to 38 mm) were analyzed. *D. sargus* consumed a great variety of prey groups, including Algae, Cnidaria, Platyhelminthes, Entoprocta, Annelida, Crustacea, Insecta, Acari, Mollusca and Teleostei. *Atherina* spp. presented a lower diversity of food groups than *D. sargus*; these included Crustacea, Insecta, Acari and Mollusca. The diet of *D. sargus* was composed mainly of harpacticoid copepods, chironomid larvae, ostracods, chironomid adults and amphipods; whereas the diet of *Atherina* spp. was composed mainly of Harpacticoid copepods, ostracods and gastropods. Along the ontogenetic development, the proportions and diversity of food items in the diet of *D. sargus* varied. The importance of harpacticoid copepods decreased with increasing size, while the importance of amphipods and isopods increased. The trophic role of larvae and juveniles of *D. sargus* and *Atherina* spp. occurring in tidal pools of rocky reefs is that of opportunistic consumers with a wide range of prey, that take advantage of these habitats' high prey availability.

Keywords: Rocky reefs, tidal pools, habitat use, trophic ecology, diet.

5.2. Introduction

Ontogenetic shifts in habitat use are a common phenomenon in marine fishes (Beck et al., 2001; Gillanders et al., 2003) and occur as an adaptive response to reduce predation, enhance food availability and reduce competition (Clark, 1974; Cushing, 1975; Lenanton, 1982; Boesch and Turner, 1984; Macpherson, 1998). Several species of fish use nursery grounds in the larval and juvenile stages and move to subtidal waters as adults (Randall, 1955; Norris, 1963; Major, 1978).

The use of tidal pools during early ontogeny is likely to enhance growth, condition and survival chances of the individuals that use them (Thompson and Lehner, 1976; Moring, 1986, 1990; Gibson, 1994; Mahon and Mahon, 1994; Macpherson, 1998; Strydom, 2008). These habitats are highly structured providing many refuges, having high biodiversity, abundance of potential prey and, during spring and summer, they warm during the ebb tide attaining temperatures that are more favorable for fish growth than those of subtidal waters (Beckley, 1985a; Moring, 1986; Amara and Paul, 2003; Cunha et al., 2007). Rapid growth enables young fishes to achieve refuge and exclude a major part of the size-selective predation (Pihl and Van der Veer, 1992; Gibson, 1994; Leggett and DeBlois, 1994; Van der Veer et al., 1994).

Various coastal habitats are well known as nurseries for juvenile marine fish (Clark, 1974; Lenanton, 1982). These include estuaries (Wallace and Van Der Elst, 1975; Day et al., 1981; Wallace et al., 1984, Costa and Bruxelas, 1989; Vinagre et al., 2010), coastal lagoons (Franco et al., 2006; Abecasis et al., 2009; Ribeiro et al., 2012), salt marshes

(Veiga et al., 2006), coral reefs (Nagelkerken et al., 2000;), mangroves (Nagelkerken et al., 2000; Dorenbosch et al., 2004), surf zone reefs (Berry et al., 1982), seagrass beds (Nagelkerken et al., 2000; Dorenbosch et al., 2004; Verweij et al., 2008) and the sandy beach surf zone (Lasiak, 1981, 1983).

Estuarine nurseries are the best documented to date, with numerous studies, from as early as the larval phase of numerous fish species (e.g. Miskiewicz, 1986; Roper, 1986; Boehlert and Mundy, 1988; Whitfield, 1994; Harris and Cyrus, 2000). Rocky tidal pools are probably the least studied nurseries for marine fish, leaving a considerable research gap worldwide.

Several studies have been developed in rocky tidal pools that include transient juvenile fish, concerning their recruitment in tidal pools of South Africa (Beckley, 2000) and New Zealand (Willis and Roberts, 1996); species diversity and seasonal changes, in Brazil (Barreiros et al., 2004), India (Tsering et al., 2012) and California (Moring, 1986); community structure, in Colombia (Castellanos-Galindo et al., 2005), Australia (Griffiths, 2003a), Azores (Santos et al., 1994), Japan (Murase, 2013), Barbados (Mahon and Mahon, 1994) and Brazil (Cunha et al., 2008); concerning spatial and temporal dynamics in Australia (Griffiths, 2003b), Japan (Arakaki and Tokeshi, 2006), spatial and vertical patterns in Hawaii (Cox et al., 2011), re-colonization after experimental elimination, in California (Thompson and Lehner, 1976; Grossman, 1982) and in South Africa (Beckley, 1985b), feeding guild structure in Colombia (Castellanos-Galindo and Geraldo, 2008) and nursery function in South Africa (Beckley, 1985a; Bennett, 1987; Gibson and Yoshiyama, 1999). Castellanos-Galindo and Geraldo (2008), studied the juveniles' diet of transient fish species in Colombia, namely *Abudefduf concolor* (Gill, 1862), *Abudefduf troschelii* (Gill, 1862), *Halichoeres aestuaricola* (Bussing, 1972), *Chaenomugil proboscideus* (Günther, 1861) and *Echidna nocturna* (Cope, 1872). In that study, *A. concolor* and *A. troschelii* were considered herbivorous and specialist feeder species, feeding mainly on macroalgae and diatoms; *H. aestuaricola* was considered a carnivorous species, feeding on bivalves, copepods and amphipods; *C. proboscideus* was considered an herbivorous and a macroalgae specialist feeder species and *E. nocturna* was considered an exclusively carnivorous species, feeding on shrimps, brachyurans and stomatopods.

It is widely acknowledged that food abundance affects organisms' distribution patterns (Van der Veer and Witte, 1993; Gibson, 1994; Vinagre et al., 2006). Fish concentrate in areas of more abundant prey even if other environmental variables are suboptimal (Lockwood, 1974; Pearcy and Hancock, 1978; Imabayashi, 1980; Poxton et al*.,* 1982; Marchand, 1988; Vinagre et al., 2006).

In southern Europe, the very high densities of early forms of marine fish in rocky tidal pools (personal observation) are indicative that these habitats may be important nurseries for various abundant marine fish species, like the white seabream *Diplodus sargus* (Linnaeus, 1758) (Garcia-Rubies, 1997) and the sand smelt *Atherina* spp.. Even though these species' diet is well-known in other habitats (Moretti et al., 1959; Castel et al., 1977; Christensen, 1978; Marfin, 1981; Ferrari and Rossi, 1983–84; Gon and Ben-Tuvia, 1983; Rosecchi, 1987; Mantilacci et al., 1990; Danilova, 1991; Rosecchi and Grivelli, 1992; Mirto et al., 1994*;* Trabelsi et al., 1994; Gisbert et al., 1996; Sala and Ballesteros, 1997; Sanchez and Norbis, 1997; Pepe et al., 1998; Scilipoti, 1998; Mariani et al., 2002; Bartulovic et al., 2004; Figueiredo et al., 2005; Sá et al., 2006; Crisafi et al., 2007; Leitão et al., 2007), there is a knowledge gap in what concerns their diet and trophic role in tidal pools. That information is important for the understanding of the reasons why such juveniles use these environments in high numbers in spring and summer.

This study aims to investigate the diet composition of the most abundant marine transient fish larvae and juveniles in tidal pools of the Portuguese coast, *D. sargus* and *Atherina* spp., and their trophic role in these habitats.

5.3. Materials and Methods

Four rocky tidal pools were selected for this study. They were located in 2 exposed rocky beaches, distanced 1.4 km, in the central coast of Portugal (Fig. 1). Each tidal pool was characterized in terms of depth and approximate area (Table 1). These pools were studied monthly, during 2 years, from January 2011 to December 2012. They were visited every month in the first spring tide of the month, when tide height was approximately 0.7 m. Tides are semi-diurnal. Water temperature was registered (Fig. 2), as well as the dominant substrata and biota. Whenever larvae and/or juveniles of marine transient fish were present they were collected with hand nets, transported to the laboratory in refrigerated bags and preserved at -20 °C.

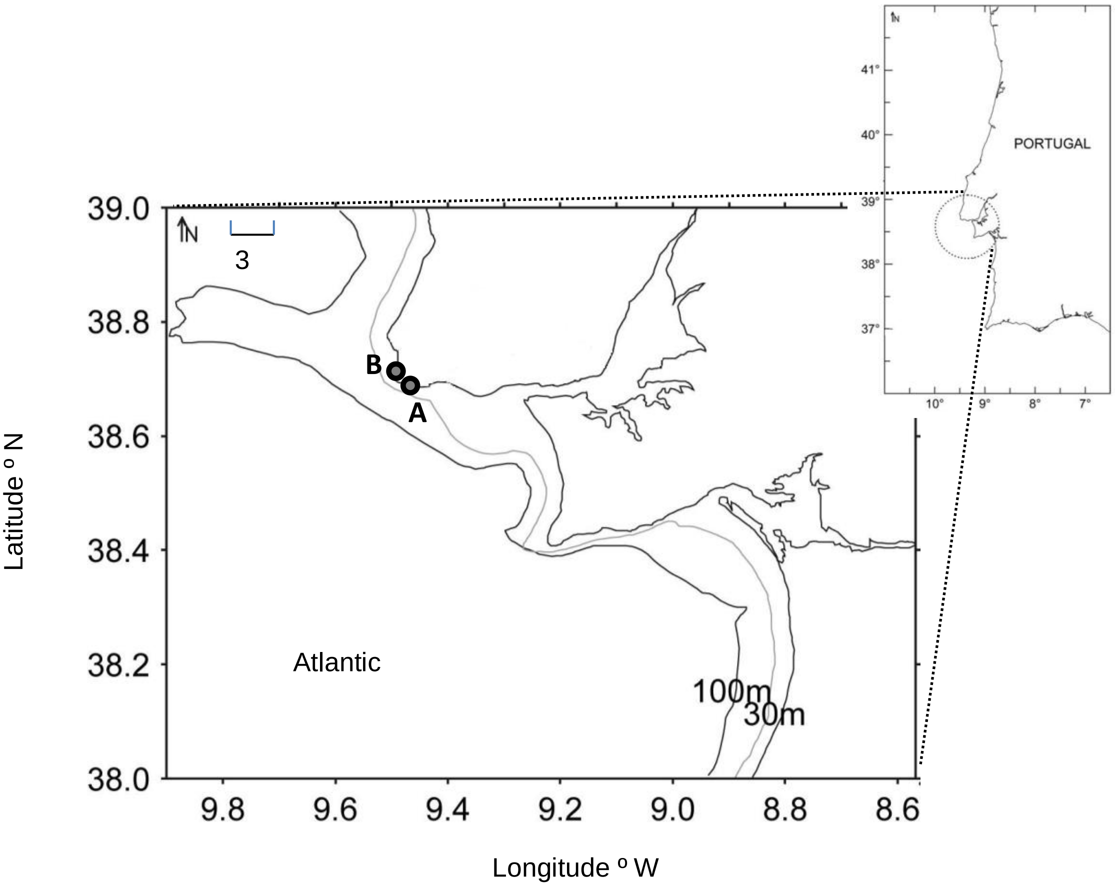

Fig. 1 – Location of the beaches (A and B) studied in the Portuguese coast.

Table 1 – Depth and approximate area of each studied tidal pool and mean densities of the transient fish species present (ind.m^{-2}). Standard deviation values of density are given in brackets

	Depth (cm)	Area (m²)	*D. sargus*	*Atherina* spp.	*L. ramada*	*S. bailloni*	*D. cervinus*	*S. pilchardus*
Beach A Pool 1	54	1.14	18.2 (24.3)	0.9 (0.0)				
Beach A Pool 2	63	1.44	21.1 (25.5)	22.9 (19.2)	2.1 (0.0)	1.4 (1.0)	1.4 (0.0)	
Beach B Pool 1	38	10.50	22.4 (52.3)	7.5 (10.7)	0.2 (0.2)		0.1 (0.0)	0.1 (0.0)
Beach B Pool 2	30	22.00	12.9 (21.0)			0.1 (0.0)	0.1 (0.0)	

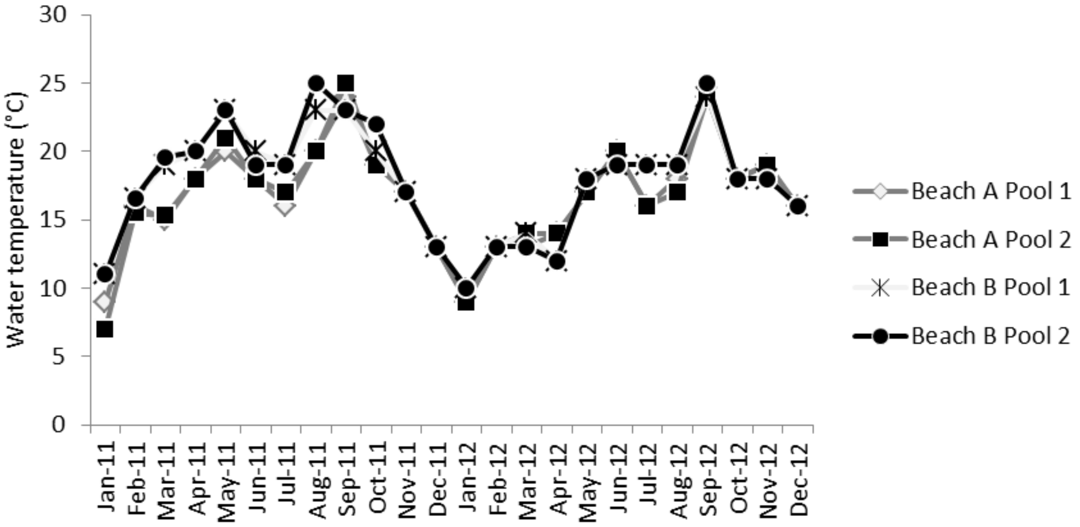

Fig. 2 – Water temperature in the pools during the sampling period.

In the laboratory, fish were identified to the species level, measured to the nearest mm, weighed with a precision of 0.1 g. The ontogenetic stage of the species was identified according to García and Moyano (1990). Post-larval stages were classified as larvae *a*, *b*, *c*, *d* and *e*, for *D. sargus*, and larvae *a*, *b* and *c*, for *Atherina* spp. and their stomach excised for the identification of its contents. Fish used for diet analysis were captured in spring and summer. The stomach contents of 483 individuals (354 *D. sargus* ranging from 7 mm to 87 mm and 129 *Atherina* spp. ranging from 10 mm to 38 mm) were analyzed. Dietary indices were calculated according to Hyslop (1980). The relative importance of each prey item in the diet was evaluated by three indices: the numerical index (NI) – percentage of the number of individuals of a prey over the total number of individuals of all prey; the occurrence index (OI) – percentage of non-empty stomachs in which a prey occurred over the total number of occurrences; and the volumetric index (VI) – percentage in volume occupied of a prey over the total volume of all prey. In order to assess diet variation throughout the ontogeny, the dietary indices were calculated for each developmental stage. Vacuity was assessed as percentage of empty stomachs.

A chi-square test was conducted to evaluate differences in both numerical and occurrence indices among different stages of development of both *D. sargus* and *Atherina* spp.. A significance level of 0.05 was considered.

5.4. Results

Pools' depth varied between 30 cm and 63 cm and their area ranged between 1.14 m^2 and 22.00 m^2 (Table 1). Temperature varied between 7 °C and 25 °C in 2011 (January and September, respectively) and between 9 °C and 25 °C in 2012 (January and September, respectively) (Fig. 2). The pools' algal communities were dominated by *Ellisolandia elongata* (J. Ellis and Solander) K. R. Hind and G. W. Saunders, 2013, *Phymatolithon calcareum* (Pallas) W. H. Adey and D. L. McKibbin, 1970, *Asparagopsis armata* Harvey, 1855 and *Ulva lactuca* Linnaeus, 1753.

The percentage of empty stomachs was very low for both species. It ranged between 0% and 6% for *D. sargus* and 0% and 15% for *Atherina* spp. (Table 2, 3). In *Atherina* spp. it decreased with development, reaching 0% in larvae *c* and in juveniles (Table 2, 3).

Table 2. - Numerical (NI – percentage of the number of individuals of a prey over the total number of individuals of all prey), volumetric (VI- percentage in volume occupied of a prey over the total volume of all prey) and occurrence (OI – percentage of non-empty stomachs in which a prey occurred over the total number of occurrences) index values of prey found in stomachs of *Diplodus sargus* (n.i. – not identified to lower systematic category)

	Larvae a			Larvae b			Larvae c			Larvae d			Larvae e			Juveniles		
Food items	NI	VI	OI	NI	VI	OI	NI	VI	OI	NI	VI	OI	NI	VI	OI	NI	VI	OI
Algae Σ	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	1.0
Ulva sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.4
Brown algae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3
Ellisolandia elongata	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3
Cnidaria Σ	0.0	0.4	1.1	0.0	0.2	0.8	0.0	0.1	0.4	0.0	0.2	0.9	0.0	1.8	3.6	0.0	0.7	1.4
Hydrozoa	0.0	0.4	1.1	0.0	0.2	0.8	0.0	0.1	0.4	0.0	0.2	0.9	0.0	1.8	3.6	0.0	0.7	1.4
Platyhelminthes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Entoprocta	0.0	0.1	1.1	0.0	0.0	0.0	0.0	0.8	2.6	0.0	0.5	0.9	0.0	0.0	0.0	0.0	0.0	0.0
Annelida Σ	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.5	0.6	1.4	2.0	1.7
Polychaeta	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.5	0.6	1.4	2.0	1.7
Artropoda Σ	100.0	99.5	94.7	99.7	99.5	97.5	100.0	99.1	97.0	99.8	97.9	95.4	99.9	97.7	95.8	98.5	95.0	94.9
Crustacea Σ	94.2	90.9	78.9	93.0	76.7	71.4	94.8	81.5	76.4	74.5	51.3	56.4	55.0	45.2	57.1	72.5	66.4	67.2
Ostracoda	3.2	6.6	12.6	2.9	3.1	13.4	1.8	3.9	11.2	4.6	2.0	5.5	2.1	1.6	4.2	11.7	2.2	6.9
Amphipoda	0.0	0.0	0.0	0.2	0.3	1.7	2.5	11.2	12.9	2.1	5.8	8.2	5.5	14.6	13.1	9.7	21.1	14.5
Decapoda Σ	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.4	0.9	0.1	1.0	0.9	0.0	0.0	0.0	2.6	6.3	6.2
Palaemonidae Σ	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.4	0.9	0.1	0.4	0.5	0.0	0.0	0.0	2.6	5.9	5.9
Palaemon elegans	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.5	1.4
Palaemon longirostris	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.3
Palaemon sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.4	0.9	0.1	0.4	0.5	0.0	0.0	0.0	2.2	5.2	4.1
Decapoda n.i.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.5	0.0	0.0	0.0	0.0	0.5	0.3
Isopoda Σ	0.0	0.0	0.0	0.1	0.5	0.8	0.7	2.3	5.2	0.5	0.8	3.2	1.1	2.0	4.8	1.7	2.5	5.9
Gnathiidae Σ	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.8	1.3	0.1	0.1	0.5	0.5	0.7	1.8	0.7	0.4	1.4
Paragnathia formica	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.8	1.3	0.1	0.1	0.5	0.5	0.7	1.8	0.7	0.4	1.4
Anthuridae Σ	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.3
Cyathura sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.3
Copepoda Σ	91.0	74.4	52.6	89.8	68.2	47.1	89.8	53.4	33.5	67.3	30.4	25.9	46.3	13.9	22.0	46.8	10.3	19.0
Harpacticoida	91.0	74.4	52.6	89.8	68.2	47.1	89.8	53.4	33.5	67.3	30.4	25.9	46.3	13.9	22.0	46.8	10.3	19.0
Crustacea n.i.	0.0	9.9	13.7	0.0	4.6	8.4	0.0	10.4	12.9	0.0	11.4	12.7	0.0	13.1	13.1	0.0	23.9	14.8
Insecta Σ	5.6	8.5	14.7	6.7	22.9	26.1	5.2	17.7	20.6	25.3	46.6	38.6	44.9	52.5	38.7	26.0	28.6	27.6
Chironomidae adults	0.0	0.0	0.0	0.3	2.1	2.5	0.7	5.0	4.7	7.8	23.9	17.7	7.6	20.2	17.9	9.5	17.7	15.5
Chironomidae larvae	5.6	8.5	14.7	6.5	20.8	23.5	4.5	12.6	15.9	17.5	22.7	20.9	37.3	32.2	20.8	16.6	11.0	12.1
Acari	0.2	0.1	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Mollusca Σ	0.0	0.0	0.0	0.3	0.3	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.3
Polyplacophora Σ	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.3
Lepidochitonidae Σ	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.3
Lepidochitona cinerea	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.3
Gastropoda	0.0	0.0	0.0	0.3	0.3	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Teleostei Σ	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Labridae Σ	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Symphodus sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Non-identified	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.4	0.0	0.0	0.0	0.0	1.7	0.7
Percentage of empty stomachs		6			0			0			2			0			0	

Table 3. Numerical (NI – percentage of the number of individuals of a prey over the total number of individuals of all prey), volumetric (VI- percentage in volume occupied of a prey over the total volume of all prey) and occurrence (OI – percentage of non-empty stomachs in which a prey occurred over the total number of occurrences) index values of prey found in stomachs of *Atherina* spp. (n.i. – not identified to lower systematic category)

	Larvae a			Larvae b			Larvae c			Juveniles		
Food items	NI	VI	OI	NI	VI	OI	NI	VI	OI	NI	VI	OI
Artropoda Σ	89.2	90.0	65.0	83.5	88.5	72.0	85.8	89.7	73.4	97.1	95.3	81.1
Crustacea Σ	88.0	80.9	60.0	81.5	86.7	64.6	85.1	86.0	69.5	95.5	89.7	69.8
Ostracoda	10.8	8.2	10.0	10.2	11.6	19.5	9.3	13.1	26.6	4.6	9.6	22.6
Amphipoda	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.7	1.6	0.2	1.5	2.8
Isopoda	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.8	0.1	0.7	1.9
Copepoda Σ	77.1	63.6	45.0	71.3	74.0	43.9	75.6	66.0	35.2	90.5	70.9	32.1
Harpacticoida	77.1	63.6	45.0	71.3	74.0	43.9	75.6	66.0	35.2	90.5	70.9	32.1
Cirripedia larvae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.6	0.9
Crustacea n.i.	0.0	9.1	5.0	0.0	1.1	1.2	0.0	6.1	5.5	0.0	6.5	9.4
Insecta Σ	1.2	9.1	5.0	1.6	1.4	4.9	0.7	3.7	3.9	1.5	5.3	10.4
Chironomidae larvae	1.2	9.1	5.0	1.6	1.4	4.9	0.7	3.7	3.9	1.5	5.3	10.4
Acari	0.0	0.0	0.0	0.5	0.4	2.4	0.0	0.0	0.0	0.1	0.3	0.9
Mollusca Σ	10.8	10.0	25.0	16.5	11.5	26.8	9.0	9.0	25.8	2.9	4.7	18.9
Bivalvia	1.2	0.9	5.0	0.9	0.7	3.7	1.2	1.2	4.7	0.3	0.9	4.7
Gastropoda	9.6	9.1	20.0	15.6	10.8	23.2	7.8	7.8	21.1	2.6	3.8	14.2
Unidentified eggs	0.0	0.0	0.0	0.0	0.0	0.0	5.2	1.3	0.8	0.0	0.0	0.0
Percentage of empty stomachs		15			3			0			0	

D. sargus consumed a great variability of prey groups, including Algae, Cnidaria, Platyhelminthes, Entoprocta, Annelida, Crustaceans, Insecta, Acari, Mollusca and Teleostei (Table 2). On the other hand, *Atherina* spp. presented a lower diversity of food groups than *D. sargus*. They included Crustaceans, Insecta, Acari and Mollusca (Table 3).

D. sargus' larvae *a* diet was comprised mainly of Harpacticoid copepods for all indices (NI = 91.0; VI = 74.4; OI = 52.6). Chironomid larvae were the second most important item according to all indices (NI = 5.6; VI = 8.5; OI = 14.7), followed by ostracods (NI = 3.2; VI = 6.6; OI = 12.6) (Table 2).

D. sargus' larvae *b* diet was also comprised mainly of Harpacticoid copepods (NI = 89.8; VI = 68.2; OI = 47.1). The second most important item was chironomid larvae for all indices (NI = 6.5; VI = 20.8; OI = 23.5) and ostracods were the third most important item, also for all indices (NI = 2.9; VI = 3.1; OI = 13.4) (Table 2).

D. sargus' larvae *c* diet was also comprised mainly of Harpacticoid copepods (NI = 89.8; VI = 53.4; OI = 33.5). The second most important item was chironomid larvae for all indices (NI = 4.5; VI = 12.6; OI = 15.9) and amphipods were the third most important item according to all indices (NI = 2.5; VI = 11.2; OI = 12.9) (Table 2).

D. sargus' larvae *d* diet was also comprised mainly of Harpacticoid copepods (NI = 67.3; VI = 30.4; OI = 25.9). While numerical and occurrence indices have chironomid larvae as the second most important food item (NI = 17.5; OI = 20.9), the volumetric index has chironomid adults at the second position (VI = 23.9), leaving chironomid larvae in the third position in terms of volumetric index (VI = 22.7) and chironomid adults as the third most important food item according to the numerical and occurrence indices (NI = 7.8; OI = 17.7) (Table 2).

For *D. sargus*' larvae *e*, Harpacticoid copepods and chironomid larvae were the most important food items according to both numerical and occurrence indices (NI = 46.3; OI = 22.0 and NI = 37.3; OI = 20.8, respectively) (Table 2). Volumetrically chironomid larvae was the most important food item while Harpacticoid copepods were less important (VI = 32.2 and VI = 13.9, respectively). Chironomid adults were the second most important group volumetrically (VI = 20.2) and the third most important item in terms of both numerical and occurrence indices (NI = 7.6; OI = 17.9). Amphipods were the third most important group volumetrically (VI = 14.6) (Table 2).

D. sargus juveniles' diet was comprised mainly of Harpacticoid copepods (NI = 46.8; VI = 10.3; OI = 19.0). While numerical and occurrence indices have Harpacticoid copepods as the most important food item (NI = 46.8; OI = 19.0), the volumetric index has amphipods at the top position (VI = 10.3 and VI = 21.1, respectively) (Table 2). The second most important item in terms of numerical index was chironomid larvae (NI = 16.6), but it had lower volumetric and occurrence indices (VI = 11.0; OI = 12.1) than amphipods and chironomid adults (VI = 21.1; OI = 14.5 and VI = 17.7; OI = 15.5, respectively). Ostracods were the third most important group in terms of numerical index (NI = 11.7) (Table 2).

Atherina spp.'s larvae *a* diet was comprised mainly of Harpacticoid copepods (NI = 77.1; VI = 63.6; OI = 45.0). Ostracods were the second most important group in terms of numerical index (NI = 10.8) and the third in terms of occurrence index (OI = 10.0). Although ostracods have a greater numerical index than gastropods (NI = 10.8 and NI = 9.6, respectively), gastropods had both greater volumetric and occurrence indices than ostracods (VI = 9.1; OI = 20.0 and VI = 8.2; OI = 10.0, respectively), occupying the second position in terms of volumetric and occurrence indices and the third position in terms of numerical index (Table 3).

Atherina spp.'s larvae *b* diet was also comprised mainly of Harpacticoid copepods (NI = 71.3; VI = 74.0; OI = 43.9). The second most important item in terms of both numerical and occurrence indices was gastropods (NI = 15.6; OI = 23.2) and the third in terms of volumetric index (VI = 10.8). Ostracods were the second in terms of volumetric index (VI = 11.6) and the third most important item in terms of both numerical and occurrence indices (NI = 10.2; OI = 19.5) (Table 3).

Atherina spp.'s larvae *c* diet was also comprised mainly of Harpacticoid copepods (NI = 75.6; VI = 66.0; OI = 35.2). The second most important item concerning all indices was ostracods (NI = 9.3; VI = 13.1; OI = 26.6), followed by gastropods (NI = 7.8; VI = 7.8; OI = 21.1) (Table 3).

Atherina spp. juveniles' diet was comprised mainly of Harpacticoid copepods (NI = 90.5; VI = 70.9; OI = 32.1). Ostracods occupied the second position for all indices (NI = 4.6; VI = 9.6; OI = 22.6). Gastropods occupied the third position in terms of numerical and occurrence indices (NI = 2.6; OI = 14.2) and chironomid larvae occupied the third position in terms of volumetric index (VI = 5.3) (Table 3).

Chi-square test's results indicated that there were significant differences among different stages of development in *D. sargus*, in both numerical and occurrence indices (p-value < 0.05) and there were no significant differences among different stages of development in *Atherina* spp., in the both numerical and occurrence indices (p-value > 0.05).

5.5. Discussion

Harpacticoid copepods were the most important prey for *D. sargus* in all the ontogenetic stages, with the exception of larvae *e*, for whom chironomid larvae were also highly relevant prey. All the indices estimated for harpaticoid copepods gradually decreased from the stage larvae *a* to the juvenile stage, which is in agreement with other studies on this species' diet that showed that along the ontogenetic development, the proportion of harpacticoid copepods in the diet decreased (Christensen, 1978; Rosecchi, 1987). This may be related with the fact that, once they are small prey, as *D. sargus* grows, it would prefer bigger food items that provide more energy.

Chironomid adults, amphipods and isopods were present in all stages of *D. sargus*, with the exception of larvae *a*. This probably happened due to their large size, since larvae *a* are small, they could not eat prey of that size. Along the ontogenetic development, their importance in *D. sargus'* diet increased.

Polychaetes, *Cyathura* sp., *Lepidochitona cinerea* (Linnaeus, 1767) and algae, namely brown algae, *Ulva* sp. and *Ellisolandia elongata*; were only eaten by juveniles, with the exception of polychaetes that were also eaten by larvae *e*. These food items presented low importance in terms of indices values, but accounted for the increase of food items diversity consumed by juveniles of *D. sargus*. On the other hand, *Palaemon* spp. had some importance in juveniles' diet, which may be related with its high energetic value (Velasco et al., 2010). Hydrozoans, entoprocts, *Paragnathia formica* (Hesse, 1864), platyhelminthes and acarids were present, but with very low importance.

Harpacticoid copepods, ostracods and gastropods were present in all stages of *Atherina* spp. with high indices values. Bivalves and chironomid larvae were also present in all stages but with low indices values, with the exception of chironomid larvae's occurrence index that was high in juveniles. Amphipods and isopods were only present in both larvae *c* and juveniles' stomachs. Cirripede larvae and acarids were present, but with low importance in terms of indices values.

As *D. sargus* and *Atherina* spp. larvae developed, they started eating prey of larger size, like amphipods and isopods, and their diet became more diversified, although statistical significant differences were only found for *D. sargus*. This behavior has been recorded for other marine fish larvae (Govoni et al., 1983; Fortier and Harris, 1989; Vinagre et al., 2008), possibly as a consequence of the increased energy requirements during larval growth (Hunter, 1981). It is also generally recognized that the size of prey consumed by most fishes increases with increasing predator size (Keast and Webb, 1966; Persson, 1990; Juanes, 1994). In addition to the faster swimming speeds and increased mouth gape size that occur as the fish grow, it is likely that both visual acuity and reaction distance to prey also markedly increase (Breck and Gitter, 1983; Wahl et al., 1993; Walton et al., 1994; Piet et al., 1998). Body size of both predator and prey have been linked directly to foraging success, since the escape response of prey is also strongly related to body size (Folkvord and Hunter, 1986; Blaxter and Fuiman, 1990).

The data presented indicates that *D. sargus* is an omnivorous and a generalist species, which is in line with previous studies in the South African marine littoral (Christensen, 1978), in the Natal Coast of South Africa (Joubert and Hanekom, 1980), in the Medes Islands (Sala and Ballesteros, 1997), in the Lakes Fogliano and Caprolace (Mariani et al., 2002), in the shallow coastal areas of Faial Island (Figueiredo et al., 2005) and in the Caprolace coastal lagoon (Costa and Cataudella, 2007). *D. sargus* is carnivorous earlier in its life and gradually increases the proportion of algae in its diet; it may ingest algae to collect their epiphyte diatoms (Joubert and Hanekon, 1980), to extract nutrients directly from the algae (Mann and Buxton, 1992) or to optimise the digestion of animals (Lobel, 1981). On the other hand, *Atherina* spp. are carnivorous and generalist species (Gon and Ben-Tuvia, 1983; Vizzini and Mazzola, 2005). Both *D. sargus* and *Atherina* spp. have been described as opportunistic feeders in other habitats, such as subtidal rocky reefs and coastal lagoons (Mariani et al., 2002; Figueiredo et al., 2005; Vizzini and Mazzola, 2005).

The food spectra characterized in the present study were in accordance with previous data on the same species in Golfe de Lion in France (Rosecchi, 1987), shallow coastal areas of Faial Island in Azores (Figueiredo et al., 2005) and in South African marine littoral (Christensen, 1978), for *D. sargus*, and in the Lagoon of Bardawil in Egypt (Gon and Ben-Tuvia, 1983), Laguna dello Stagnone in Sicilia (Mirto et al., 1994) and Trichonis lake in Greece (Chrisafi et al., 2007), for *Atherina* spp..

Resident fishes of intertidal rock pools of the Portuguese coast, like *Gobius paganellus* Linnaeus, 1758, *Gobius cobitis* Pallas, 1814, *Lipophrys pholis* (Linnaeus, 1758) and *Coryphoblennius galerita* (Linnaeus, 1758) are omnivorous (Couch, 1877; Wheeler, 1960, 1969; Velasco et al., 2010), and as they grow larger their diet changes to include larger organisms (Gibson, 1970), as observed in *D. sargus.* Their diet is similar to both *D. sargus* and *Atherina* spp.'s diet, including harpacticoid copepods, amphipods, isopods and gastropods. This suggests that resident fish in rock tidal pools are opportunistic feeders like *D. sargus* (Mariani et al., 2002; Figueiredo et al., 2005; Leitão et al., 2007) and *Atherina* spp. (Castel et al., 1977; Gon and Ben-Tuvia, 1983; Bartulovic et al., 2004; Chrisafi et al., 2007), that take advantage of the environment they inhabit since benthonic prey like Copepoda Harpacticoida, Amphipoda and Isopoda are very common in rocky tidal pools (Vizzini and Mazzola, 2005).

The present study found that intertidal feeding habits of *D. sargus* change considerably as larvae and juveniles grow, consuming larger and more diverse prey along the ontogenetic development. In rocky intertidal pools, sessile invertebrates, algae, and plants provide habitat and food for a wide variety of mobile invertebrates, including amphipods, isopods, crabs, shrimp, polychaetes, gastropods, and chitons, that are major components of the diets of rocky intertidal fishes, which take advantage of the high prey availability of these habitats.

Given that such small fish typically have very fast gastric evacuation rates (Bromley, 1994) it is likely that the food found in the stomachs was consumed in the pool, during the ebbing tide, and not in the surrounding environment. The very low percentage of empty stomachs found indicates that these fish use tidal pools as feeding grounds and not just as refuge from predators or because of the higher temperatures attained.

Future studies should look at the differences in prey availability in tidal pools and adjacent subtidal environments, in order to further clarify the role of food abundance in the selection of tidal pools as habitats by transient marine fish larvae and juveniles.

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6. General conclusions and Future work

D. sargus and *Atherina* spp. were the most abundant transient marine fish within the rocky tidal pools. Other species of coastal marine fish also used tidal pools, but more inconsistently and in lower numbers (*L. ramada, S. bailloni, D. cervinus and S. pilchardus*). The use of tidal pools is seasonal, being the highest density peaks registered in spring due to the entering of high numbers of post-larvae in the pools. The density and species richness of rockpool fish is maximum in spring-summer due to the favorable conditions of this habitat and minimum in autumn.

D. sargus and *Atherina* spp., continuously use tidal pools during the period when these habitats are favorable, we can see that in the change from larvae to juveniles, as well as the gradual increase in mean size and enhancement of the overall condition, along the time spent in the pools (spring-autumn). Exception made to *Atherina* spp. whose overall condition was maintained throughout the months.

Abiotic factors, like rainfall patterns, may affect the time of arrival and the number of the transient fish encountered in tidal pools and explain the differences in species composition, densities, temporal patterns of colonization and habitat use found between 2011 and 2012.

D. sargus consumed a greater variety of prey groups than *Atherina* spp.. It consumed Algae, Cnidaria, Platyhelminthes, Entoprocta, Annelida, Crustacea, Insecta, Acari, Mollusca and Teleostei, while *Atherina* spp. only consumed Crustacea, Insecta, Acari and Mollusca. These food items are very common within tidal pools. Intertidal feeding habits of *D. sargus* change considerably as larvae and juveniles grow, consuming larger and more diverse prey along the ontogenetic development, only consuming algae in the juvenile stage. In *D. sargus*, the importance of harpacticoid copepods decreased along the development; whereas the importance of amphipods and isopods increased. *D. sargus* is an omnivorous, generalist and opportunistic species; whereas *Atherina* spp. are carnivorous, generalist and opportunistic species.

The food items consumed for both *D. sargus* and *Atherina* spp. were similar to the ones consumed by the resident fish in the pools, which are also opportunistic species that take advantage of the high prey availability of these habitats. It was concluded that it is likely that the food found in the stomachs was consumed in the pool, during the ebbing tide, and not in the surrounding environment. Given that such small fish typically have very fast gastric evacuation rates, the very low percentage of empty stomachs found indicates that these fish use tidal pools as feeding grounds and not just as refuge from predators or because of the higher temperatures attained.

Future works should include long-term data time-series to allow the investigation of inter-annual patterns of tidal pool colonization and how they relate to environmental factors. Investigations into the differential survival and recruitment to adult stocks of the individuals that use tidal pools *versus* the ones that do not, or that use estuaries as nurseries, should clarify how important tidal pools are for the maintenance of adult stocks of important commercial species.

It is also important to know if the individuals visiting the pools during the ebb tide are always the same. Tagging and release studies would reveal if these species have site fidelity to each pool or if the occupation of these habitats is occasional, and if individuals that inhabit coastal subtidal waters occasionally use tidal pools during ebb tide.

The study of prey availability and diversity in tide pools and adjacent subtidal environments, should further clarify the importance of high food availability in the selection of tidal pools by transient marine fish larvae and juveniles.