UNIVERSIDADE DOS AÇORES
DEPARTAMENTO DE OCEANOGRAFIA E PESCAS

Recruitment dynamics and early life history of the blackspot seabream, *Pagellus bogaraveo* (Perciformes: Sparidae)

João Pedro Natário Teixeira

Dissertação apresentada à Universidade dos Açores para obtenção do Grau de Mestre na
Área Científica de Ecologia Marinha

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por

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Tese orientada por
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ABSTRACT

The blackspot seabream, *Pagellus bogaraveo*, is ecologically and commercially one of the most important demersal species in the Azores. However, for the past decades researchers have focused primarily on the species biology, population dynamics and population structure, and as consequence little is known about the early life stages and recruitment dynamics. Recently, several studies have been conducted with the goal of investigating the species lifecycle, including adult habitat use, movements and dispersion patterns as well as recruitment dynamics. This work focused essentially on the understanding and characterization of it´s early life history, recruitment and post-settlement dynamics. To achieve these goals I used two approaches: 1) underwater visual census (UVC), i.e., recruit counts, to investigate the temporal dynamics of recruitment, and 2) the analysis of juvenile otolith microstructure to investigate the early life history traits of the blackspot seabream. UVC surveys were conducted monthly from April 2011 to May 2012 in three sites along the south and east shores of Faial island (Azores). The results showed clear patterns regarding the seasonality of recruitment. Recruitment peaks were observed in April and May, in both years, followed by a clear succession in the juvenile size structure over the following months.

Otolith daily increment formation was validated which allowed me to use otolith daily increment analysis to retrospectively study the early life history of blackspot seabream. After interpreting the otolith microstructure and identifying the settlement mark, I was able to determine age, length-at-age relationship, pelagic larval duration (37.37±0.28 d), estimated size at settlement (13.50±0.17 mm), and average growth rates. Growth rates were significantly higher after settlement (16.82±0.47 μm d^{-1}) compared to the larval stage (7.11±0.08 μm d^{-1}). After back-calculating birth dates and grouping individuals by cohort, comparison tests were used to investigate if there were significant differences in ELHT among cohorts. The results showed significant differences in PLD among cohorts.

The present study provides a basis for establishing the seasonal patterns of recruitment of the blackspot seabream, and after reviewing the advantages and limitations of the UVC method, I discussed the possibility of a monitoring program for recruitment at the archipelago scale as tool for providing recruitment information necessary to improve conservation and fisheries management plans.

RESUMO

O goraz, *Pagellus bogaraveo*, é ecologicamente e comercialmente, uma das espécies demersais mais importantes nos Açores. Contudo, nas últimas décadas, a investigação focou-se sobretudo na biologia da espécie e na dinâmica e estrutura da população, e consequentemente, pouco se conhece acerca dos primeiros estágios de vida e da dinâmica de recrutamento. Recentemente, vários estudos têm vindo a ser realizados com o objectivo de investigar e caracterizar o ciclo de vida da espécie, incluindo o uso de habitat, movimentos e padrões de dispersão dos adultos e a dinâmica de recrutamento. Este trabalho focou-se essencialmente na caracterização e compreensão da biologia e ecologia da fase larvar (*ELHT*)e da dinâmica de recrutamento e pós-recrutamento. Para atingir estes objectivos, recorreu-se a dois tipos de estudo: 1) aos censos visuais subaquáticos para as contagens de recrutas, de forma a investigar a dinâmica temporal de recrutamento, e 2) à análise da microestrutura de otólitos de juvenis, de forma a investigar retrospectivamente os *ELHT* do goraz. As amostragens realizaram-se mensalmente desde Abril de 2011 até Maio de 2012 em três locais ao longo da costa sul e este da ilha do Faial (Açores). Os resultados mostraram padrões claros relativamente à sazonalidade do recrutamento, tendo sido detectados picos de recrutamento durante Abril e Maio, em dois anos consecutivos. Para além disso, a sucessão na estrutura de tamanhos das agregações de juvenis, durantes os meses seguintes, foi também evidente.

A formação de incrementos diários dos otólitos foi validada através de uma experiência de marcação de juvenis, o que permitiu que fossem utilizados com sucesso para caracterizar, retrospectivamente, a fase de vida larvar do goraz. Após, a interpretação da microestrutura do otólito e identificação da marca de recrutamento (*settlement mark*) , determinou-se a idade, a relação entre a idade e o comprimento, a duração da fase larvar (37,37±0,28 d), o tamanho de recrutamento (*settlement*) estimado (13,50±0,17 mm) e as taxas de crescimento médio para duas fases diferentes do ciclo de vida. As taxas de crescimento foram significativamente superiores na fase pós-recrutamento (*post-settlement*) (16,82±0,47 μm d^{-1}) comparativamente à fase larvar (7,11±0,08 μm d^{-1}). Após calcular as datas de nascimento foi possível agrupar os juvenis recolhidos por coorte, e compara as fases de vida larvar entre coortes sucessivas. Os resultados revelaram diferenças significativas entre coortes apenas para a duração da fase larvar

Assim, este estudo fornece a base de conhecimento acerca dos padrões sazonais de recrutamento do goraz, e depois de rever as vantagens e limitações do método de censos, é discutida a possibilidade de implementação de um programa de monitorização de recrutamento do goraz em todo o Arquipélago dos Açores, como ferramenta para obter informação sobre a dinâmica de recrutamento da espécie, necessária para melhorar os planos de conservação e gestão.

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CHAPTER 1

GENERAL INTRODUCTION

The depletion of fish stocks associated with the seas of the continental shelves has led to increasing fishing efforts directed at deep-water species (e.g. Haedrich, 1995; Koslow et al, 2000), especially on seamounts, continental slopes, and the slopes of oceanic islands (Stockley et al, 2005). Most deep-sea fisheries in the Northeast Atlantic originated as artisanal fisheries, particularly in the Azores, mainland Portugal and southern Spain, where deep water is close to land (Gordon et al, 2003). However, rapid depletion of seamount and other deep-sea fisheries, even within managed Exclusive Economic Zones, has taken place because of a lack of understanding of the biology of target species and the intense fishing pressure that can be placed on stocks using modern technology (Rogers, 1994). Most deep-water catches in the Northeast Atlantic today result from highly mechanized long-line fisheries (Gordon et al, 2003) and Azores is not an exception, over the last three decades, traditional fishing methods have been replaced by the use of modern fishing vessels using large arrays of long lines (Menezes et al, 2001; Stockley et al, 2005). The exploitation dynamics of the blackspot seabream, *Pagellus bogaraveo* (Brünnich, 1768), Teleostei, Sparidae, (Fig. 1.1) one of the main target species of the Azores long-line fishery (Stockley et al, 2005), during the last decades illustrates this trend, attending to an increase in pressure on the island population after the collapse of the fishery in continental waters (Krug, 1989; Gordon et al, 2003, Chilari et al, 2006; Erzini et al, 2006; Menezes et al, 2006; Lorance, 2011). Blackspot seabream is found in the Eastern Atlantic Ocean occurring in western coasts of Europe and Africa and in the Mediterranean Sea. The latitude and longitude ranges for this specie are from 65ºN, Norway, to 27ºN, Canary Islands, and from 30ºW, West of the Azores, to 22ºE, Mediterranean, respectively (Krug, 1994). The species has been exploited in the Azores, at least since the XVI century as part of the demersal fishery (Pinho, 2003). Historically, landings increased from 400 t at the start of the eighties to approximately 1000 t at the start of the nineties, between 1990 and 2009 the annual landings have fluctuated around 1000 t, with a peak in 2005 (ICES, 2012). During the last two years the landings decreased to 687 t, and 624 t. In general a continuous decrease has been observed since 2005

(ICES, 2012).

Over the past decades researchers have focused on the biology, ecology and habitat use of adult blackspot seabream in Azores (e.g. Krug, 1989, 1998; Menezes et al, 1998; Stockley et al, 2000, 2005; Estácio et al, 2001; Morato et al, 2001, Afonso et al 2012). The blackspot seabream can be found from inshore waters to about 700 m (Krug, 1989). The bigger fishes are found at depths greater than the juveniles, indicating an ontogenetic migration toward deeper waters (Morato et al, 2001; Lorance, 2011). The juveniles live near-shore occurring at depths generally lower than 50 m and as they grow, they gradually move away to offshore (Desbrosses, 1938), whereas the adults can be found in the continental slope (Bauchot and Hureau, 1986) and seamounts (Morato et al, 2001), usually above sandy and rocky bottoms (Menezes et al, 1998). The blackspot seabream is mainly carnivorous and its diet is opportunistic depending on habitat and prey availability (Morato et al, 2001). It is considered a slow growing species (ICES, 2012) and in the Azores, the maximum age record observed was 15 years and 56 cm length (Krug, 1994). The spawning season occurs once a year between January and April with peak activity in February and March (Krug, 1990). According to some aquaculture studies (Peleteiro et al, 1997; Olmedo et al, 1998) the blackspot seabream exhibits external fertilization and both eggs and larvae are pelagic. Despite the importance of recruitment processes, research has been focused primarily on the biology of this species and population dynamics, and as consequence little is known about the early life stages in the wild, including larval and juvenile stages.

Objectives

The main goals of this work were, to (1) characterize the temporal recruitment dynamics of *P. bogaraveo*, (2) describe relevant early life history traits, (3) describe the relationship between age and length of recruits, and (4) characterize larval and post-settlement growth. To pursue these goals I combined the following methodologies:

1) monitoring of recruit populations during a full year by means of underwater visual census;
2) otolith microstructure analysis to investigating larval traits and determine the age-at-length relationship.

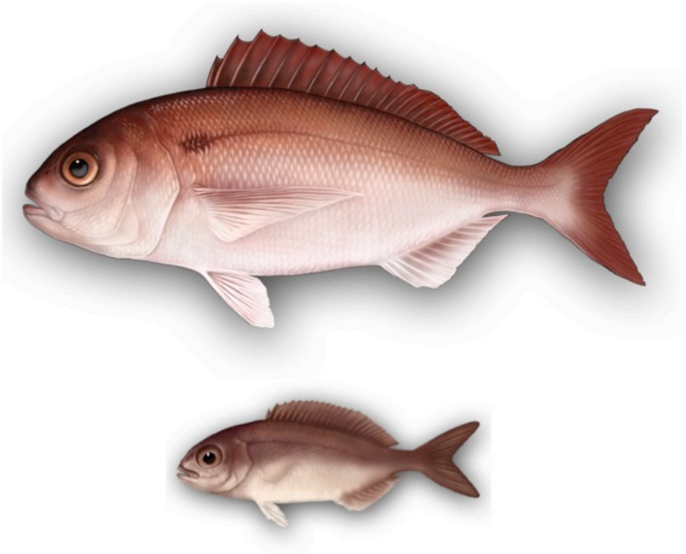

Figure 1.1. *Pagellus bogaraveo* illustration of the adult phase (above) and the juvenile phase (below) (L. Gallagher/FishPics/ImagDOP.

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CHAPTER 2

TEMPORAL DYNAMICS OF RECRUITMENT OF THE BLACKSPOT SEABREAM, *Pagellus bogaraveo* (PERCIFORMES: SPARIDAE)

Abstract

In order to study the temporal dynamics of recruitment of the *Pagellus bogaraveo*, I conducted monthly underwater visual census (UVC) surveys, between April 2011 and May 2012 in three sites along the south and east shores of Faial island (Azores). Recruit counts were recorded and attributed a size class. After surveys were completed, individuals were collected from areas adjacent to the transects for subsequent validation of both species and size. The PERMANOVA tests results revealed significant differences between sampling period and study site, as for the interaction between both ('Interaction'). Despite the heterogeneity among sites, the three replicated of the island showed clear patterns regarding the seasonality of the recruitment dynamics of and post-recruitment juvenile age structure present at the monitored sites. Peaks in recruitment (i.e. density of class 0-3) were detected in April and May in both years, in accordance to what is described for the *P. bogaraveo* spawning season. It was also visible a succession in size structure of juvenile assemblages over time, which culminated with the disappearance of the juveniles from sampling areas in January, supporting the ontogenetic vertical migration theory.

Introduction

Recruitment is defined as the number of individuals that reach a specified stage of the life cycle (e.g. metamorphosis, settlement or joining the fishery) (Jennings et al, 2001). Once this term can refer to functionally distinct events (e.g. planktonic survivorship only, survivorship of a cohort through planktonic and post-settlement life stages until vulnerable to a specific fishing gear, the actual event of larval settlement, post-settlement survivorship until initial census observation, immigration of any life history stage to any new habitat/population; etc), it is also relevant to clarify the specific process or event actually under investigation (Richards and Lindeman, 1987;

Cowen and Sponaugle, 1997). For coral reef fishes, “settlement” is considered to be the arrival of new individuals from the pelagic habitat, and “recruitment” is typically defined as the survival of newly settled juveniles up to the time of sampling (Richards and Lindeman, 1987; Forrester 1990; Levin, 1994). Thus, after the pelagic stage, variable mortality rates shortly after settlement of habitat selection by young juveniles may further influence the distribution and abundance of the new recruits (Cowen and Sponaugle, 1997). Although the previous definition encompasses early post-settlement mortality, when juveniles are sampled very shortly after settlement, ‘recruitment’ becomes functionally equivalent to ‘settlement’ (Sponaugle and Cowen, 1997). As such, recruitment represents the end product of a series of dynamic physical and biological processes that vary spatially and temporally. The juvenile fishes generally occur at sites that provide refuge from predation and feeding areas (Walthers and Juanes, 1993). Unlike many temperate species of importance to fisheries, tropical reef fishes undergo a distinct habitat change at the end of larval life, settling from the plankton into the benthic habitats of adults. This allows recruitment patterns to be easily measured (Bergenius et al, 2002). Normally, fishery biologists working on deep-sea species have to deal with juveniles that are inaccessible to sampling gears so they measure recruitment as the abundance of youngest year class entering the fishery (Jennings et al, 2001). Even though *Pagellus bogaraveo* is a temperate demersal fish species, it is a suitable candidate for studying its dynamic of recruitment through underwater visual census (UVC) techniques used by reef fish ecologists., since least part of the juvenile population inhabits near-shore littoral coastal areas during their first year of life on the Azores islands (ICES, 2012). Brock (1954) pioneered the first UVC method and stated that under proper circumstances (relatively clear and shallow waters) a diver could enumerate fishes seen so that estimations of abundance and size could be made. Since then several variants of the UVC technique have formed the basis of most studies of reef fish ecology (Jones and Thompson, 1978; Willis, 2001). The basic technique involves counting individuals within a predetermined (sometimes estimated) distance either side of a line transect (Bell et al, 1985). This method requires relatively little expenditure of field time, it is inexpensive to employ and non-destructive in nature, which makes it appealing to workers conducting repeated observations (Sale and Sharp, 1983; Watson and Quinn II, 1997; Willis, 2001). However, it is not 100% accurate (Sale and Douglas, 1981), obvious errors include problems in species identification, and in counting and estimating size of fishes seen (Brock, 1982; see Harmelin-Vivien et al 1985 for review). Since the 80s, several studies have begun to analyze the limitations in terms of precision and accuracy together with the probable sources of biases (see Sale and Douglas, 1981; Brock, 1982; Sale and Sharp, 1983; Bell et al, 1985; McCormick and Choat, 1987; Bellwood and Alcala, 1988; Buckley and Hueckel, 1989; Smith, 1989; St. John et al, 1990; Watson et al, 1995; Cheal and Thompson, 1997; Watson and Quinn II, 1997; Kulbicki, 1998 and Samoilys and Carlos, 2000). The several sources of biases that have been identified and investigated are listed in Harvey (2002). Nevertheless, according to Harmelin-Vivien et al (1985), given some necessary precautions and adequate training, underwater counts can produce highly reliable results and has been widely used over the past decades to estimate settlement rates and recruitment level of other species, including other sparid (e.g. Sponaugle and Cowen, 1994; Caselle and Warner, 1996; Nemeth, 2005; Caselle et al, 2010; Fontes et al, 2010, 2011; Cheminee et al, 2011).

It is known that the pattern and magnitude of recruitment can strongly influence conservation and management options (Dunstan and Johnson, 1998) and it has been suggested that an understanding of the ecology and dynamics of juvenile stages should be an integral part of assessing and managing fish populations (Walters and Juanes, 1993). Thus, the aims of this work were, to (1) validate both visual identification and size estimates of blackspot seabream recruits observed in UVC, (2) characterize the temporal dynamics of recruitment of the blackspot seabream and (3) describe the dynamics of post-recruitment residency and evolution size structure of assemblages in nursery areas.

Methods

Study Area

This study took place on Faial Island where I surveyed putative costal nursery areas. All sampling sites were established in sheltered bays or harbors, two on the south shore and one on the east shore. The sites are dominated by shallow habitat (depth<-10 m) with sandy bottoms with small and medium size boulders covered with turf algae (Fontes et al, 2011). First site was Castelo Branco harbor's bay (C. Branco) (38º31.092 N; 28º43.390 W), the second site is located along the west margin of the

Porto Pim bay (P. Pim) (38º31.477 N; 28º37.757 W) and the last one, Doca, is the area along the outer wall of the old marina break-water (38º31.995 N; 28º37.463 W) (Fig. 2.1).

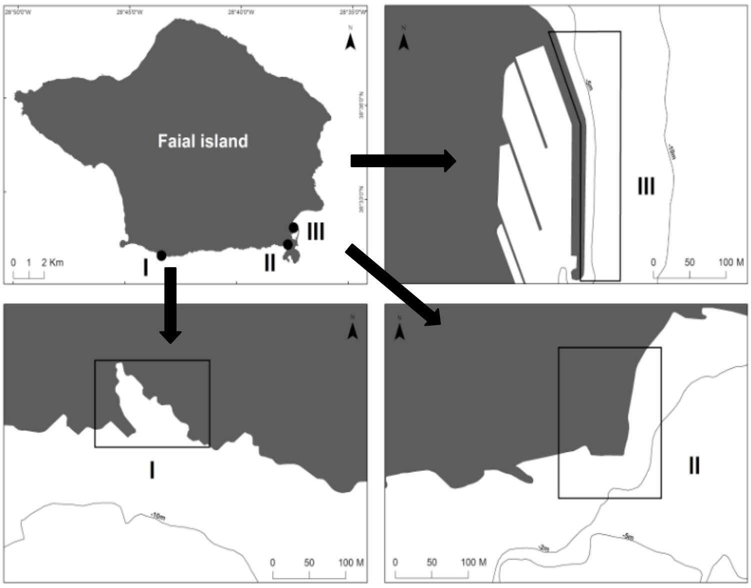

Figure 2.1. Map of Faial Island, showing the locations of the three study sites: Castelo Branco harbor's bay (I), west margin of the Porto Pim's bay (II) and Doca (III).

Recruitment surveys

Recruitment counts were performed on four random 50x5 m linear transects (Halford and Thompson, 1994; Harmelin-Vivien et al, 1995) by snorkeling and freediving (depth < -10 m). Recruit counts were conducted by the same diver, swimming at a constant slow speed (3.33 m min^{-1}) along the transect counting all juveniles along the 250 m^2 area. Monthly surveys were performed from April 2011 to May 2012, and over the last three months additional surveys were also done weekly, weather permitting, at Castelo Branco harbour's bay. To minimize the effects of temporal variation in recruitment between the three sites, surveys for a given month were completed at all sites in a ≤5 d period, weather permitting (Caselle and Warner, 1996). Surveys were done between 1000 and 1600 h and under similar conditions (i.e. considering underwater visibility, cloudiness, swell conditions, wind and presence of human disturbance). Inclusion criteria for individuals and fish schools in underwater visual census surveys were as follows, if an individual entered a transect ahead of the observer it was counted; if a school or part of it entered a section of a transect ahead of the observer, all members of the school were counted; individuals or schools whose repeated entry in the transect was detected were ignored (Brock, 1954; Bodkin, 1986; Halford and Thompson, 1994; Schmiing et al, 2013). Recruit size was estimated and individuals assigned to one of three-predefined centimeters size classes, 0-3, 3-6, 6-9, 9-12 and >12cm total length (TL). Data were recorded on to a plastic slate containing a reference scale bars of 3, 6, 9 and 12 cm, to support the size estimations. If multispecific fish schools (e.g. *Pagellus bogaraveo* and *Pagellus. acarne*) were encountered a percentage for each species was estimated after a later close inspection or collection if necessary.

Fish collection

Recruits were collected adjacent to the transects following the UVC surveys. Specimens were collected according to their size with three different methods in May, July, August, October, and November 2011 and in April and May 2012. The recruits ranging from 0 to 3 cm were captured by diver using aquarium dip nets, the individuals ranging from 3 to 6 cm were captured by drop netting and those larger than 6 cm were collected with fishing rod. Fish specimens were immediately stored in plastic jars with 96% ethanol with the exception of larger specimens (>6 cm) that were sacrificed in ice. The recruits were identified using a number of relevant morphometric traits, including the number of spines and rays of the anal fin, three spines and 10-12 rays (Bauchot and Hureau, 1986). This criterion avoided the possibility of miss identification with other co-occurring sparid species (e.g. congener specie *P. acarne*). Both total length (TL) and standard length (SL) of each recruit was measured in mm to the nearest 0.1 mm.

Statistical Analysis

Fish count data were converted to densities by dividing the recruit counts by transect area (250 m^2). Recruit densities were plotted on a log scale, in order to minimize the error on the largest estimates (see Harmelin-Vivien et al., 1985; Kulbicki, 1998).

To further analysis, each study site had equal number of observations (C. Branco, n=56; P. Pim, n=56; Doca, n=56), i.e. C. Branco, in order to be comparable to the sampling in the other two places, I only took into consideration the sampling which were correspondent in time.

Before standardization I added a dummy variable (0.0001) due to zero values in some transects (Clarke and Gorley, 2006). Density data were standardized and after removing the dummy variable, converted to a resemblance matrix based on Euclidean distance. To test for potential differences in recruit densities, a two-way crossed design, PERMANOVA (permutational multivariate analysis of variance; Anderson et al, 2008) main test was used to assess simultaneous response of variables (recruit size classes) to following factors: 'Sampling period' (fixed factor with fourteen levels: April/11, May/11, June/11, July/11, August/11, September/11, October/11, November/11, December/11, January/12, February/12, March/12, April/12 and May/12), 'Study site' (Fixed factor with three levels: C. Branco, P. Pim and Doca) and the interaction between both ('Interaction'). If PERMANOVA main test detected significant differences, pos-hoc pairwise tests were performed to obtain comparisons among all pairs of levels of each factor (Anderson et al, 2008).

For the following analysis, it was necessary to remove two levels of factor Sampling Site (April/11 and May/11) from the data array, in order to obtain only a full year context. RELATE routine was performed to investigate the occurrence of a significant temporal pattern in each study site by calculating Spearman rank correlations (rho-values; $\rho=1$, a perfect match) between each study site's resemblance matrix (Euclidean distance) and a cyclicity model matrix provided by the routine. The cyclicity model is formed by the inter-point distances of equally spaced points around a circle, which expects adjacent sampling periods to be the most similar, sampling periods two steps apart less similar and so on, but as the year progresses the pattern gradually returns to that at the start of the year (e.g. June/11 and May/12 are only 1 step apart, not 11) (Clarke and Gorley, 2006). Temporal patterns between study sites were also compared using RELATE routine by calculating Spearman rank correlations for each resemblance matrix against each other (Clarke and Gorley, 2006). Statistical analyses were run using PRIMER 6 (version 6.1.12) & PERMANOVA+ (version 1.0.2) software package (Plymouth Marine Laboratory).

Results

The juvenile samples collected were identified as *Pagellus bogaraveo* and the total body size measurements were in accordance with visual estimations during underwater visual census (UVC). A total of 196 transects were conducted from April 2011 to May 2012; 84 transects in C. Branco, 56 in P. Pim bay and 56 in Doca. Surveys were completed at all sites in less than 4.5±1.2 d (mean±s.e.) for any given sampling period, (except in weekly sampling for C. Branco).

Regarding the seasonality of recruitment, recruit density has varied over time, i.e. between sampling periods. The statistical analysis showed significant differences for factors Sampling period, Study site, as for the interaction between both (Interaction) (Table 2.1). In other words, apart from the effect of seasonality, the results have also showed differences between the study sites and apparently, a significant interaction between the other two factors, which has affected the variation in recruit density. Together the three factors explained almost 60% of the total variability; the Sampling period contributed the most (30.26%), followed by the Interaction (19.97%) and also the Study site with a contribution of 9.62% (Table 2.1). Besides the PERMANOVA tests, I also tested the hypothesis that recruitment would be equal throughout the year, i.e. if there would be no cycle in the temporal dynamics of recruitment for blackspot

Table 2.1. Summary of PERMANOVA main test results for the analysis of differences in recruit density across the different factors. *p<0.05.

Source	DF	SS	MS	Pseudo-F	p(perm)
Sampling period	13	230220	17709.00	7.82	0.001*
Study site	2	19094	9547.00	4.21	0.001*
Interaction	26	117160	4506.30	1.99	0.001*
Res	126	285470	2265.60		
Total	167	651950			

Estimates of components of variation

Source	Estimate	Sq. root	% Contribution
S(Sampling period)	1287.00	35.87	30.26
S(Study site)	130.03	11.40	9.62
S(Interaction)	560.16	23.67	19.97
V(Res)	2265.60	47.60	40.15

seabream. The results with the RELATE routine showed that comparisons between each study site (resemblance matrix) and all sites combined (resemblance matrix) against cyclicity models indicated significant correlations ($p<0.05$) but with low correlation coefficient (ρ) values (Table 2.2.). This suggested that there is a trend over time for recruit density but the cycle was not evident when matched against the cyclicity model. The best match was found for C. Branco with a rho-value of 0.32 (Table 2.2). Comparisons between the resemblance matrices of each study site against each other revealed no significant matches ($p>0.05$), i.e. the temporal pattern of recruit density was different between sites (Table 2.2).

Table 2.2. Summary of RELATE routine results showing Spearman rank correlation (ρ) and significance level (p) for each pair of matched resemblance matrices. $\rho=1$ represents a perfect match; *$p<0.05$. Dashes (-) mean that is an inappropriate match.

Resemblance matrices	Cyclicity model	Castelo Branco	Porto Pim	Doca
Castelo Branco	$\rho = 0.32$; $p = 0.01$*	-	$\rho = 0.17$; $p = 0.05$	$\rho = 0.13$; $p = 0.37$
Porto Pim	$\rho = 0.17$; $p = 0.01$*	$\rho = 0.17$; $p = 0.05$	-	$\rho = 0.006$; $p = 4.14$
Doca	$\rho = 0.23$; $p = 0.01$*	$\rho = 0.13$; $p = 0.37$	$\rho = 0.006$; $p = 4.14$	-
All sites	$\rho = 0.25$; $p = 0.01$*	-	-	-

From now on, I refer to density as the logarithm of recruit counts divided by transect area. After combining the UVC data from the three sites and treated as replicates from the island as a spatial unit, I clearly identified recruitment peaks (high density of class 0-3) between April and June in 2011 and April and May in 2012. Average density of class 0-3 was highest in May 2011, 0.14±0.06 (mean±s.e.), and in May 2012 0.12±0.05 (Fig. 2.2). Figure 2.2 also showed a succession of size structure over time. The size class 3-6 had the maximum density in June 2011 (0.18±0.11), followed by class 6-9, which had a maximum density in July 2011 (0.34±0.11). Maximum densities of size classes 9-12 and >12 were observed in April 2011 and July 2011, respectively. The class 6-9 stood out with highest densities observed in all sampling periods. From December 2011 to January 2012 the overall juvenile density decreased and during February and March 2012 the densities declined even further and were close to zero. (Fig. 2.2).

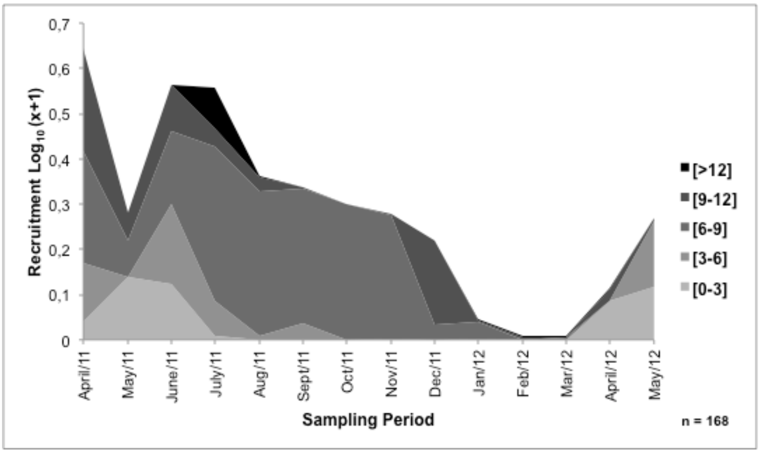

Figure 2.2. Average recruit density of *P. bogaraveo* for all sites combined (stacked area chart). Size classes: 0-3, 3-6. 6-9, 9-12 and >12 cm are represented by the gradient from lighter grey to black, in which class 0-3 corresponds to lighter grey and class >12 corresponds to black.

The first recruits were detected at C. Branco in April 2011. The recruits showed some pigmentation and had the appearance of some early stage of a sparid species. Species identification had to be confirmed in the laboratory and in the following months (May and June 2011) the surveys at C. Branco showed a successive increase in density of this size class (0-3) (Fig. 2.3). In May and June 2011, class 0-3 aggregations increased in density, peaking in June 2011 (0.37±0.25). Recruits were bigger and appeared to be more developed than the ones observed in April 2011. In August 2011 and from then on, I didn't detect any individual from class 0-3 until the end of 2011. However, the succession of size classes was clear from July to December 2011. The class 3-6 succeeded to class 0-3 in July 2011, followed by class (6-9) that overcame the previous class in August and September, reaching a maximum value of 0.20±0.006 in October 2011. Class 9-12 succeeded to class 6-9 in November and December 2011 (Fig. 2.3). In January there was no record of recruits of any size class at C. Branco. In March(II)/2012 some transparent early stage of a sparid fish were observed outside the sampling areas but it was not possible to collect samples and species identity could not be confirmed. In April(I)/2012 small schools of recently settled recruits (TL<1.5 cm) were found. The individuals with no pigmentation showed at least some limited control of their movements in water column, and after identification in the laboratory, part of the sample was identified as *P. bogaraveo* and other as *Diplodus* sp. For the next four weeks I saw aggregations of class 0-3 increasing in density, peaking in (0.21±0.21) in April(V)/12 (Fig. 2.3). Except for April(I)/2012 I didn't detect any further arrival of settlers. In May 2012 a successive increase in density of class 3-6 was observed, peaking in May(II)/12 (Fig. 2.3).

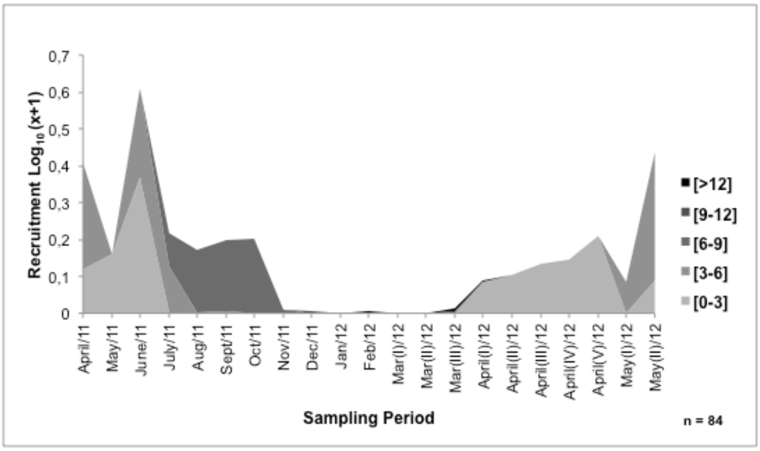

Figure 2.3. Average recruit density of *P. bogaraveo* at C. Branco (stacked area chart). Size classes: 0-3, 3-6, 6-9, 9-12 and >12 cm are represented by the gradient from lighter grey to black, in which class 0-3 corresponds to lighter grey and class >12 corresponds to black.

In P. Pim, the blackspot seabream reruits were observed during all sampling periods. Class 0-3 fish were most abundant in May 2011 (0.09±0.09), even though small schools of recent settlers (0-3) were observed, in March, adjacent to the transects and thus were not included in this analysis. The maximum density values of 0.49±0.22 and 0.52±0.25 for classes 6-9 and 9-12, respectively, were both observed in April

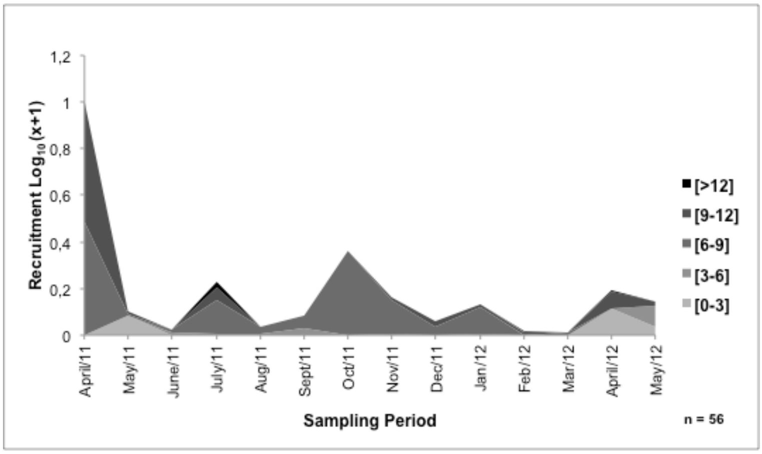

Figure 2.4. Average recruit density of *P. bogaraveo* at P. Pim (stacked area chart). Size classes: 0-3, 3-6, 6-9, 9-12 and >12 cm are represented by the gradient from lighter grey to black, in which class 0-3 corresponds to lighter grey and class >12 corresponds to black.

2011. The highest density of class 6-9 fish (0.36±0.13) was recorded later that year in October. In 2012 the highest density of class 0-3 fish (0.12±0.08) was observed in April followed by a peak of class 3-6 density (0.09±0.05) in May (Fig. 2.4).

The third study site, Doca (III), showed maximum density of 0.17±0.10 for class 0-3 in May 2011 and 0.28±0.25 for class 3-6 in June 2011. The classes 6-9 and 9-12 had maximum density in July 2011 (0.78±0.17) and in December 2011 (0.52±0.17), respectively. The class >12 had a maximum density value of 0.25±0.17 in July 2011. In January 2012 was detected the absence of the large school of recruits (9-12) (that was observed in the previous month) and from this month to April 2012 there were no blackspot seabream recruits observed. Peak recruitment occurred in May 2012 with class 0-3 showing a value of 0.23±0.14 (Fig. 2.5). Similarly to what happened in P Pim, also small schools of recent settlers (0-3) were detected in March 2012 but since they were observed outside the transect area they were not included.

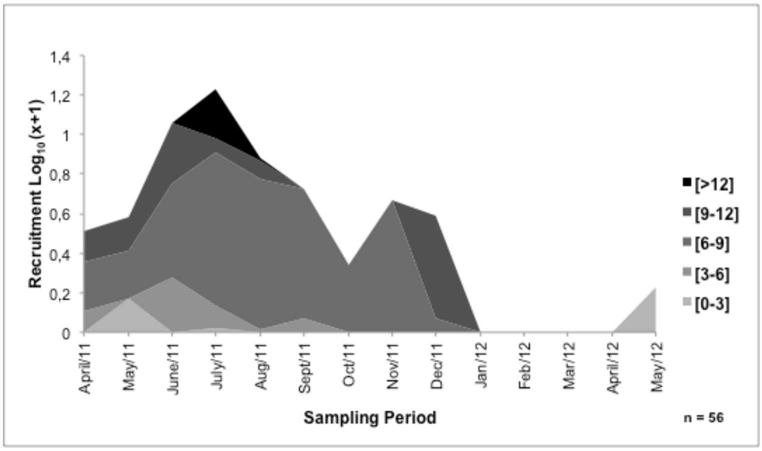

Figure 2.5. Average recruit density of *P. bogaraveo* at Doca (stacked area chart). Size classes: 0-3, 3-6, 6-9, 9-12 and >12 cm are represented by the gradient from lighter grey to black, in which class 0-3 corresponds to lighter grey and class >12 corresponds to black.

Discussion

Temporal dynamics

The results show a clear temporal trend in recruitment dynamics of *Pagellus bogaraveo*. Both the combined data from the three study sites and the data from each site separately showed the existence of recruitment peaks in two consecutive years. Recruitment patterns were consistent with the known seasonality of blackspot seabream spawning (see Krug, 1990). The density of recent settlers reached a peak between April and May and the observations of smaller recruits in late March 2012, together with the individuals collected in early April, probably resulted from the spawning season peak activity in February and March (Krug, 1990). These observations suggested that blackspot seabream is a total spawner, but spawning events may not be synchronized. Generally, if we look to the recruitment dynamics from all sites combined (Fig. 2.2), a seasonal cycle in recruit densities can be observed, i.e. the results show a clear recruitment signal over time. The highest intensity of recruitment appeared to occur in the months following the arrival of first recruits. The evolution in size structure of recruits present in the study areas over time was clear, with a wide range of juvenile size classes co-esixting over this period. Typically, after recording the highest densities (all size classes combined) around June/July a overall recruit density gradually decreased over the next few months, culminating with the disappearance of recruits from the study areas during winter months. Posteriorly the cycle restarted with the arrival of new individuals, beginning another recruitment season.

A few hypotheses could be put forward to explain the absence of recruits during the winter months at C. Branco and Doca. First, this demographic change could be the result of high mortality, which is unlikely given the high densities of recruits observed in December 2011 at Doca. In addition, post-setlement mortality is expected to be inversely proportional do recruit size/age, thus one would not expect disproportionally higher mortality of largest size classes late in the recruitment season. Secondly, fish movements within the habitat, i.e. they were not in the sampling area when surveys took place could produce this pattern, and thirdly, and most likely, ontogenically driven vertical migrations, probably cause the observed pattern. This hypothesis is in agreement with the known size specific vertical distribution in the region (Menezes et al 2006). Furthermore, the observed highest recruit densities (patchy schools), at Doca, in September, October and November 2011, followed by the observation of a single massive school of thousands of individuals in December 2011 could also suggest a signal for collective ontogenetic migration/habitat shift. In January, February and March 2012, recruits were no longer detected at the study area, providing extra support for the ontogenetic migration hypothesis. On the other hand, despite the significance of factor ‘Sampling period’, the ‘Study site’ and the ‘Interaction’ term also contributed to explain some of the observed variability. For instance, the fact that most newly settled fish were only detected at C. Branco may be explained by the fact that it is a relatively small bay, making it easier to monitor and detect changes in the fish community structure as four 250 m^2 transects covered a significant portion of the suitable habitat. Secondly, at P. Pim the evolution of size structure was not so clear, when compared to the other sites. The differences of recruitment dynamics for this study site could be related to its habitat features. Porto Pim bay is a sheltered area providing a rare shallow water habitat in the Azores (Morato et al, 2003). Such conditions make it an important nursery area for several fish species (Nash et al., 1994; Santos et al., 1994; Santos & Nash, 1995). Besides, the results from the beach-seine surveys performed between July 2011 and June 2012 also confirmed the importance of Porto Pim bay as a nursery ground for blackspot seabream (Paulino, unpublished data).

Despite the general pattern observed relative to the evolution of the size structure over time, some exceptions were observed. For instance, in April 2011 recruits ranging in size from 3 to 12 cm co-occurred and most of the time could be seen in multiple size class aggregations, indicating that recruitment is continuous within the highly seasonal recruitment period. The high density of class 6-9 observed in 2011 probably resulted from settlement events prior to the beginning of sampling. In opposition to the general trend in which older individuals probably migrate to deeper waters or other habitat a few months after settling, recruits bigger than 12 cm, though at lower densities, were observed in July and August 2011 and January, February, March and April 2012. This could indicate that some of these bigger and older recruits may have different strategies of habitat use. Apparently, some recruits when reaching a certain size, may be capable to choose their habitat and by that, extending their presence in near-shore areas contrary to the recruits that supposedly migrated. At this stage it is not clear what could be the potential advantage of such an alternative strategy. One simple explanation could be that when the juveniles attain a certain age or competence the large aggregation that where formed in during the recruitment season start to break up as it may no longer be beneficial to live in very dense aggregations where competition may increase (Forrester, 1990).

Assuming that recruits are more or less resident over the next few months after recruitment the observed evolution in the size structure of assemblages observed over time can be explained by growth, variability in recruitment intensity (consecutive addition of new cohorts or individuals) may be related to multiple processes. Pre-settlement and post-settlement processes can control recruitment variability, by acting separately or in concert (Levin, 1996; Andrews and Anderson, 2004). Recruitment variability remains an understood problem and it is accepted that all early stages (e.g. eggs, yolk-sac larvae, larvae and juveniles) can be significantly affected by variable mortality (see Houde, 1987 for review). Survival in the marine environment is a complex process (Anderson, 1988). High mortality occurs in the plankton, Cushing (1983) said that fish larvae die quickly (at 5-10% d^{-1}); either through predation, starvation, offshore larval transport, or inability to find suitable habitat before settlement competency is lost (Booth and Brosnan, 1995). The co-occurrence and subsequent impact on survivorship of a variety of physical processes that can influence larval dispersal or retention had been emphasized in various temperate systems (Richards and Lindeman, 1987). The physical transport processes that can affect availability of larvae are listed in Caselle and Warner (1996). Hamer and Jenkins (1996) found significant relationships between larval supply and short-term recruitment, suggesting that post-settlement processes, such as mortality and migration, were not strong enough, in the period shortly after settlement. Generally, it is accepted that variation in settlement is determined primarily by availability of competent larval supply (Caselle and Warner, 1996), and the rapid settlement probably offers the protection of immediate shelter, with consequent lowering of high predation rates in the water column, and the certainty of having located a suitable settlement site (Shapiro, 1987). The observations at C. Branco regarding the increase of density for class 0-3 during 2011 without detecting the arrival of new settlers and the fact that recruits were getting bigger and appeared to be more developed in May and June, than the ones observed in April 2011, could suggest that settlement (transition from oceanic pelagic to nursery habitat) occurs randomly. It is possible that the first priority for late stage blackspot seabream larvae is to settle to any coastal habitat and exit the high mortality larval stage. Then, in a second step, they may have better ability and mobility to select better quality nursery habitat and potentially converge to form larger aggregations over time. In addition they may also be mimicking the choices of older recruits (Levin, 1993) or seeking protection in numbers by aggregating with other conspecifics (White and Warner, 2007).

Such strategy, in which larvae would metamorphose and settle as rapidly as possible, would remove larvae from the water column quickly and provide immediate shelter (Shapiro, 1987). As to the second step, it is known that larvae do not behave as passively as once assumed (Caselle and Warner, 1996), they have capacity to locate suitable settlement sites, showing habitat preference (William and Sale, 1981; Cowen and Sponaugle, 1997), and are very active swimmers (Stobutzki and Bellwood, 1994). In conclusion, I speculate that the successive increase of density of class 0-3 may be the result of the addition of post-settlers that randomly settled at an adjacent location and then converged to C. Branco potentially reflecting some sort of habitat choice or other unknown selection or simply mimicking the choices of other fish.

Regarding the factors which affect recruit density, there are several natural causes of death of fish, e.g. starvation, lethal environmental conditions, and disease (Sissenwine, 1984). According to Houde (1987), predation is common to all stages. However, during surveys I directly observed post-settlers (TL<3 cm) being predated by other juvenile predatory fishes, e.g. *Pseudocaranx dentex* (Carangidae). Predation on this particular size, suggest that shortly after settlement, the young individuals were more vulnerable to this agent of mortality. Thus, schooling behavior and large post-settlement aggregations (class 0-3) may act as defense mechanisms, for instance when they had just recently settled and were more dispersed in the habitat, the opportunity of integrating in other species school, along with individuals of equivalent development stage (size), may worked as an important strategy to survive a critical post-settlement period of predation. Sale et al (1984), experimental data suggested that while mortality can be quite high during the first five days after settlement, rates of mortality decrease rapidly after that to levels not much higher than that among adults (Sale et al, 1984). Additional studies will be required to assess the impact of predation on blackspot seabream recruit mortality.

Spatial patterns

Although the main goal of this work was to describe the general temporal dynamics of blackspot seabream recruitment, the results showed that both recruit density and temporal dynamics may also vary by site as shown by the PERMANOVA analysis. More over, the interaction between the sampling period and study site was also significant, which means there was a contribution to the variability of recruit density by the way they interact (Table 2.1.) The heterogeneity in recruit density among sites was not surprising because of the different habitat features. The choice of the three replicates was not intended to make comparisons between sites, rather I seek represent better the blackspot seabream recruitment dynamics in Faial island. It is likely that part of the spatial variability observed in recruitment patterns is related to microhabitat use and by the patchy nature of recruits spatial distribution which may affect the efficiency of the sampling method.

Studies in other temperate systems have also noted that the dispersion of recruits appears to be influenced by habitat patchiness (Levin, 1993). Besides, occasionally coastal man-made structures (e.g. harbors, artificial beaches) are suggested to increase the recruitment of some littoral fish species (e.g. sparids) (Harmelin-Vivien et al, 1995), which could explain why Doca exhibited the higher densities of recruits. Apparently, appropriate modifications in habitat structures of the shallowest zones may enhance the recruitment potential of some areas. At C. Branco recruits were preferentially along the man-made wall, especially during peak recruitment (class 0-3).

Regarding, the depth distribution I observed that blackspot seabream recruits, similarly to other members of the family Sparidae, lived primarily in the shallowest zone (0 to 2 m), normally in areas sheltered from the prevailing winds (García-Rubies and Macpherson, 1995). The smallest individuals were located mostly a few centimeters (0.3 - 0.5 m) under the surface as for individuals from other size classes (>3 cm) were usually between 1 to 1.5 m under the surface, which was also observed in other sparid species (Harmelin-Vivien et al, 1995). Often, the individuals of a same fish species are differentially distributed in the environment depending on their size (Harmelin-Vivien et al, 1985), for instance at Doca, the zero values of densities in March and April 2012, could be explained by microhabitat segregation. During those months, recruits (<3 cm) were observed and collected in other shallower and sheltered areas (e.g. Horta harbor’s SE corner). However, in May 2012, recruits were observed in the sampling area (along the outer wall of the old marina break-water, just few meters from the harbor’s corner; individuals were bigger and appeared more developed). These observations, size related, suggest that as they grow over time, their habitat needs change, probably due to density-dependent mechanisms (Forrester, 1990; Levin, 1993).

Cheal and Thompson (1997) stated that no sampling program can hope to remove all sources of bias and the use of methodological pilot studies, as a precursor to visual census studies of fish, are required in order to gather the most accurate and precise data. Considering the ecology and behavior of the blackspot seabream recruits the selected monitoring method (UVC) can be considered suitable. Recruits are non-cryptic, diurnally active species and had neutral behavior in the presence of the observer, all necessary features for the UVC efficiency according to Jennings et al (2001). Besides, as a schooling species, recruits were more obvious as the school provides a much larger target image. Once one fish was sighted it was unlikely that other members of the school would be overlooked (Cheal and Thompson, 1997). Variations in transecting methods, and determinations of the inherent accuracies, precisions, and biases, have been discussed for various types of reef habitats (Buckley and Hueckel, 1989). McCormick and Choat (1987) provided one of the few studies to examine the reliability of a range of sizes to determine the optimal size for a survey. The workers found that precision of all transect sizes was reasonably close and the most precise estimates were generated by the 20x5 m strip-transects. According to their study, there was a 70% saving in time using a 20x5 m rather than a slightly smaller 15x5 m transect to obtain the same level of precision. The transect area that I adopted (50x5 m), recommended by Samoilys and Carlos (2000), is used as the basic sampling unit for many reef species (see Halford and Thompson, 1994; Harmelin-Vivien et al, 1995; Jennings et al, 2001) and it seemed suitable both to sampling areas dimensions and underwater visibility (from 5 to 15 m). Watson and Quinn II (1997) advised that if we do not have information about the fish movement, transect direction should be randomly chosen to reduce bias. As to replication and site selection, since I observed patchy distributions and some indication of microhabitat segregation, it may be important to improve survey design and increased replication. Exploratory surveys must be conducted to assess the most effective set number of transects per site, to reduce the variance and to set a standard number for data to be comparable between sites. Samoilys and Carlos (2000) described a consistent pattern of reduction in variability with increasing level of replication, but with no appreciable change in precision of estimates beyond 10 to 15 replicates. However, it must be considered that their reality was different, their work consisted in estimating the abundance of several species of coral reef fishes, and the conclusions about replication level were probably related with study sites. I suggest that the sampling frequency should be weekly, weather permitting, at least during the recruitment season (from March to July) to detect peak recruitment and collect recently settled individuals so that coupled with otolith analysis, bridges may be built between early life history traits and juvenile demography. For the other months, sampling may be monthly, to assess how post-settlement processes affect recruit densities and to gather evidences that support the ontogenetic migration theory. Regarding the sources of bias (systematic errors) around abundance estimates that cannot be reduced by additional replication, it is necessary to accept that they exist and try to ensure they are consistent from site to site or month to month by employing the same sampling technique in the same way (Jennings et al, 2001). An important principle applied to quantitative ecological fieldwork is the standardization of procedures (Smith, 1989). Establishing the specific rules by which fish are counted is likely to be one of the most important mechanisms for standardizing bias (Thompson and Mapstone, 1997). Still even when consistently biased abundance estimates are recorded they would still adequately describe temporal trends in relative recruitment rates (Jennings et al, 2001).

A major advantage of UVC method is that habitat data can be collected at the same time and that the divers gain an understanding of the fished ecosystem that is often missed by other sampling methods (Jennings et al, 2001), e.g. through UVC, Vigliola et al (1998) demonstrated that settlement for 3 sparid species occurred at about 10 mm TL, before that, sparid settlement had been studied with other methods that only caught juveniles larger than 20 to 25 mm TL. Through this study, the UVC method allowed us to observed juveniles behavior, microhabitat segregation (probably looking for refugee), school aggregations with other related species in early post-settlement stage (when they are more vulnerable) and predation events by other juvenile fishes.

Reliable estimates of population size are important in many types of ecological study, especially those concerned with the management of exploited species (McCormick and Choat, 1987). Given the quick, inexpensive and non-destructive nature of the UVC method together with the commercial importance of the blackspot seabream in the Azores, the data that could result from a recruitment monitoring program for this species, extended to other areas of the archipelago, could be of great value if incorporated in decision-support tools, i.e. prediction models of fish stock assessment and fisheries management reports. UVC as a management tool depends on the accuracy of estimating length frequency distributions and estimating fish underwater is difficult, for this reason it had been referred in the past, as an ignored fisheries management tool (Bell et al, 1985). For the past decades, several studies have been performed using UVC method to evaluate the effects of the nursery grounds, artificial reefs and fishery reserves (marine reserves) on fish communities (see Buckley and Hueckel, 1989; Tupper and Juanes, 1999; Cheminee et al, 2011; Arceo et al, 2012). I think that if included in a monitoring program, the UVC surveys would be useful for management policies but it would be essential to ensure long-term consistency in data collection and that previous considerations be incorporated in the design, especially if multiple observers would be involved in surveys over large spatial scales or long periods (see Thompson and Mapstone, 1997).

Finally, the results of the present study provide a basis for establishing the seasonal patterns of recruitment of the blackspot seabream. Further work is needed to elucidate if the patterns observed in Faial island are common to other islands of the archipelago and what additional aspects may influence the distribution and abundance of recruits. Such information would be a major step forward in our understanding of recruitment dynamics for this species.

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CHAPTER 3

EARLY LIFE HISTORY OF BLACKSPOT SEABREAM, *Pagellus bogaraveo* (PERCIFORMES: SPARIDAE)

Abstract

In this chapter I characterized the early life history traits (ELHT) of *Pagellus bogaraveo* larvae and post-settlers through the analysis of otolith microstructure of individual fish (ranging from 11.50 to 109.72 mm), collected at Castelo Branco bay in the south shore of Faial island (Azores). Prior to otolith microstructure analysis daily increment formation was validated through an otolith marking experiment, confirming the usefulness of otolith daily increment counts to determine age, size and growth of blackspot seabream early stages. The otolith microstructure analyses and the identification of the settlement mark allowed to determine total age, length-at-age, pelagic larval duration (37.37±0.28 d), estimated size at settlement (13.50±0.17 mm), and pre and post-settlement average growth rates. Average daily growth rates were twice as high during the early post-settlement stage (16.82±0.47 μm d^{-1}) than over the larval stage (7.11±0.08 μm d^{-1}). After back-calculating birth dates, which were in accordance with blackspot seabream known spawning season, comparison tests were used to compare ELHT among cohorts. Overall I found low variability in the ELHT among cohorts.

Introduction

The concept of using the underwater visual census (UVC) techniques on juveniles coupled in some cases with otolith analyses and ageing techniques to investigate the early life, dispersal and recruitment dynamics is not new (see Sponaugle and Cowen, 1994, 1996, 1997; Caselle and Warner, 1996; Bergenius et al, 2002; McCormick and Hoey, 2004; Sponaugle and Pinkard, 2004; Nemeth, 2005; Sponaugle et al, 2006; Fontes et al, 2010, 2011). For the past decades, this approach has allowed to better understand the temporal patterns of recruitment and discover some clues about the early life history period, which historically, has been referred as a "black box" for marine fishes (Cowen and Sponaugle, 1997).

Otoliths are stone-like calcium carbonate accretions situated within the semicircular canals in the heads of teleost fishes, which assist in equilibrium maintenance and sound perception (Victor, 1982). Although ages are encoded on virtually all hard body parts of bony fish, otoliths are especially reliable at recording the timing of important life-history events. The early formation of otoliths during fish development (i.e. they first appear in the embryonic stage), their protected location, and their nonresorptive qualities, allow accurate annual and daily age determinations (Jones, 1992; Campana, 2001). Thus, in the temperate regions, fisheries management largely relies on this technique for aging and the otoliths emerged as the most-often preferred anatomical structure (Fowler, 1990; Kayama et al, 2007).

Daily age information is a valuable tool for studies of early life history and factors affecting recruitment. It is necessary for back-calculating cohort-specific spawning dates and represents the best approach for estimating mortality and growth rates in young fish (Jones, 1992; Ahrenholz et al, 1994). A prerequisite, however, is the validation of the temporal periodicity of otolith increment formation (see Geffen, 1992; Ahrenholz et al, 1994). The mechanism behind daily increment formation is not well understood, and the existence of the phenomenon among all fish is undetermined. Validation studies are achieved by examining either otoliths from larvae or juveniles of known age, or by examining otoliths that have been marked on a predetermined date. In order to support studies of age validation and growth measurement, chemical compounds such as oxytetracycline (OTC), have been used to mass-mark fish at all developmental stages, including eggs, larvae, juveniles and adults (Reinert et al, 1998).

The early life of fishes involves a variety of ecological, physiological and behavioral traits that represent both adaptation and response to the pelagic environment (Cowen and Sponaugle, 1997). The interaction between these traits (e.g. benthic versus pelagic spawning, egg size, larval duration, growth rate, size and age at settlement, metamorphosis, larval behavior, etc) will determine the survival of pelagic stages and ultimately, their successful settlement and subsequent dynamics of juvenile growth and survival (see Cowen and Sponaugle, 1997 for review). According to Wilson and McCormick (1999), discrete habitat shifts such as hatching, first-feeding and settlement have been observed to coincide with changes in otolith microstructure (see Brothers et al, 1976; Victor, 1982; Sponaugle and Cowen, 1994, 1997; Bergenius et al, 2002; Raventós and Macpherson, 2001; Fontes et al, 2011). The discovery of daily increments and settlement marks on the otoliths (Panella, 1971; Wilson and McCormick, 1997, 1999) provided means of ageing fishes on a daily basis, determining the amount of time they have spent in the plankton prior to settlement, and also making possible back-calculations, which provide interesting insights into birth dates and settlement patterns (Raventós and Macpherson, 2001).

Similar to what has been done to many other temperate and tropical reef fish species and given the fact that *Pagellus bogaraveo* represents, commercially and ecologically, an important species in the Azores, it is relevant to fill this gap regarding the information about the ecology of larval and juvenile stages. Thus, the present study aims, to (1) validate otolith daily increment formation for *P. bogaraveo* juveniles, (2) determine early life history traits, namely: pelagic larval duration (PLD), i.e. the interval from hatching to settlement, and post-settlement age (PSA), size at settlement (SAS), birth dates, average larval growth (ALG) and average post-settlement growth (APSG), (3) determine the age-to-length relationship, and lastly (4) compare early life history traits among cohorts.

Methods

Study Area

The fish were collected at Castelo Branco harbor's bay (38º31.092 N; 28º43.390 W) located in the south shore of Faial Island (Azores). This location was chosen due to a number of factors. Castelo Branco harbor's bay is a sheltered easy access shallow (depth <-10 m) bay with rocky reef habitat with patches of sandy bottom small and medium size boulders covered with turf algae.

Fish collection

Recruits were collected according to their size, with three different methods, in May, July, August, October and November 2011 and April 2012. The smallest juveniles (0 to 3 cm) were captured by diver using aquarium dip nets, the individuals ranging from 3 to 6 cm by drop net and those bigger than 6 cm by fishing rod. Fish specimens were immediately stored in 500 ml plastic jars with 96% ethanol with the exception of bigger specimens (>6 cm) that were sacrificed in ice and frozen.

Otolith preparation

In the laboratory, all individuals were identified using a number of relevant morphometric traits, including the number of spines and rays of the anal fin. Which for this particular sparid species is three spines and 10-12 rays (Bauchot and Hureau, 1986). This procedure eliminated identification errors and confusion with other sparid species co-existing in the Azores (e.g. congener specie *Pagellus acarne*). Both total length (TL) and standard length (SL) of each recruit was measured in mm to the nearest 0.1 mm, and sagittal otoliths were dissected. Each pair of otoliths was separated and stored individually in micro plate wells submerged in medium viscosity immersion oil. Otoliths were kept in oil for no less then two weeks (to enhance ring clarity) (Fontes et al, 2010). After removing excess oil, left otoliths were embedded in thermoplastic resin and mounted on glass slides. Otolith preparations were later polished using (4 and 9 μm) diamond microfilm. The level of the polish was frequently checked under the microscope to determine the amount of material removed and to avoid fracturing the otolith (see Secor et al, 1991).

Otolith daily increment validation

The validation of otolith increment formation periodicity consisted in two approaches. The first method was based on a oxytetracycline (OTC) marking experiment in which 26 individuals were collected (November 2011) and 21 of them were injected with a concentration of 5 mg OTC/ ml sterile saline. The concentration administered was calculated according to the minimum value recommended (50 mg/kg) (see Brothers, 1985, 1990). The remaining 5 specimens were kept apart as the control group. Recruits were maintained alive during 14 d inside a cage (100x50x50 cm) submerged in Horta harbor. Both TL and SL of each recruit were measured to the nearest 0.1 mm in the beginning and in the end of the experiment. In order to distinguish each fish an external tag T-Bar Anchor (Floy® Small Fish Tags) was inserted below the dorsal fin using a grip pistol. Each tag had a serial number that allowed distinguishing each recruit after the OTC treatment. These procedures were completed in approximately 10 s to reduce the stress (Kayama et al, 2007). In the end of the experiment, sagittal otoliths were removed and analyzed with a fluorescence stereo microscope (Leica MZ 16 FA) equipped with external ultraviolet light source (Leica EL6000). Two examiners independently viewed the otoliths for fluorescent OTC mark presence or absence (Unkenholz et al, 1997). To avoid losing the OTC mark, the otoliths were not polish and thus increments analysis was not possible, instead I measured the length from OTC mark-to-the outer edge of the otolith. Post marking otolith increment was divided by the experiment duration (14 d) to determine recruits post-settlement average growth rate (APSG). Assuming no variation in the growth rate of marked individuals and control, post-settlement AGR of marked otoliths were then compared to post-settlement AGR from 10 individuals of the same total body size caught in the same site, whose otoliths were polished and analyzed, obtaining AGR by dividing the width of last 14 increments near of the outer edge of the otolith by the number of increments. However, it was not possible to validate the periodicity of increments before capture, therefore I have assumed that they were also daily.

The second approach consisted in the analysis of larvae otoliths provided by the Centro Oceanográfico de Vigo del Instituto Español de Oceanografia that were dissected, read and after compared to the individuals known age (days).

Otolith analysis and microstructure

For each otolith, the following measurements were determined: from rostrum to post-rostrum, from dorsal edge to ventral edge, the radius from core to the edge of post-rostrum and, from core to the settlement mark. Otolith increments were counted along the longest axis of the otolith, i.e. from core to outer edge, using transmitted light microscopy, a video camera connected to a PC and *ImagePro* 4.5 Image analysis tool kit. A single reader read otoliths once after calibration by repeatedly reading a random set of 30 otoliths until the difference between counts was less than 10% (Fontes et al, 2010). The settlement mark (SM) was identified for each otolith analyzed, using literature descriptions (see Wilson and McCormick, 1999). SM was assumed as the most well defined increment in a zone where post-settlement increments were wider than pre-settlement increments with the indicator of optical contrast between the two zones. Pre-settlement increments were defined as those occurring from the core to the SM (Raventós and Macpherson, 2001). Post-settlement increments were considered as those from SM out to the edge of post-rostrum. Pelagic larval durations (PLD) and post-settlement age (PSA) were determined for each size class. PLD determination consisted in counting the increments from core out to SM plus the addiction of 6 days.. According to Peleteiro et al (1997) the vitelline sac takes five days to be consumed and larval specimens only open the mouth on the sixth day of life. The estimation of juvenile size at settlement (SAS) was determined by back-calculation method through a linear regression of total length-on-radius of the otolith. Juvenile's birth dates were estimated using recruit's age (d) and sampling dates. Average growth rates were calculated for each life stage, average larval growth (ALG) was estimated by dividing the distance from the core to the SM by the PLD, and average post-settlement growth (APSG), dividing the distance from the SM out to the edge of post-rostrum by the post-settlement age.

Statistical Analysis

Student's *t*-test for independent samples by groups was used to evaluate the differences in AGR between OTC treatment and class 90-120 (95% confident interval). Early life history traits data were grouped by cohorts using estimated birth date as criterion (Table 3.5). The assumptions of normality and homogeneity of variances of data were tested for each response variable (PLD, SAS, ALG and APSG) with the Shapiro-Wilk test and Levene test, respectively (Quinn and Keough, 2002). If tests were positive, data were analyzed for the effect of cohort using a one-way ANOVA. When significant differences in ELHT were detected among cohorts, the post-hoc Unequal N HSD comparison test was used to determine which cohort differed from one another. If tests were negative, data were transformed in order to meet the assumptions of normality and homogeneity of variances. If not satisfactory, non-parametric Kruskal-Wallis test and multiple comparisons p-values were perfomed. All the data analyses were conducted with STATISTICA software (version 10, Statsoft).

Results

The OTC marking experiment revealed the daily periodicity of increment formation, since there was no significant difference between average growth among marked individuals and the 90-120 size class group (t-value = -1.801; df = 28; p-value = 0.082) (Table 3.1). The second approach was inconclusive, the increment clarity and definition of larvae otoliths, from the Centro Oceanográfico de Vigo del Instituto Español de Oceanografia, proved to be very difficult to examine due the absence of clear growth rings comparable to wild juveniles.

Table 3.1. Summary results of daily increments validation method. TL – Total length (mm); AGR – Average growth rate. AGR (OTC) = length from the OTC mark-to-the edge of the otolith divided by 14 days of experiment (μm d^{-1}); AGR (Class 90-120) = length of 14 increments divided by the number of increments (μm inc^{-1}).

	Mean TL (mm; s.e.)	**Mean AGR (*μm d^{-1}; **μm inc^{-1}; s.e.)**	**Range**	**CV**	**n**
OTC	99.29 (1.32)	11.56 (0.53)*	8.07 – 16-50	20.67	20
Class 90-120	103.66 (1.41)	13.00 (0.37)**	10.55 – 14-39	8.93	10

The daily increment clarity and definition were very visible on the sagittae from recent settlers. It was also visible the presence of the subdaily increments (i.e. faint increments occurring between dark, well-defined increments) (Fig. 3.1).

The type of settlement mark (SM) identified was a combination of type II and type III descriptions (see Wilson and McCormick, 1999). SM was identified as the most well defined increment in a zone where post-settlement increments were wider than pre-settlement increments with the indicator of optical contrast between the two zones. I found SM on 78 otoliths from post-settled individuals (Fig. 3.2). Sagittal otoliths from recent settlers (n=6) did not show SM (Fig. 3.1).

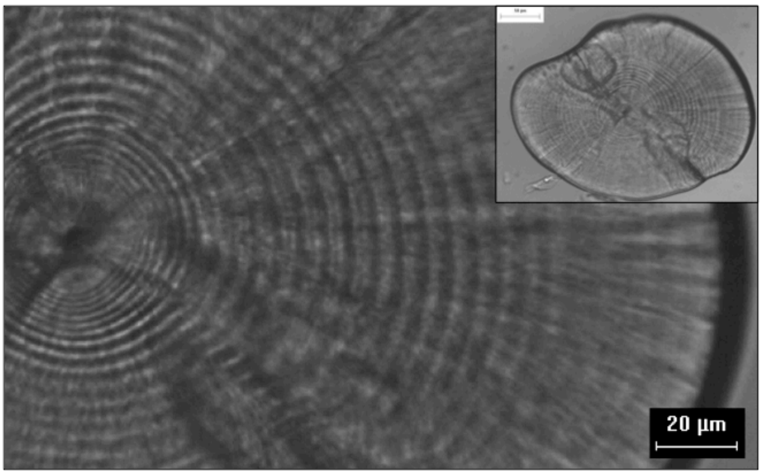

Figure 3.1. Sagitta from *P. bogaraveo* recently settler collected at Castelo Branco harbor's bay (Faial Island, Azores), showing the otolith increments definition. White background scale bar = 50 µm, black background scale bar = 20 µm.

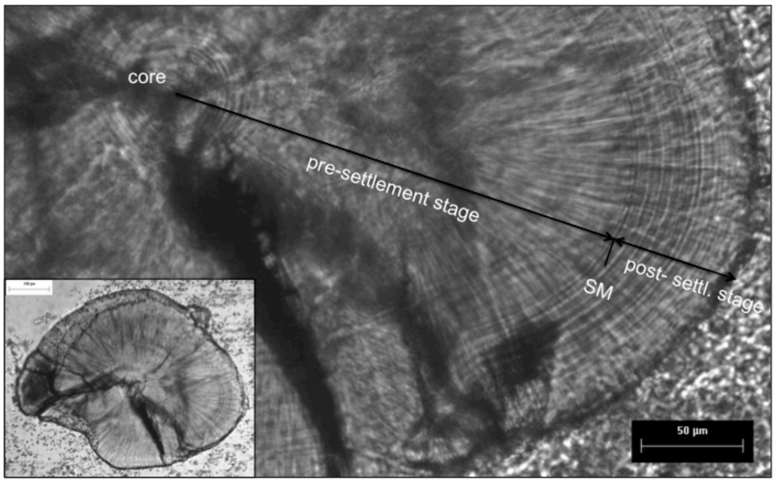

Figure 3.2. Sagitta from *P. bogaraveo* recruit collected at Castelo Branco harbor's bay (Faial Island, Azores). SM = Settlement mark. White background scale bar = 100 µm; black background scale bar = 50 µm.

The pelagic larval duration (PLD) was determined for 84 individuals. Average PLD was 37±0.28 days ranging from 32 to 42 days (CV = 6.84). Size at settlement (SAS) was estimated by back-calculation method through a linear regression, where y is radius of the otolith and x is the total body size of the recruit (see Fig. 3.4). Estimated average size at settlement was 13.50±0.17 mm (CV = 11.21). Average larval growth rate was 7.11±0.08 µm d^{-1} and average post-settlement growth rate was 16.82±0.47 µm d^{-1} (Table 3.2).

Table 3.2. Early life history traits for *P. bogaraveo* juveniles collected at Castelo Branco harbor's bay (Faial Island, Azores). PLD – Pelagic larval duration (d); SAS – Size at settlement (mm); ALG – Average larval growth (μm d^{-1}); APSG – Average post-settlement growth (μm d^{-1}).

	Mean value (s.e.)	**Range**	**CV**	**n**
Length from core-to-SM (μm)	264.78 (3.56)	200.70 – 338.14	11.88	78
PLD (d)	37.37 (0.28)	32 – 42	6.84	84
Estimated SAS (mm)	13.50 (0.17)	10.35 – 17.03	11.21	84
ALG (μm day^{-1})	7.11 (0.08)	5.15 – 8.69	10.55	84
APSG (μm day^{-1})	16.82 (0.47)	2.83 – 30.18	24.83	78

Otoliths from each size class were analyzed and ELHT are reported in Table 3.3 and 3.4. Mean PLD was not significantly different among size classes. PLDs ranged from 35 to 36 days for younger juveniles (0–15) (CV = 1.46) and from 38 to 41 days for oldest juveniles (90–120) (CV = 2.41). The mean post-settlement age (PSA) for the smallest size class (0–15) was considered 1 day by default, since individuals did not exhibit SM but were already considered post-settlers. PSA for the remaining size classes ranged from 5 days for the 15 – 20 mm size class and 141 days (4 months and 21 d) for the oldest/largest size class sampled. Individuals from the class 0–15 settled at 14.22 mm total length. For the following size class (15 – 20) the mean estimated SAS was 12.84 mm, for the 30 – 60 class was 14.17 mm and for the largest size class (90 – 120) was 13.30 mm (Table 3.3). It is also interesting to note that the variation associated with SAS estimates for the two smaller size classes and youngest post-settlers is larger than the variability associated with the remaining classes.

The back-calculated birth dates (month/year) were determined for each size class (Table 3.4). Since it was not possible to measure the distances between individual increments, average growth rates were calculated for both pre and post-settlement stages (Table 3.4).

Table 3.3. Summary of early life history traits for each size class of *P. bogaraveo* juveniles collected at Castelo Branco harbor's bay (Faial Island, Azores).

Year	Month	Size Class (mm)	Mean TL (mm; s.e.)	Range	CV	Mean PLD (d; s.e.)	Range	CV	Estimated Mean SAS (mm; s.e.)	Mean PSA (d; s.e.)	n
2012	April	0-15	12.18 (0.28)	11.50 - 13.40	5.65	35 (0.21)	35 - 36	1.46	14.22 (0.51)	1 (0)	6
2011	May	15-20	18.70 (0.41)	17.34 - 19.63	5.37	35 (0.60)	33 - 37	4.19	12.84 (0.73)	5 (0.61)	6
	May	20-25	22.53 (0.35)	20.14 - 24.98	6.89	37 (0.61)	33 - 42	7.40	13.12 (0.38)	8 (0.76)	20
	May	25-30	26.78 (0.48)	25.03 - 29.45	6.27	35 (0.62)	32 - 39	6.07	12.98 (0.35)	15 (0.65)	12
	May. July	30-60	44.79 (1.82)	30.16 - 58.41	18.19	38 (0.53)	35 - 42	6.11	14.17 (0.37)	45 (3.42)	20
	July. August	60-90	66.68 (1.19)	61.94 - 71.52	5.63	39 (0.49)	37 - 42	3.95	13.86 (0.29)	75 (2.11)	10
	October	90-120	103.66 (1.41)	95.07 - 109.72	4.31	39 (0.30)	38 - 41	2.41	13.30 (0.40)	141 (4.65)	10

Table 3.4. Age, back-calculated birth dates, average larval growth (ALG) and average post-settlement growth (APSG) for each size class of *P. bogaraveo* juveniles collected at Castelo Branco harbor's bay (Faial Island, Azores). Dashes (-) mean that individuals did not exhibited the settlement mark.

Year	Month	Size Class (mm)	Mean Age (d; s.e.)	Range	CV	Mode Birth Date	ALG (μm day^{-1}; s.e.)	CV	APSG (μm day^{-1}; s.e.)	CV	n
2012	April	0-15	36 (0.21)	36 - 37	1.42	February 2012	7.77 (0.27)	8.59	-	-	6
2011	May	15-20	40 (0.95)	37 - 44	5.82	April 2011	7.18 (0.47)	16.15	15.86 (2.71)	41.83	6
	May	20-25	45 (0.83)	38 - 51	8.34	April 2011	6.98 (0.17)	10.67	20.08 (1.13)	25.18	20
	May	25-30	50 (1.00)	45 - 56	6.89	March 2011	7.22 (0.20)	9.56	17.49 (0.67)	13.35	12
	May. July	30-60	83 (3.64)	53 - 107	19.52	April 2011	7.26 (0.17)	10.34	15.65 (0.58)	16.46	20
	July. August	60-90	114 (2.15)	100 - 124	5.92	May 2011	6.97 (0.17)	7.53	15.85 (0.74)	14.78	10
	October	90-120	180 (4.77)	156 - 200	8.38	April. May 2011	6.65 (0.18)	8.43	13.36 (0.49)	11.58	10

The length-at-age relationship obtained for 84 recruits followed a linear regression model ($R^2 = 0.98$), data are best described by the equation: L= (A-9.91)/1.63 (Fig. 3.3). Also, relationships between the total length and the radius of the otolith (Fig. 3.4), and between the age and the radius of the otolith (Fig. 3.5) followed a linear regression model.

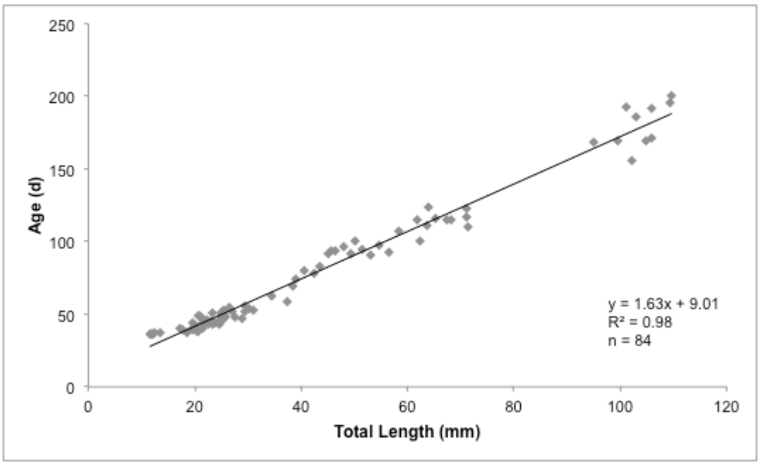

Figure 3.3. Length-at-age relationship for 84 recruits of *P. bogaraveo*.

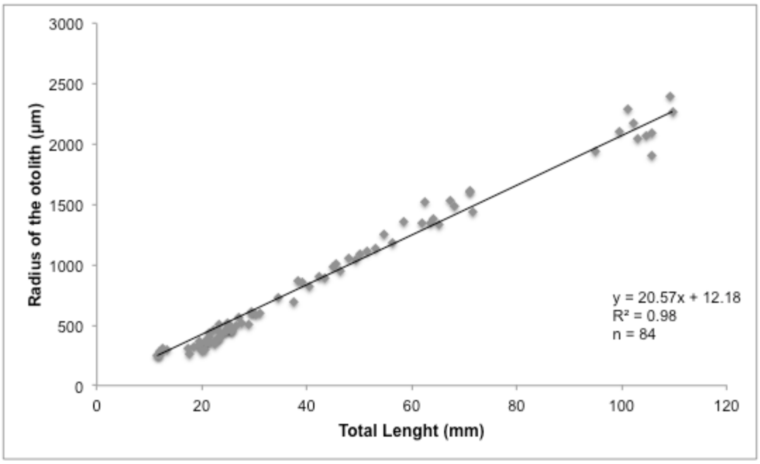

Figure 3.4. Relationship between the total length (mm) and the radius of the otolith (µm) for 84 recruits of *P. bogaraveo*.

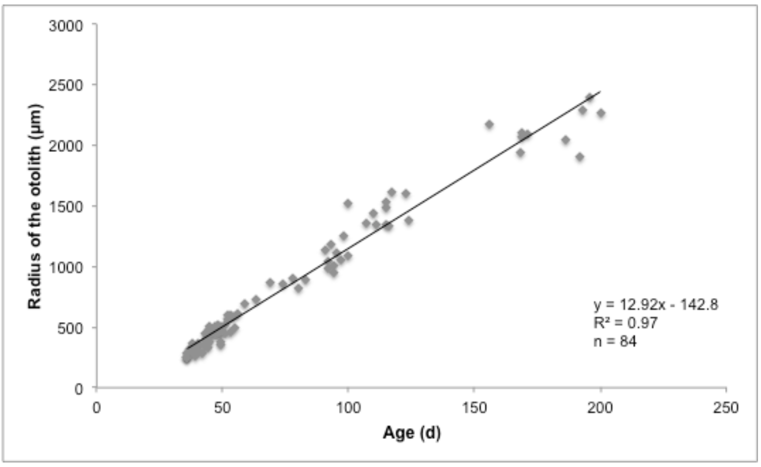

Figure 3.5. Relationship between the age (d) and the radius of the otolith (μm) for 84 recruits of *P. bogaraveo*.

After the otoliths were analyzed, individuals were grouped into cohorts according to their back-calculated birth dates. I obtained seven groups (one otolith was excluded in order to avoid having a cohort composed of a single individual) (Table 3.5).

Table 3.5. Cohorts of *P. bogaraveo* obtained from the estimated birth dates.

Cohorts	Estimated birth dates	n
5MAR11	27 February - 11 March 2011	4
26MAR11	20 March - 31 March 2011	18
6APR11	1 April - 10 April 2011	25
16APR11	11 April - 20 April 2011	17
26APR11	21 April - 30 April 2011	4
7MAY11	2 May - 11 May 2011	9
29FEV12	28 April - 29 April 2012	6

PLD data did not conform the assumptions of normality and homogeneity of variance, even after several attempts of data transformation. Therefore, non-parametric Kruskal-Wallis test was performed and the results showed significant differences (Kruskal-Wallis: H=15.39; p=0.017; d.f.=6) for PLD (Table 3.6.). The multiple comparisons p values test examined that significant differences occurred between cohort 7MAY11 and 29FEV12 (Table 3.6, Fig. 3.6). As to other ELHT (estimated SAS, ALG and ln-transformed APSG), the results from one-way ANOVA tests revealed no significant differences (p>0.05) among cohorts (Appendix 3.1, 3.2 and 3.3).

Table 3.6. Summary of Kruskal-Wallis test with multiple comparison p values results comparing *P. bogaraveo* PLD among cohorts. *p<0.05.

Kruskal-Wallis test: H (6, N= 83) =15.38 p=0.017*

Multiple Comparisons p values (2-tailed)

Cohorts	5MAR11	26MAR11	6APR11	16APR11	26APR11	7MAY11	29FEV12
	R: 49.13	R: 35.03	R: 43.52	R: 37.68	R: 58.88	R: 62.56	R:22.00
5MAR11		1.00	1.00	1.00	1.00	1.00	1.00
26MAR11	1.00		1.00	1.00	1.00	0.11	1.00
6APR11	1.00	1.00		1.00	1.00	0.89	1.00
16APR11	1.00	1.00	1.00		1.00	0.26	1.00
26APR11	1.00	1.00	1.00	1.00		1.00	0.37
7MAY11	1.00	0.11	0.89	0.26	1.00		0.03*
29FEV12	1.00	1.00	1.00	1.00	0.37	0.03*	

Cohorts exhibited relatively greater variation in PLD than estimated SAS (Fig. 3.6). The results showed a linear relationship between the PLD and SAS for five cohorts (5MAR11, 26MAR11, 6APR11, 16APR11 and 26 APR11), i.e. as the mean SAS increased with mean PLD (Fig. 3.6.). However, both 7MAY11 and 29FEV12 contradicted the previous pattern because both showed two different means of PLD for an approximated mean SAS. In addition 29FEV12 cohort exhibited the minimum value for mean PLD (35±0.21 d), whereas 7MAY11 showed the maximum value (40±0.44 d). The cohort 29FEV12 had also the smallest dispersion in PLD values compared to all other cohorts, and cohort 5MAR11 showed the highest variation for both traits (mean PLD, 38±1.49 d; mean SAS, 14.38±0.66 mm).

Considering the average growth rates prior and after settlement, I obtained higher variation for APSG than in ALG (Fig. 3.7). Most interesting was the significantly higher growth rates observed in the post-settlement stage in relation to the larval stage, particularly in the two earlier size classes for which average post settlement age ranged between 5 and 8 days, that is, growth rates roughly doubled in the first week following settlement in respect to previous larval growth (Table 3.3 and 3.4). Cohorts 16APR11, 26APR11 and 5MAR11 showed higher dispersion for APSG compared to others. Cohort 26APR11 showed the minimum value (14.27±1.14 µm d^{-1}) for APSG, whereas cohort 06MAR11 had a maximum value of 18.45±0.86 µm d^{-1}. Both 5MAR11 and 26APR11 exhibited the highest values for ALG but their APSG were lower than others, except for 7MAY11, which had the minimum value of 6.82±0.18 µm d^{-1} for ALG (Fig. 3.7).

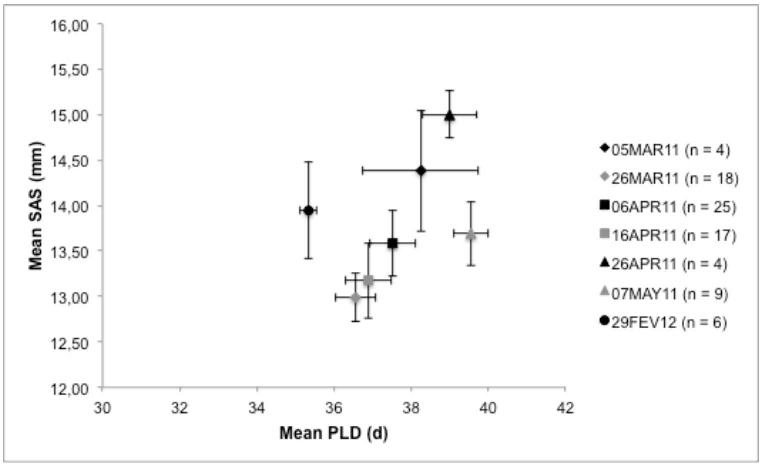

Figure 3.6. Mean larval duration (PLD) vs. mean estimated settlement size (SAS) for each cohort of *P. bogaraveo*. Error bars indicate ± SE.

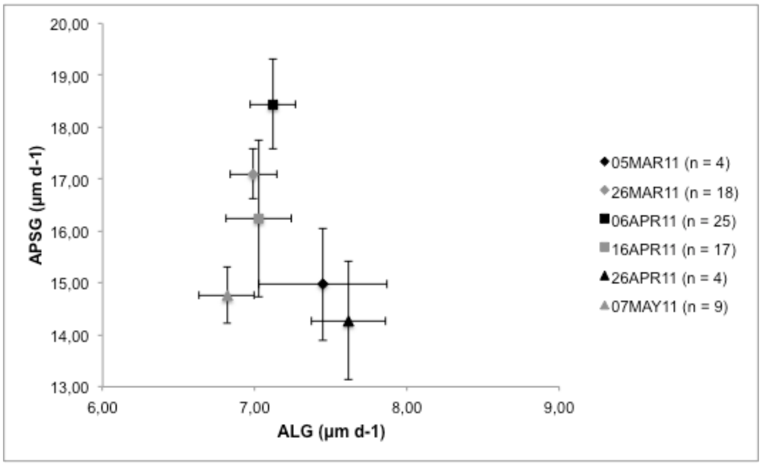

Figure 3.7. Average larval growth (ALG) vs. average post-settlement growth (APSG) for each cohort of *P. bogaraveo*. Cohort 29FEV12 was not included because individuals were recently settlers without SM. Error bars indicate ± SE.

Discussion

The daily age information obtained from larval and juvenile fish otoliths is a valuable tool for studies of early life history and factors affecting recruitment (Jones, 1992; Ahrenholz et al, 1994). However, it is of critical importance to validate the rate of increment formation (Geffen, 1992). Therefore, the results from the validation experiment by OTC marking for *P. bogaraveo* juveniles were essential to proceed to ELHT analysis. The conditions in which juveniles were kept during the fourteen days of experiment, conformed to what is described as the optimum environment for validation studies, where photoperiod and temperature cycles reflect natural conditions (see Geffen, 1992). The reason why I chose to validate through the comparison growth rates from control individuals was to prevent the loss of the OTC mark during otolith processing, i.e. otolith preparation methods can sometimes destroy the mark if overpolishing or cracking occurs (Reinert et al, 1998). For future studies, I propose to conduct an OTC marking experiment with newly settled individuals (e.g. using the immersion method, Bergenius et al, 2002) in order to examine the OTC mark and otolith increments without requiring prior preparations. On the other hand, the difficulties in examining the otoliths from larvae of known age, provided by the Centro Oceanográfico de Vigo del Instituto Español de Oceanografia, were probably related to larval culture conditions. Individuals were grown under artificial light 24 hours of the day and available food 24 hours of the day. The differences in the increment clarity and definition became evident when comparing to the sagittae from the most recently settled juveniles (Fig. 3.1).

The SM was defined on the basis of the abrupt changes in increment width and ring pattern and definition (Wilson and McCormick, 1999). The SM position and properties was highly consistent among all otoliths analyzed (CV = 11.88) (Table 3.2). The SM type identified for *P. bogaraveo* combined the traits of three different SM types described in Wilson and McCormick (1999). In addition, the otolith radius from the recently settled fish were an appropriate means of confirming settlement marks, since the total number of increments of the newly settled individuals was similar to the number of increments before the SM on the otoliths of post-settlers. The fact that otoliths from newly settlers did not show SM corroborates other studies, in which the SM was never observed in newly settled individuals (Raventós and Macpherson, 2001). Therefore, I assumed that the microstructural mark identified, corresponded to the settlement transition (Fig. 3.2). The presence of SM on otoliths has proved to be valuable in determining larval duration in many species (Wilson and McCormick, 1997; Raventós and Macpherson, 2001), which perhaps is one of the most extensively studied characteristics of the ELH (Cowen and Sponaugle, 1997). Within the range of larval durations, certain groups have consistently low (and relatively invariant) larval duration (e.g. pomacentrids, Wellington and Robertson, 2001), while other taxa express considerable variability among and even within species (e.g. gobiids, Sponaugle and Cowen, 1994). The mean PLD of 37.37±0.28 d (CV = 6.84) was very similar to those obtained by Raventós and Macpherson (2001) for some species of the family Sparidae (Table 3.2), which also supported the daily increment validation. The authors found PLD of 38, 31.2 and 38 d for *Pagrus pagrus*, *Sarpa salpa* and *Spondyliosoma cantharus*, respectively. Comparably to *P. bogaraveo*, all previous sparid species have also pelagic spawning and all settle in winter-spring, contrary to other sparids, which settle in summer and showed shorter PLD (e.g. *Diplodus* sp.) (Raventós and Macpherson, 2001). Since the initiation of increment formation was unknown for *P. bogaraveo*, I chose to add 6 days to the total number of larval increments to take into account the first-feeding period described by Peleteiro et al (1997). However, the time of embryonic development was not accounted, so I recommend that future research give emphasis in determining the time at which the first otolith increment is formed because it is important for accurate age interpretations as well as back-calculating the date of hatch.

Through retrospective age determination I was able to demonstrate that the individuals collected in October 2011 (class 90-120 mm), despite their total body size, belong to the same recruitment season as the smaller individuals sampled in May 2011 (TL<30 mm) (Table 3.3), which supports the idea that blackspot seabream juveniles are resident in the same area during their first months post-settlement. The back-calculated birth dates were all in accordance to what is described for *P. bogaraveo* spawning season (see Krug, 1990) (Table 3.4.).

According to the literature, PLD is typically more variable than the SAS (Cowen and Sponaugle, 1997). However, the PLD did not show much variation (CV = 6.84) among individuals, which may be related to differences in the pelagic conditions experienced by larvae and the strength of selection occurring before settlement, as demonstrated in a study by Meekan and Fortier (1996). Also, the estimated SAS did not show much variation (CV = 11.21) for most of the aged recruits, I found that the smallest and most recently settled recruits (April 2012) sampled were smaller (12.18±0.28 mm TL) than the average SAS estimated for the remaining cohorts sampled (13.50±0.17 mm). This difference could be the result of bias in SM estimates from otolith analysis in large recruits. Another possibility is that selective mortality systematically removed the smaller, possibly lower condition, settlers from the population. Selection over some arbitrary period of time after settlement would result in the preferential survival of larger settlers producing higher average SAS. In fact, post-settlement selection has been documented for a number reef species and, in some cases, selection was shown be most intense in the short period following settlement (Booth, 1995; Carr and Hixon, 1995; Tupper and Boutilier, 1995; McCormick, 1999;

Meekan et al., 2006; Fontes, 2009).

The growth histories recorded in otoliths of fishes provide a means to open and explain the interior of the larval "black box" (Vigliola and Meekan, 2002). For instance, studies analyzing otoliths of temperate marine fish larvae have reported that differential larval growth rates can persist throughout the larval period and suggest that faster growing larvae metamorphose earlier and at a larger size, and have a survival advantage (Nemeth, 2005). Given the disproportionality between the time that blackspot seabrem larvae spend in plankton (from 32 up to 42 days) and the time of post-settlement stage (from 5 up to 141 days) of analyzed fish, I obtained a higher variation for APSG than in ALG (Fig. 3.7), and APSG was twice as high when compared to the ALG (Table 3.2). Thorrold and Milicich (1990) also found in two damselfish species that growth after settlement was significantly faster than growth prior to settlement. According to the authors this growth trend has been showed in *Haemulon flavolineatum* (Brothers and McFarland, 1981), *Thalassoma bifasciatum* (Victor, 1986c), *Abudefduf abdorninalis* (Radtke, 1985), and *Pomacentrus nagasakiensis* and *Pomacentrus wardi* (Pitcher, 1988). Values of 0.34 and 0.28 mm d^{-1} for *Chromis atripectoralis* and *Pomacentrus coelestis,* respectively are in the same range as growth in post-settlement *Thalassoma bifasciatum* (0.31 mm d^{-1}; Victor 1986b), and *Pomacentrus nagasakiensis* and *Pomacentrus wardi* (0.15 to 0.25 mm d^{-1}; Pitcher, 1988), but are slower than in newly settled *Abudefduf abdominalis* (0.57 mm d^{-1}; Radtke, 1985). After converting the otolith APSG to fish body size APSG I obtained a mean of 0.18±0.032 mm d^{-1} for the six cohorts of blackspot seabream, which is within the prior growth ranges. This difference between larval growth and post-settlement growth may be an important clue to the understanding of blackspot seabream life strategy. Although, there is not much information about juvenile growth in sparid species or other demersal species whose life cycle is analogously complex. The circumstantial evidence available suggests that the blackspot seabream has a complex life cycle where the mature demersal populations, some of them inhabiting seamounts, release pelagic eggs and larvae, which recruit in coastal nurseries. In this context, the enhanced juvenile growth, and probably lower mortality, in nursery areas compared to pelagic larval stage could be one of the advantages of such a complex life cycle, involving large scale movements and dramatic habitat shifts.

The relationship between the total body length and the radius of the otolith was highly correlated (Fig. 3.4), indicating that at least during the juvenile stage, the otolith growth is an accurate index of somatic growth. Besides, both relationships between the total body length and age (Fig. 3.3), and between age and the otolith radius (Fig. 3.5), also suggested that during this development stage, both otolith growth and somatic growth are linear to the age of fishes.

After back-calculating birth dates, I was able to group *P. bogaraveo* juveniles into cohorts, which could play an important role for future research and fisheries management of the species. The analysis of results indicated that estimated SAS, ALG and APSG did not differ significantly between cohorts (Appendix 3.1, 3.2 and 3.3), which could be related to the fact that all specimens that were examined, were the survivors, i.e. the product of selection. Alternatively, they may all have had a very similar early life history path until the moment of sampling, regardless of the development stage or the month when were collected. From the moment of conception, individuals vary in development and growth rates, which will pre-dispose some individuals to a lower probability of surviving later developmental stages (McCormick and Hoey, 2004). On the other hand, once I obtained cohorts from February to May, I could have expected obtain differences in growth rates similar to Oxenford (1994). The author found that individuals that have hatched in warmer months (i.e. April-July, toward the end of the spawning season, similarly to blackspot seabream) grew significantly faster than those hatched in colder months (i.e. November-March, toward the beginning of the spawning season), according to him, those patterns were consequence of temperature effects on metabolic rate.

Regarding the test results for larval duration among cohorts, the significant differences between cohort 7MAY11 and 29FEV12 (Table 3.6, Fig. 3.6) may be related to fact that both cohorts were from different seasons, and one was from the beginning of the spawning season (29FEV12) as the other was from the end of the spawning season (7MAY11), which may implicate different conditions in plankton, i.e. variations in temperature and/or available food. Overall, there was not much variation between PLDs and the positive linear relationship between the PLD and SAS for five cohorts (Fig. 3.6) is in accordance to the strong correlation between PLD and SAS found in Fontes (2009). Cowen and Sponaugle (1997) stated that PLD could be determined by a tradeoff between settling rapidly in order to initiate higher growth rates as juveniles, versus extending the larval stage beyond competency to settle in order to maximize chances of settling under optimal conditions. While short PLD should reduce larval mortality (Leggett and Deblois, 1994), larvae spending longer periods in the plankton tend to settle at larger sizes (Denit and Sponaugle, 2004). Even though my experimental design was not been planned to link larval history to juvenile demography, I believe that is the next step towards the understanding of recruitment of blackspot seabream. In order to accomplish it, future work should incorporate shorter sampling interval, possibly every other day, at least during the peak recruitment period, for both census surveys and recent settlers collection. That way it would be possible to investigate potential links between early life history, such as larval growth rate, size at age and size at settlement, and recruitment magnitude as well as post-settlement survival and selection. Such a sampling design should allow testing the ‘stage-duration’ hypothesis, as well as the ‘bigger-is-better’ hypothesis. The 'stage duration' hypothesis predicts that larvae which experience favorable feeding conditions, and therefore grow quickly, will achieve metamorphosis at earlier ages and experience lower cumulative mortality due to predation during the larval stage when mortality rates are known to be high, while the 'bigger is better' hypothesis holds that larger larvae are less susceptible to predation. Hence the prediction is that larvae, which hatch at larger sizes, or grow at faster rates, thereby achieving larger body size at a given age, should be less vulnerable to predation (Leggett and Deblois, 1994; Miller et al, 1988).

In summary, I have made important excursions into the “black box” of early life history of blackspot seabream, providing a basis from which future studies should continue to investigate by testing the prior hypotheses and understanding the underlying mechanisms, which should improve our ability to make more reliable predictions concerning recruitment variability. In addition, understanding and exploring the relationships between the ever changing pelagic environment and both the early life traits and recruitment dynamics, i.e. integrating biological and environmental sciences, could potential support the use of widely available remote sensing tools to access pelagic conditions and predict the larval survival. Our ability to make reliable predictions over wide spatial scales could significantly contribute to our understanding of population dynamics and ultimately improve resource management strategies.

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SYNTHESIS AND CONCLUSIONS

Until now most researchers agreed that, at least part of the juvenile blackspot seabream population, spent most of their first year in inshore waters while high resolution information about the temporal dynamics of recruitment and early life history information from wild fish were nonexistent. Through this work a relevant basis of information regarding the larval and early juvenile stages of the blackspot seabream life cycle is now available as well as high resolution data on the temporal dynamics of recruitment.

The first part of this study allowed me to describe the seasonal pattern of recruitment and determine when recruitment peaks are expected as wells as size structure evolution of post-recruitment, namely the succession of recruit size structure overtime. The recruitment peak was detected in April and May in two consecutive years and observations regarding the older recruits are consistent with the hypothesis of ontogenetic vertical migration starting sometime during the winter months. Regarding the early life history, the second approach confirmed the usefulness of otolith microstructure analysis to retrospectively investigate the pelagic larval and juvenile histories, particularly the pelagic larval duration period (PLD), size at settlement (SAS), total age, birth date, larval growth, post-settlement growth and determine the length-at-age relationship. The contrast observed between the larval growth and post-settlement growth may represented the most important findings concerning the early life history, since it could be an important clue to the understand of the blackspot seabream complex life strategy.

For future directions, I would recommend that the next step should focus on investigating the link between larval history, environmental conditions and recruitment. Understanding the pre and post-settlement processes, which may affect juvenile demography, i.e. recruitment magnitude, post-settlement survival and population dynamics. Finally, given the economic and ecological relevance of this species I fell that implementing a recruitment monitoring program, at the archipelago scale, to determine spatial patterns and recruitment magnitude would potentially improve the performance of conservation and management plans. Knowledge of spatial patterns of recruitment and identification of important nursery areas are particularly important for spatially explicit management and conservation tools such as marine protected areas designed to protect important nursery areas from where adult populations can be replenished. These actions can potentially allow us to, in the future, make reliable predictions about recruitment and over wide spatial scales and ultimately improve resource management and conservation strategies.

APPENDIXES

Appendix 2.1. Summary of PERMANOVA pairwise tests results for term ‘Interaction’ for pairs of levels of factor ‘Sampling period’ within each level of factor ‘Study site’. The results presented are only the pairs from the 91 groups obtained for each level of factor ‘Study site’, whose significance level had meaning, i.e. the pair corresponded to consecutive months (*p<0.05).

Within level 'C. Branco' of factor 'Study site'			
Groups	**t**	**P(perm)**	**perms**
October/11, November/11	1.46	0.029**	35
Within level 'P. Pim' of factor 'Study site'			
Groups	**t**	**P(perm)**	**perms**
September/11, October/11	2.14	0.04*	35
November/11, December/11	2.65	0.026*	25
December/11, January/12	2.84	0.025*	25
January/12, February/12	3.88	0.024*	25
Within level 'Doca' of factor 'Study site'			
Groups	**t**	**P(perm)**	**perms**
June/11, July/11	1.47	0.048*	35
November/11, December/11	3.66	0.035*	18
December/11, January/12	44.39	0.032*	8

Appendix 2.2. Summary of PERMANOVA pairwise tests results for term 'Study site'. *p<0.05.

Term 'Study site'			
Groups	**t**	**P(perm)**	**perms**
C. Branco, P. Pim	2.25	0.001*	998
C. Branco, Doca	1.73	0.027*	998
P. Pim, Doca	2.08	0.002*	998

Appendix 3.1. Summary of the one-way ANOVA results comparing *P. bogaraveo* estimated SAS with cohorts. SS – Some of squares; DF – Degrees of freedom; MS – Mean squares; *p<0.05.

	SS	**DF**	**MS**	**F**	**p**
Intercept	10045.96	1.00	10045.96	4556.66	0.00
Cohort	20.38	6.00	3.40	1.54	0.18
Error	167.56	76.00	2.20		

Appendix 3.2. Summary of the one-way ANOVA results comparing *P. bogaraveo* ALG with cohorts. SS – Some of squares; DF – Degrees of freedom; MS – Mean squares; *p<0.05.

	SS	**DF**	**MS**	**F**	**p**
Intercept	2766.04	1.00	2766.04	5203.94	0.00
Cohort	5.23	6.00	0.87	1.64	0.15
Error	40.40	76.00	0.53		

Appendix 3.3. Summary of the one-way ANOVA results comparing *P. bogaraveo* APSG (ln-transformed data) with cohorts. SS – Some of squares; DF – Degrees of freedom; MS – Mean squares; *p<0.05. Cohort 29FEV12 was not included because individuals were recently settlers without SM.

	SS	**DF**	**MS**	**F**	**p**
Intercept	740.62	1	740.62	2723.14	0.00
Cohort	2.31	5	0.46	1.70	0.15
Error	19.31	71	0.27		